JARQ 56 (4), 313-320 (2022) https://www.jircas.go.jp

REVIEW

Blast Disease of Ryegrass in Japan: The Characteristics, Geographical Distribution, and Control by Fungicide-coated Seeds

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Abstract

The blast of Italian ryegrass (*Lolium multiflorum*) and perennial ryegrass (*L. perenne*) is a serious disease, especially in the southwestern region of Japan, and it causes seedling blight and directly affects crop yield. Isolates of the pathogenic fungus were obtained from ryegrass of Miyakonojo (31°N), Kyushu to Osaki (38°N), Tohoku, Japan from 1999 to 2010, and 126 isolates were identified as *Pyricularia oryzae* based on the sequences of rDNA-ITS and the *β-tubulin* gene. The pathogen was inoculated into the seedlings of Italian ryegrass under different temperatures and showed the most rapid lesion enlargement at 31°C and more abundant sporulation from 22°C to 31°C within the tested range of 19°C and 34°C, which suggested that the pathogen favored medium–high temperatures. Italian ryegrass seeds were coated with fungicides, and this method was found to be effective in controlling the disease in the field. Orysastrobin, a permeable QoI fungicide, was more effective than benomyl or probenazole in suppressing disease occurrence. The effect was clear regardless of the Italian ryegrass cultivar used. Orysastrobin most suppressed lesion enlargement and sporulation in the seedling inoculation tests. Especially in warm and wet regions, the fungicide-coated seeds method is an effective technique for suppressing the disease.

Discipline: Agricultural Environment
Additional key words: genetic phylogeny, plant pathogen, *Pyricularia*, sporulation, temperature

Introduction

Because of their high productivity, palatability, and digestibility for livestock, Italian ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*Lolium perenne* L.) are considered important forage grasses worldwide. They are used in hayfields and grazing lands across Japan, and it is estimated that nearly 70,000 ha existed in 2019, which is the largest share of forage grass cultivation. Of the 36 diseases of ryegrasses reported in Japan (PSJ 2021), the blast disease caused by the fungus, *Pyricularia oryzae* Cavara, is one of the most serious, especially in the southwestern regions such as the Kyushu and Chugoku districts. It is also known that the wheat isolates originate from ryegrass isolates by host jumping (Tosa et al. 2016). The causal fungus was not pathogenic to rice (Kusaba et al. 1998). The disease was first reported in the Miyazaki Prefecture, Kyushu, in 1979, which spread quickly until the 1990's, mainly in the southwestern region, and the increased damage spread to the Kanto district in central Japan (Nishimi et al. 2009). As the disease aggravates relatively high temperatures (approximately 25°C) and causes seedling death and dieback soon after seeding in Italian ryegrass, particularly in early seedings from late summer to early autumn, late seeding after October is recommended to avoid the damage caused by the disease (Sumida et al. 2003). However, the detailed reaction to the temperatures of the pathogen is unknown. The pathogen is the same species that causes rice blast, and the rice pathogen attacks ryegrass under favorable conditions (Nishihara 1981). Seedborne transmission is evidenced by the presence of the same ryegrass lineage of the pathogen in the USA and Japan (Tosa et al. 2007).

This study aimed to isolate the blast pathogen of

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Received 28 September 2021; accepted 11 January 2022.

forage crops, including ryegrass, from a wide area of Japan and to identify the genetic differences among the pathogens by phylogenetic analyses. The reactions at different temperatures were evaluated to determine the pathogenicity and to further understand the characteristics of the pathogen. Additionally, this study aimed to develop a suitable method to control the disease of ryegrass seedlings by sowing fungicide-coated seeds in late summer.

Materials and methods

1. Collection of the isolates

Isolates of *Pyricularia* spp. causing blast on Italian and perennial ryegrasses were obtained from the diseased samples collected in 12 prefectures including the Miyazaki, Kumamoto, Yamaguchi, Ishikawa, Toyama, Nagano, Niigata, Shizuoka, Gunma, Tochigi, Fukushima, and Miyagi Prefectures from 1999 to 2010 (Fig. 1). Single conidia isolation was conducted as previously reported (Tsukiboshi et al. 2005), and the obtained isolates were cultured on V8 juice agar (V8A: 180 mL of Campbell's V8 juice, 3 g of $CaCO_3$, and 15 g of agar per liter) slants at 20°C until use. In total, 126 isolates, 119 from Italian ryegrass and 7 from perennial ryegrass, were obtained, and representatives are shown in Table 1.

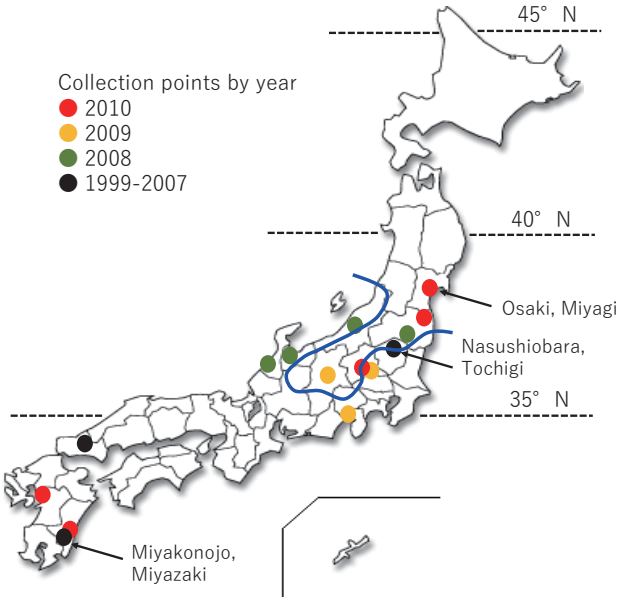

Fig. 1. Geographical distribution of *P. oryzae* isolated from ryegrass collected from 1999 to 2010 in Japan
The blue line indicates the boundary of 22°C of daily average temperatures in September 2008 based on the Automated Meteorological Data Acquisition System (AMeDAS, https://www.jma.go.jp/jma/en/Activities/amedas/amedas.html).

Table 1. Representative isolates of *Pyricularia* spp. from grasses collected in Japan [a]

Isolate	Fungal species	Host plant	Geographical origin	Isolation date	MAFF
IRBY1-1	*Pyricularia oryzae*	*Lolium multiflorum*	Atou, Yamaguchi	2003/10	511461
T-1	*Pyricularia oryzae*	*Lolium multiflorum*	Nasushiobara, Tochigi	2005/8	511463
T-4	*Pyricularia oryzae*	*Lolium multiflorum*	Nasushiobara, Tochigi	2007/9	511490
T-8	*Pyricularia oryzae*	*Lolium multiflorum*	Nasushiobara, Tochigi	2007/9	
Shirakawa-1	*Pyricularia oryzae*	*Lolium multiflorum*	Shirakawa, Fukushima	2008/9	511489
N-11-1	*Pyricularia oryzae*	*Lolium multiflorum*	Kahoku, Ishikawa	2008/10	511486
N-29-1	*Pyricularia oryzae*	*Lolium multiflorum*	Toyama, Toyama	2008/10	511487
N-50-1	*Pyricularia oryzae*	*Lolium multiflorum*	Sanjo, Niigata	2008/10	511488
F-10	*Pyricularia oryzae*	*Lolium perenne*	Osaki, Miyagi	2010/8	
OG-1	*Pyricularia oryzae*	*Dactylis glomerata*	Nasushiobara, Tochigi	2007/7	511492
OG-2	*Pyricularia oryzae*	*Dactylis glomerata*	Nasushiobara, Tochigi	2007/7	511493
ECOT5	*Pyricularia oryzae*	*Eleusine coracana*	Nasushiobara, Tochigi	1999/8	511465
C-2-1	*Pyricularia oryzae*	*Hordeum vulgare*	Miyakonojo, Miyazaki	2007/10	511494
C-2-2	*Pyricularia oryzae*	*Hordeum vulgare*	Miyakonojo, Miyazaki	2007/10	511495
ZMJ136	*Pyricularia oryzae*	*Secale cereale*	Nasushiobara, Tochigi	2008/7	511496
To-14	*Pyricularia grisea*	*Digitaria ciliaris*	Shikano, Tottori	2009/8	
MBT1-3	*Pyricularia grisea*	*Zea mays*	Nasushiobara, Tochigi	2004/8	511464
I-247-2	*Pyricularia penniseticola*	*Chloris gayana*	Ishigaki, Okinawa	2002/2	306640
O-19	*Pyricularia pennisetigena*	*Pennisetum purpureum*	Ishigaki, Okinawa	2006/10	
I-233	*Pyricularia urashimae*	*Panicum maximum*	Iriomote, Okinawa	2002/2	306671
I-246	*Pyricularia urashimae*	*Panicum maximum*	Ishigaki, Okinawa	2002/2	306672

[a] The isolates used for the analysis of the *β-tubulin* gene were chosen and deposited in the NARO Genebank with MAFF numbers (https://www.gene.affrc.go.jp).

A total of 13 isolates were obtained by the same method from other grasses and were also used for comparison in phylogenetic analyses: orchardgrass (*Dactylis glomerata* L.), finger millet (*Eleusine coracana* (L.) Gaertn.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), southern crabgrass (*Digitaria ciliaris* (Retz.) Koeler), corn (*Zea mays* L.), Rhodes grass (*Chloris gayana* Kunth), Napier grass (*Pennisetum purpureum* Schum.), and Guinea grass (*Panicum maximum* Jacq.).

2. Phylogenetic analysis

As described previously (Tsukiboshi et al. 2005), the whole genomic DNA of all the isolates was extracted from the mycelia grown on V8A. Polymerase chain reaction (PCR) amplification of the complete internal transcribed spacer regions with 5.8S ribosomal DNA (rDNA-ITS) and parts of the *β-tubulin* gene, known as informative regions for molecular classification, was performed using the primer pairs ITS1–ITS4 and Bt1a–Bt1b, respectively, using the method described by Hirata et al. (2007). As described previously, the PCR products were purified and sequenced (Tsukiboshi et al. 2005). The rDNA-ITS regions and *β-tubulin* gene sequences of standard isolates of *Pyricularia* species were also used for phylogenetic analyses. All the sequences were aligned using the multiple sequence alignment program CLUSTAL X, and the alignment gaps were treated as missing data. The nucleotide variation was analyzed by the neighbor-joining method, and bootstrap analysis was implemented using 1,000 replicates of the heuristic searches to determine the confidence levels of the inferred phylogenies.

3. Seedling inoculation test under different temperatures

The tetraploid Italian ryegrass cultivar, Hitachihikari bred in Ibaraki Prefecture (Snow Brand Seed Co., Ltd., Sapporo, Japan), which is moderately blast-resistant, was used in the seedling inoculation tests. An aliquot (0.5 g) of seeds was sown in a plastic pot of 1/10,000a size filled with commercial granulated culture soil. The seedlings were cultivated to the 7-8-leaf stage for approximately 3 weeks in a greenhouse at 20°C-25°C. The isolate T-1 (Table 1), which showed abundant sporulation by the above-described method, was grown under BLB (FL-20S; Toshiba, Japan) on V8A for 7 days to produce conidia. The plants were inoculated by atomizing with approximately 10 mL of conidial suspension (10^5 conidia/mL). The inoculated plants were kept in a wet chamber for 24 h at 25°C and transferred to a light incubator at controlled temperatures of 19°C, 22°C, 25°C, 28°C, 31°C, and 34°C. After 4-8 days of incubation, lesion areas were estimated by measuring the long and short diameters of the produced lesions, considering them as oval since the produced lesions were oval to spindle shaped. To estimate the conidial production, 10 lesions from an inoculated plant were cut into pieces and put on water agar under white fluorescent light (FL-20W, Toshiba) with a photoperiod of 12 h/12 h light/dark cycles. After 3 days, the leaf pieces were placed in 0.5 mL-distilled water amended with 0.1% Tween 20, and treated using an ultrasonic washing machine for 1 min. The number of conidia produced on a lesion was assessed under a light microscope using a hemocytometer. The experiment was repeated thrice for each temperature.

4. Seedling and field tests using fungicide-coated seeds

Seedling tests using fungicide-coated seeds were conducted using the cultivar Hitachihikari, as described above. Fungicide-coated seeds were prepared using the pellet coating procedure reported by Scott (1975) with some modifications, whereby the fungicides orysastrobin (BASF Japan, Tokyo, Japan), probenazole (Meiji Seika Pharma, Tokyo, Japan), and benomyl (Sumitomo Chemical Garden Products Inc., Tokyo, Japan) were finely powdered and mixed with 2.5-g talc and 1.5-mL glycerin per 5-g seeds. The effective content of each fungicide was adjusted to 2% of the seed weight. The control seeds were coated with only talc and glycerin. As described above, seeds of the cultivar were treated with the aforementioned coating procedures, and the obtained seedlings were investigated for the virulence of the fungal isolate, T-1, except that the incubation period of excised lesions was adjusted to 2 days.

Field tests were conducted using the diploid Italian ryegrass cultivars Minamiaoba bred in Ibaraki Prefecture and Sachiaoba bred in Yamaguchi Prefecture (Snow Brand Seed Co., Ltd.) and the cultivar Hitachihikari in a field at the National Institute of Livestock and Grassland Science (36.915° N, 139.933° E), Nasushiobara, Tochigi, in central Japan. A total of 10 grams of the dried fungicide-coated seeds was sown on September 3, 2008 and August 26, 2009, in a 3 m long row for each treatment. The tests were performed under natural disease conditions, and each plot was triplicated where the incubated inoculum (barley grains) was applied in late summer of 2007. The suggested fertilizer rates (6 kg each of N–P–K/10a) were applied to rows in the field. Plant stands were estimated on October 17, 2008 and October 9, 2009 for their disease index (DI): 0 = no symptoms, 1 = low-level occurrence on lower leaves, 2 = high-level occurrence on lower leaves, 3 = high-level occurrence on middle leaves, 4 = high-level occurrence on upper leaves, and 5 = whole plant infection and death (Fig. 2). The disease severity index

(DSI) was calculated as DSI = [(1 × frequency of DI 1) + (2 × frequency of DI 2) + (3 × frequency of DI 3) + (4 × frequency of DI 4) + (5 × frequency of DI 5)] / total number of observations.

Results

1. Phylogenetic analysis of the collected isolates

All 126 isolates collected from ryegrass were identified as *P. oryzae* based on the sequence of rDNA-ITS regions, with similarities from 99.7% to 100% with the standard accession (AB274423). The sequence data of the representative isolates are shown in the NARO GenBank database as MAFF isolates (https://www.gene.affrc.go.jp/databases-micro_search.php). The pathogen of ryegrass blast was placed into the cultivated crop pathogen (CC-) group of *P. oryzae* as proposed by Tosa et al. (2004) clustered with the isolates from barley, rye, orchardgrass, and finger millet (Fig. 3) based on the phylogenetic analysis of the *β-tubulin* gene. The pathogens of rice and wheat appeared to belong to different groups than ryegrass, but this was unclear in the phylogeny. The isolates of *P. oryzae* from ryegrass were distributed from Miyakonojo (31°N), Miyazaki, Kyushu to Osaki (38°N), Miyagi, Tohoku (Fig. 1). The isolates obtained from southern crabgrass and corn were identified as *P. grisea* (Cooke) Saccardo based on the sequences of rDNA-ITS and the *β-tubulin* gene. The fungus from southern crabgrass causes blast disease in corn (Nishihara 1987). The isolates of *P. grisea* were not found in ryegrass, although Suzuki et al. (2015) reported it in Yamagata in Tohoku. The isolate from Rhodes grass was identified as *Pyricularia penniseticola* Klaubauf, Lebrun & Crous, that from Napier grass as *Pyricularia pennisetigena* Klaubauf, Lebrun & Crous, and those from Guinea grass, previously identified as *Pyricularia* sp. LS-group (Tsukiboshi et al. 2009), as *Pyricularia urashimae* Castroag., Crous & Ceresini.

2. Virulence of the pathogen at different temperatures

T-1, the isolate, showed distinct virulence to Italian ryegrass seedlings at temperatures of 19°C-34°C. Lesion enlargement was the most rapid at 31°C, showing a growth of 1.6 mm^2/day with a maximum lesion area of 11.4 mm^2, 4 days after inoculation (Table 2). The lesion enlargement at 19°C and 22°C was 0.5-0.6 mm^2/day and was significantly suppressed compared to that at 25°C-31°C. The lesions enlarged slowly at 34°C (0.8 mm^2/day), showing a maximum area of 8.8 mm^2, 5 days after inoculation. The amount of sporulation per square millimeter lesion reached a maximum of 2.07×10^4 spores at 22°C, but no significant differences were discovered among those between 22°C and 31°C. Sporulation per square millimeter lesion was 4.30×10^3 at 19°C and 1.70×10^3 at 34°C, which was significantly lower than those between 22°C and 31°C. The degree of sporulation per lesion reached 1.12×10^5 at 28°C, but no significant differences were detected among those between 22°C and 31°C. The amount of sporulation per lesion was 2.00×10^4 at 19°C and 7.30×10^3 at 34°C, which was significantly lower than those between 22°C and 31°C.

The geographical distribution of the ryegrass isolates was restricted to the south of the boundary line with a 22°C daily average temperature in September 2008; however, isolates were found north of the line and in the Tohoku district in 2010 (Fig. 1). Severe damage by the disease was observed in Fukushima, Tohoku in 2010.

3. Effect of fungicide-seed coating on disease occurrence in the field and seedlings

Blast disease was first observed in the field test on September 30, 2008. The disease in Italian ryegrass sown by fungicide-coated seeds was suppressed regardless of the fungicide and cultivar used (Fig. 4). Orysastrobin, a QoI fungicide, was the most suppressive to disease

Fig. 2. Symptoms of blast in Italian ryegrass in the field at Nasushiobara, Tochigi Prefecture
Disease occurrences were estimated 6 weeks after seeding with the disease index of 0: no symptoms (left), 1: low-level occurrence on lower leaves, 2: high-level occurrence on lower leaves, 3: high-level occurrence on middle leaves (center), 4: high-level occurrence on upper leaves, and 5: whole plant infection and death (right).

occurrence among the fungicides, with a DSI range of 0.00-0.15, which was significantly lower than that of the other fungicides. Benomyl showed a DSI range of 0.04-0.56, which was significantly lower than that of the control. The suppressive effects of probenazole were lowest among the fungicides with a DSI range of 0.00-1.58, which was significantly lower than that of the control (0.56-2.75). Among the three cultivars of Italian ryegrass, Sachiaoba showed the lowest DSI range (0.56-0.84) in the control, whereas Hitachihikari ranged from 0.73 to 1.57 and Minamiaoba ranged from 1.75 to 2.75. The field test in 2009 showed similar results, and the disease was suppressed by coating the seeds with the fungicides (data not shown). As the disease occurred in autumn, the fungicide treatment restrained seedling blight, which is the most severe problem in Japan.

In the seedlings of the cultivar Hitachihikari, orysastrobin was most effective in controlling lesion enlargement and sporulation by the pathogen (Table 3). Lesion enlargement was 0.6 mm^2/day, which was significantly lower than that of 1.1 mm^2/day in the control. Although not significantly, ratios of sporulating lesions were lowered by the fungicides. Sporulation was suppressed by the fungicide, and orysastrobin significantly lowered it to 1.24×10^3 spores per mm^2 lesion and 3.88×10^3 per lesion, compared with 2.13×10^3 and 1.43×10^4 in the control, respectively. The amount of sporulation by treatment with benomyl and probenazole was lower than that of the control, but no significant suppression was detected.

Discussion

The blast fungus of ryegrass was restricted to the south of the Kanto district in central Japan (Nishihara 1981, Nishimi et al. 2009) until the 2000's. Here, the pathogen was newly collected from the Tohoku district, north of Kanto, where blast disease had not previously been reported. All the isolates from Italian and perennial ryegrasses and those from rye, orchardgrass, barley, and finger millet were identified as *P. oryzae* based on the phylogenetic analyses. Recently, a new species recognition system for *Pyricularia* was developed (Klaubauf et al. 2014, Crous et al. 2016), and the group

Fig. 3. A neighbor-joining tree inferred from the sequences of the *β-tubulin* gene of *Pyricularia* spp.
Numbers in front of the branches represent bootstrap values (1,000 replicates). The isolates used are shown in bold as the isolate numbers and original hosts in bold. Isolate data (Hirata et al. 2007) provided with the GenBank accession numbers are shown with the original hosts. Scale bar represents the genetic distance (0.01), showing the number of base changes.

infecting rice, wheat barley, and ryegrass, including the CC-group (C_3 crop isolate group) suggested by Tosa et al. (2004), was classified as *P. oryzae*. The results of species identification in this study coincided with the species recognition system. The ryegrass isolate used in this study was pathogenic to ryegrass, orchardgrass, rye, and oat, but not to rice (Tsukiboshi 2011) as previously stated. Kusaba et al. (2006) indicated that the Japanese isolates of Italian ryegrass form a distinct group close to the wheat group, but different from rice or finger millet, based on PCR-RFLP (Restriction Fragment Length Polymorphism) analysis and their pathogenicity. The ryegrass pathogen could not be separated from the groups of other hosts based on the sequences of rDNA-ITS and the *β-tubulin* gene; however, the isolates from ryegrass in this study were likely part of the ryegrass group suggested by other researchers (Kusaba et al. 2006, Farman 2007) because of their phylogeny and pathogenicity. Although the pathogenicity to fescue (*Festuca* spp.) by the ryegrass isolates was not estimated in this study, the blast pathogen of ryegrass was reported to be pathogenic in fescue and festulolium in other countries (Vincelli et al. 2008, Makaju et al. 2016). As reported previously (Tsukiboshi 2011), the isolate OG-1 of *P. oryzae* from orchardgrass was obtained in a greenhouse and assumed to infect orchardgrass from nearby ryegrass due to its strong pathogenicity to ryegrass. To the best of my knowledge, the natural occurrence of blast disease in orchardgrass has never been reported.

The virulence of the ryegrass isolate was greatest at 31°C for lesion enlargement and significantly higher at 22°C-31°C for sporulation per lesion than those at 19°C and 34°C in the seedling inoculation test. Trevathan et al. (1994) and Li et al. (2014) indicated that wet and humid conditions and the resulting increase in sporulation are contributing factors to the disease progression in ryegrass

Table 2. Lesion enlargement and sporulation of *P. oryzae* on Italian ryegrass under different temperatures [a]

Temperature (°C)	Lesion enlargement (mm^2/day)	Sporulation (/mm^2 lesion) [b]	Sporulation per lesion [b]
19	0.6 a	4.30×10^3 a	2.00×10^4 a
22	0.5 a	2.07×10^4 b	8.45×10^4 b
25	1.2 b	1.52×10^4 b	9.02×10^4 b
28	1.3 b	1.64×10^4 b	1.12×10^5 b
31	1.6 c	1.29×10^4 b	8.03×10^4 b
34	0.8 a	1.70×10^3 a	7.30×10^3 a

[a] All the experiments used the Hitachihikari cultivar. Figures in each column with different letters are significantly different according to Tukey's HSD test ($P < 0.05$).
[b] Figures were statistically analyzed after $\log_{10}$-transformation.

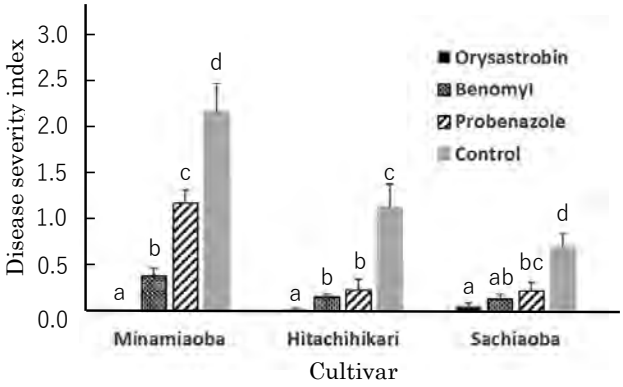

Fig. 4. Suppression of disease severity by sowing fungicide-coated seeds in the field
Fungicide-coated seeds were sown on September 3, 2008, and disease severity was estimated on October 17, 2008. Data for each cultivar were statistically analyzed by Welch's multiple *t*-test, and different letters indicate significant differences ($P < 0.05$). Vertical bars indicate the standard errors.

Table 3. Lesion enlargement and sporulation of *P. oryzae* on Italian ryegrass grown from fungicide-coated seeds [a]

Fungicide	Lesion enlargement (mm^2/day)	Ratio of sporulating lesions (%) [b]	Sporulation [c] (/mm^2 lesion)	Sporulation [c] (/lesion)
Orysastrobin	0.6 a	61.1 a	1.24×10^3 a	3.88×10^3 a
Benomyl	1.0 b	60.9 a	1.54×10^3 ab	7.81×10^3 b
Probenazole	0.9 ab	77.8 a	2.10×10^3 bc	1.08×10^4 b
Control	1.1 b	86.2 a	2.13×10^3 bc	1.43×10^4 b

[a] All the experiments performed at 25°C using the Hitachihikari cultivar. Different letters indicate significant differences ($P < 0.05$, Tukey's HSD test).
[b] Figures were statistically analyzed after arc-sine transformation.
[c] Figures were statistically analyzed after log10-transformation.

blast. The results of this study indicate that rapid lesion enlargement and increased sporulation at medium–high temperatures promote disease progression in the field. Moss and Trevathan (1987) reported that the optimum temperature for blast disease development is 26°C in perennial ryegrass turf, which also supports the results of this study. Although the results were obtained using an isolate collected in central Japan, the isolates collected in southwestern Japan were also reported to cause severe damage to ryegrass at 25°C (Sumida et al. 2003), and the difference in pathogenicity between them was assumed to be small. The distribution of the disease in Japan is anticipated to expand north, accompanied with global warming because of the temperature characteristics of the pathogen.

The disease occurrence of Italian ryegrass grown from seeds coated with fungicides was suppressed compared to that of the control. Particularly, orysastrobin has a strong effect on controlling disease severity in the field. Lesion enlargement and sporulation in the seedlings were more restricted by orysastrobin than by the other fungicides and the control. Orysastrobin is a permeable and systemic fungicide that is assumed to translocate from coated seeds to seedlings and inhibit lesion enlargement and sporulation. Orysastrobin-coated seeds of Italian ryegrass, produced using the method in this study, were sown in Miyazaki, Kyushu in September 2010, and the disease occurrences were substantially reduced with a 6%-83% increase in the yield that year (Higashi et al. 2011). Benomyl was also effective in controlling the disease in the field, but the effects on virulence in the seedlings were unclear, compared with those of orysastrobin. In contrast, probenazole was not distinctly effective in controlling the disease in the field or seedlings. Probenazole is a plant activator that induces resistance to the disease; however, it was not sufficient to induce resistance in young seedlings by seed coating of the fungicide. Bred in Japan (Mizuno et al. 2003), the resistant cultivar, Sachiaoba, was the most effective among the cultivars used to control the disease, and the fungicide-coated seeds of the cultivar showed the lowest disease severity in the field.

The damage caused by blast disease in Italian ryegrass seedlings sown from late summer to early autumn is very severe, and seed coating with orysastrobin is a promising control method. Although the fungicide cannot be used in Japan because of the revocation of its registration, it could be used in other countries where it is permitted. However, since QoI fungicides, including orysastrobin, cause rapid development of fungal tolerance (Kim et al. 2003), the continuous use of the fungicide should be avoided. Further research on the temperature reactions is necessary to predict the expansion of the disease to northern Japan. Other controlling methods, including breeding with resistant cultivars, should be performed to achieve a more effective and sustainable control of the disease in the future.

Acknowledgements

I thank Drs. Koya Sugawara and Takako Kiyoshi, Institute of Livestock and Grassland Science, NARO, for their helpful suggestions and advice during this study.

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