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Overwintering of Rice Sclerotial Disease Fungi, *Rhizoctonia* and *Sclerotium* spp. in Paddy Fields in Japan

Inagaki Kimiharu, [1]Guo Qingyuan and Arakawa Masao
Laboratory of Plant Pathology, Faculty of Agriculture,
Meijo University, Tenpaku, Nagoya 468-8502, Japan
[1]Department of Plant Protection, Agricultural College,
Xinjiang Agricultural University, Urumuqi, Xinjiang 830052, China

Abstract: Rice sclerotial diseases are caused by sclerotium-forming fungi including *Rhizoctonia fumigata*, *R. oryzae-sativae*, *R. oryzae*, *R. solani* AG-2-2 (IIIB) and *Sclerotium hydrophilum*. In the early March pretransplanting season of rice plants in 3 paddy fields (area: 6 to 8 a), rice stubble and surface soil containing plant debris and sclerotia were collected at ca. 5 m intervals from each field, followed by fungal isolation in order to examine the survival rates of these *Rhizoctonia* and *Sclerotium* species. Isolates of these fungi from surface soil and stubble numbered 565 and 1120, respectively. *S. hydrophilum* and *R. oryzae-sativae* were isolated significantly more frequently from stubble than from surface soil, while there was no significant differences in the isolation frequency of *R. fumigata* between surface soil and stubble, indicating that the survival of *S. hydrophilum* and *R. oryzae-sativae* is highly related to the availability of stubble, whereas *R. fumigata* relates to both isolation sources. *R. fumigata,* which had caused sclerotial disease over a wide area (25 to 30%) of paddy fields in the previous year, overwintered at a higher rate in the fields, as compared with fungi such as *R. oryzae-sativae, R. oryzae* and *R. solani* AG-2-2 (IIIB), which had caused disease only within a limited area (0-15%) of those same fields.

Key words: Rice sclerotium-forming fungi, survival, disease occurrence, paddy fields

INTRODUCTION

Sclerotium-forming fungi belonging to *Rhizoctonia* and *Sclerotium* species are associated with diseases of the leaf sheath of rice plants (*Oryza sativa* L.), which are known generically as rice sclerotial diseases[1]. Sclerotial diseases, which are prevalent in rice growing areas of Japan, Taiwan, Thailand, the Philippines, Colombia and the United States[2-6], include globular sclerotium disease caused by *S. hydrophilum* Saccardo, gray sclerotium disease by *R. fumigata* (Nakata and Hara) Gunnel and Webster, brown sclerotium disease or aggregate sheath spot by *R. oryzae-sativae* (Sawada) Mordue, bordered sheath spot by *R. oryzae* Ryker and Gooch, brown sheath blight by *R. solani* Kuhn AG-2-2 (IIIB) and sheath blight by *R. solani* AG-1(IA)[1,6]. Among these various sclerotial diseases, bordered sheath spot and brown sclerotium disease as well as sheath blight cause significant damage to the quality of rice when they reach the top leaf sheath of the plant[3,7] . Sheath blight has been recognized as an economically significant disease that is sometimes equated with blast caused by *Pyricularia grisea* (Cooke) Saccardo. These sclerotial diseases are symptomatically similar to each other, though slightly differing in size, color and the existence of a belt-shaped margin of lesions[1,8]. Moreover, their pathogens produce morphologically different sclerotia on and in leaf sheaths, usually during the maturation stage of rice[1].

In the rice growing areas extending from central to southern Japan, the transplanting of rice seedlings into paddy fields is usually carried out from May to June, while the harvest occurs from September to October. In paddy fields, therefore, rice sclerotial disease fungi overwinter in the form of sclerotia or plant debris on and in surface soil and stubble from autumn until the following spring. After transplanting of the seedlings, elongated hyphae from these sources infect the leaf sheaths. Lesions of sclerotial diseases (except those of sheath blight) generally appear around the heading stage and gradually increase as the rice plants mature[7]. Throughout the growth stages of rice plants in paddy fields, these pathogens are isolated from paddy water as well as from the rice plants, although they differ in frequency at specific stages of the plants[9]. However, little information

Corresponding Author: Dr. Inagaki Kimiharu, Laboratory of Plant Pathology, Faculty of Agriculture, Meijo University, Tenpaku, Nagoya 468-8502, Japan E-mail:inagakik@ccmfs.meijo-u.ac.jp

is yet available to explain the actual survival conditions of these fungi from the disease-occurring season in the previous year to the spring of the following year. The present study describes an investigation into the survival of rice sclerotial disease fungi, except that of *R. solani* AG-1 (IA) which is already well known, in rice stubble and plant debris on/in the surface soil of paddy fields at the pretransplanting season in March. Moreover, we report on the relationship between the survival of sclerotial disease fungi and disease occurrence during the previous maturation stage of rice plants.

MATERIALS AND METHODS

Sclerotial diseases: The sclerotial diseases of rice plants focused in the present study are: globular sclerotial disease caused by *Sclerotium hydrophilum*, gray sclerotium disease by *Rhizoctonia fumigata* [= *S. fumigatum* Nakata and Kawamura], brown sclerotium disease by *R. oryzae-sativae* [= *S. oryzae-sativae* Sawada], bordered sheath spot by *R. oryzae* [= *Waitea circinata* Warcup and Talbot] and brown sheath blight by *R. solani* AG-2-2-IIIB [= *Thanatepholus cucumeris* (Frank) Donk][10-13].

Paddy fields: A sampling of isolation sources and a survey of sclerotial disease occurrence were conducted from 1990 to 1993 in 3 paddy fields, i.e., TW (area: 9 a), TM (8 a) and TO (7 a), in Togo, a town near Nagoya in Central Japan. In the 3 adjacent paddy fields, a late cultivar of rice known as Aichi-no-kaori (heading stage: late August, harvest stage: mid-October) was planted throughout the test periods. These paddy fields, in which monoculture of rice plants had been conducted over the last two decades, were first plowed around late March to early April and again in mid-May for the watering and transplanting of rice seedlings that is usually conducted in late May.

Sampling: For the sampling of 2 isolation sources, i.e., surface soil and rice stubble, 37 to 40 sites in each paddy field were established at around 5 m intervals, as described previously. In the early March pretransplanting season, around a 20 cm^2 surface soil and 2 samples of stubble were collected from each site, desiccated at 10 to 18 for 15-20 days and stocked in a room at 15 until fungal isolation.

Fungal isolation: In order to collect plant debris from surface soil, 200 g of soil that had first been finely pounded was put into 500 mL of distilled water in a 1000 mL Erlenmeyer flask, stirred for 60 sec and sieved with a Tyler mesh (No. 60: 250 m openings)[14]. These procedures were repeated 5 times to ensure a sufficient collection of plant debris. After 4 to 5 washings in distilled water, the plant debris was disinfested with 1% sodium hypochlorite for 2 to 3 min, rinsed in sterilized water and placed on 2% water agar (WA)-plates (Petri dishes, 9 cm in diameter) which contained 50 ppm of streptomycin. One hundred pieces of plant debris were used for fungal isolation. Three to five days after incubation at 28, hyphal tips elongated from the pieces of plant debris were transplanted to potato sucrose agar (PSA)-slants. In stubble composed of 10-20 withered rice stems, the stems were cut into 100 small segments ca. 1 cm in length, washed in distilled water 4-5 times and prepared for fungal isolation according to the above-mentioned procedures. As the segments were liable to disintegrate into even smaller pieces during washing, these pieces were carefully collected with the Tyler mesh and were then also prepared for fungal isolation. Lesions of sclerotial diseases on the leaf sheaths were cut into 2 segments per lesion. After washing in distilled water 4-5 times, the segments were prepared for fungal isolation in the same manner used for the other isolation sources.

All isolates were identified as belonging to the respective species of *Rhizoctonia* and *Sclerotium* mainly on the basis of a comparison of cultural characteristics between field isolates from isolation sources and a tester isolate of each pathogen, which were grown on PSA plates at 28 for 14 days. However, when it was difficult to identify on the basis of cultural characteristics, a face-to-face culture between field isolates and a tester one, conducted on WA-plates at 28 for 2-5 days, was prepared to observe the occurrence of hyphal anastomosis[13].

Field survey of disease occurrence: During the maturation stage of rice plants, sclerotial diseases were surveyed for their occurrence at all sites in TW and TM fields from 1991 to 1993 and 1991 to 1992, respectively. Each site for the disease survey occupied a 20-25 rice hill area, i.e., around 1 m square within a field. In paddy fields, sclerotial diseases were visually diagnosed according to their lesion type on leaf sheaths, but lesions were also collected, followed by the isolation of pathogens described above in those cases where discrimination among sclerotial diseases was difficult, especially between bordered sheath spot and brown sheath blight. Globular sclerotial disease was exempted from this survey because its pathogen produces indistinct yellowish lesions due to its weak virulence[1].

Data analysis: Ratios (%) of isolation and dispersal of each fungus were calculated as follows: isolation (%)

= (the number of fungal isolates from surface soil or stubble. the number of total fungal isolates from both surface soil and stubble)×100 and dispersal (%) = (the number of total sites with surface soil or stubble from which each fungal species was isolated . the number of total sites with surface soil and stubble from which each fungal species was isolated)×100. Each of these fungal ratios was compared between surface soil and stubble at p = 0.05 using the Student's t-test.

RESULTS

Number of fungal isolates: Isolates from surface soil collected 6 times during the pretransplanting season in 3 paddy fields from 1990 to 1993 totaled 565, while those from stubble totaled 1120 (Table 1). The isolation ratio of *S. hydrophilum* in stubble was 74%, against 26% in surface, indicating a significant difference between these isolation sources. *R. oryzae-sativae* ratios showed the tendency similar to those of *S. hydrophilum*. However, the ratios of *R. fumigata* in soil and stubble were 47 and 53%, respectively, reflecting an insignificant difference. Since the number of isolates of *R. oryzae* and *R. solani* AG-2-2 (IIIB) was so small, it was difficult to account for the significant difference between the 2 isolation sources.

Number of sites infested with fungi: In surface soil, all 3 paddy fields abounded in sites infested with *S. hydrophilum* and *R. fumigata*, ranging from 3 to 24, as compared with 0 to 13 infested with *R. oryzae-sativae, R. oryzae* and *R. solani* AG-2-2 (IIIB) (Table 2). In stubble, more sites were infested with the former fungi than with the latter ones. Dispersal ratios of *S. hydrophilum* and *R. oryzae-sativae* were significantly higher in stubble compared with surface soil, whereas the ratios of *R. fumigata*, *R. oryzae* and *R. solani* AG-2-2 (IIIB) showed no such significant difference.

Table 1: Isolation of rice sclerotial disease fungi from surface soil and stubble in 3 paddy fields (TW, TM and TO) during the pretransplanting season (early March)

	Surface soil							Stubble							
	TW			TM	TO			TW			TM	TO			
Fungus	1991	1992	1993	1992	1990	1991	Total (%)[b]	1991	1992	1993	1992	1990	1991	Total (%)	Total[c]
S. hydrophilum	5[a]	25	15	64	76	7	192 (25.9)b[d]	117	53	38	217	38	86	549 (74.1)a	741
R. fumigata	19	77	20	38	91	35	280 (47.0)a	28	55	15	61	29	128	316 (53.0)a	596
R. oryzae-sativae	5	2	0	1	0	6	14 (7.1)b	4	73	37	39	10	21	184 (92.9)a	198
R. oryzae	4	0	0	1	9	1	15 (26.3)a	14	1	17	0	5	5	42 (73.7)a	57
R. solani AG-2-2 (IIIB)	7	40	2	9	6	0	64 (688.0)a	0	26	1	2	0	0	29 (31.2)a	93
Total	40	144	37	113	182	49	565 (33.5)	163	208	116	319	82	240	1120 (66.5)	1685

[a] No. of isolates from 40, 40 and 37 filed sites in TW, TM and TO field, respectively
[b] %: [No. of isolates of each fungus from residues/No. of total isolates of each fungus from both residues and stubble]×100
[c] The total sum of isolate from both residues and stubble
[d] Two values in the same row, e.g., 280 and 316 for *R. fumigata*, indicated by the same letter do not significantly differ according to Student t-test (p=0.05)

Table 2: Distribution of rice sclerotial disease fungi infesting surface soil and stubble in 3 paddy fields (TW, TM and TO) in the pretransplanting season (early March)

	Surface soil							Stubble							
	TW			TM	TO			TW			TM	TO			
Fungus	1991	1992	1993	1992	1990	1991	Total (%)[b]	1991	1992	1993	1992	1990	1991	Total (%)	Total[c]
S. hydrophilum	5[a]	15	9	24	13	5	71 (36.2)b[d]	26	18	14	24	26	17	125 (63.8)a	196
R. fumigata	9	20	3	15	9	16	81 (50.9)a	10	15	5	8	15	25	78 (49.1)a	159
R. oryzae-sativae	4	2	0	1	0	5	12 (30.0)b	3	7	4	4	7	3	28 (70.0)a	40
R. oryzae	2	0	0	1	2	1	6 (30.0)a	3	1	3	0	4	3	14 (70.0)a	20
R. solani AG-2-2 (IIIB)	6	13	2	7	2	0	30 (66.7)a	0	13	1	1	0	0	15 (33.3)a	45
Total[e]	40	40	40	37	35	35	227.	40	40	40	37	35	35227	.	.

[a] No. of field sites where fungi were detected
[b] %: [No. of total sites with residues from which fungi were detected / no. of total sites with residues and stubble from which fungi were detected]×100
[c] The total sum of sites with surface soil and stubble from which fungi were detected
[d] Two values in the same row indicated by the same letter do not significantly differ according to Student t-test (p=0.05)
[e] Total of field sites surveyed in each respective field

Table 3: Distribution of rice sclerotial disease fungi in 3 paddy fields (TW, TM and TO) in the pretransplanting season (early March)

	TW			TM	TO	
Fungus	1991	1992	1993	1992	1990	1991
S. hydrophilum	70.0[a]	62.5	57.5	78.4	60.0	54.3
R. fumigata	42.5	67.5	20.0	48.6	62.9	80.0
R. oryzae-sativae	15.0	22.5	10.0	10.8	11.4	20.0
R. oryzae	10.0	2.5	7.5	2.7	11.4	11.4
R. solani AG-2-2 IIIB	15.0	50.0	7.5	18.9	5.7	0.0

[a] % : [(No. of total sites with infested stubble or surface soil) / no. of total sites surveyed]×100

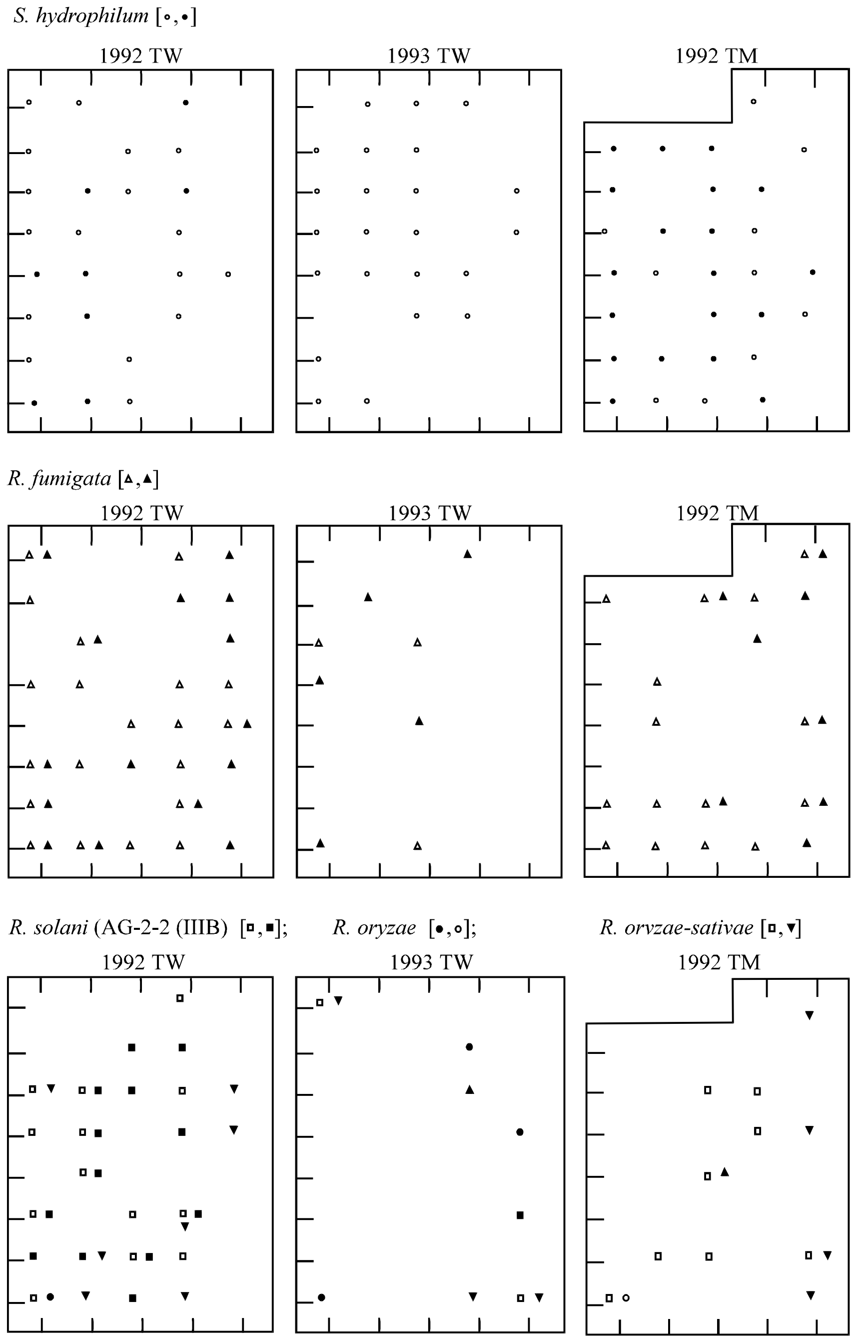

Fig. 1: Spatial distribution of rice sclerotial disease fungi infesting surface soil and stubble in 2 paddy fields (TW and TM, area: 6-7 a) in the pretransplanting season (early March). In each fungus, the white ground symbol and the dark shading one show surface soil and stubble, respectively

Table 3 shows the percentage of infested sites among all those surveyed. *S. hydrophilum* had the highest ratios (from 54 to 78%) among the 5 sclerotial disease fungi, followed by *R. fumigata* (from 20 to 80%), while the other 3 sclerotial disease fungi showed lower ratios (from 0 to 50%).

Dispersal of fungi in paddy fields: The number of fungi per site ranged from 1 to 4 in soil and 1 to 3 in stubble, averaging 1.5 in soil and 1.4 in stubble, which was not a significant difference (Table 4). The dispersal of fungi in fields was shown in Fig. 1, omitting TO field-data similar to those from TW and TM fields. In *R. fumigata*, which

Table 4: Frequency of appearance of rice sclerotial disease fungi on/in surface soil and stubble per paddy field site

Isolation source	No. of fungi per site 1	2	3	4	Total	Mean
Surface soil	78[a]	37	7	2	124	1.5a[b]
Stubble	108	52	9	0	169	1.4a

[a] No. of sites infested with 1 to 4 sclerotial disease fungi
[b] Values followed by the same letter within a column do not significantly differ at Student t-test (p=0.05)

Table 5: Occurrence of 4 sclerotial diseases at the mature stage (late September) of rice plants in 2 paddy fields (TW and TM)

Disease	Causal fungus	Disease occurrence (%) disease TW 1991	TW 1992	TW 1993	TM 1991	TM 1992
Gray sclerotium disease	*R. fumigata*	-[b]	25.0	37.5	29.7	78.4
Brown sclerotium disease	*R. oryzae-sativae*	0.0	0.0	0.0	5.4	2.7
Bordered sheath spot	*R. oryzae*	0.0	0.0	0.0	5.4	0.0
Brown sheath blight	*R. solani* AG-2-2(IIIB)	15.0	7.5	5.0	0.0	8.1

[a] [No. of field plots where disease occurrence was detected / total plots surveyed]×100, [b] Not surveyed

was detected at 8 to 27 sites in TW field and 18 in TM field, there were many cases where the fungus was detected from one isolation source at one site but from 2 isolation sources at another. Such a dispersal detected in *R. fumigata* was similarly found in the other sclerotial disease fungi. As detected in TW and TM fields for *S. hydrophilum* and *R. fumigata*, sclerotial disease fungi distributed little in clusters in paddy fields. In the TW field, the location of the fungus-detected-sites in many cases differed from year to year, e.g., *S. hydrophilum* was detected at the same 16 sites but also at 16 different sites between 1992 and 1993.

Disease occurrence: In TW and TM fields, gray sclerotial disease occurred at 10 to 15 sites (25 to 38%) out of 37 and 11 to 29 sites (30 to 78%) out of 40, whereas the other 3 sclerotial diseases, especially bordered sheath spot, occurred at fewer sites, I. e., 0 to 6 sites (0 to 15%) in each field (Table 5).

DISCUSSION

The present study demonstrated that the *Rhizoctonia* and *Sclerotium* spp. causing sclerotial diseases on rice plants differ as to their place for survival, when overwintering in paddy fields from the harvest season until the pretransplanting season of the following spring. Thus, *S. hydrophilum* and *R. oryzae-sativae* proved to survive at a higher rate in stubble compared with surface soil, while *R. fumigata* survived in both stubble and surface soil. As for fungal isolation from sclerotial disease lesions, globular sclerotial and brown sclerotial disease fungi, i.e., *S. hydrophilum* and *R. oryzae-sativae*, respectively are reported to be more frequently isolated from lesions produced on the lower part of rice leaf sheaths than those on the upper part and gray sclerotial disease fungus, *R. fumigata,* is isolated frequently from lesions on both the lower and upper parts[15]. These isolation results indicated that *S. hydrophilum* and *R. oryzae-sativae* are likely to cause sclerotial diseases at the base of rice plants. The survival of sclerotial disease fungi is, therefore, considered to be closely related to the locus of occurrences of the respective disease within rice hills. As to the formation of sclerotia on rice plants, *R. oryzae-sativae* forms in the leaf sheath tissues and the inside of stems and *S. hydrophilum* forms in the leaf sheath tissues, the inside of stems and also on leaf sheath surfaces[1,6]. These characteristics of sclerotial formation and disease development at the basal parts of plants within hills are probably of advantage to *R. oryzae-sativae* and *S. hydrophilum* in surviving at a higher rate in stubble after the harvest of rice. On the other hand, *R. fumigata* produces sclerotia on the leaf sheath surface and inside leaf sheaths[1,6], indicating that its sclerotia are likely to fall on to the surface soil as well as into the stubble and consequently the fungus can survive almost equally well on/in both surface soil and stubble. Although for *R. oryzae* and *R. solani* AG-2-2 (IIIB) it is unclear which one of 2 isolation sources is more effective for their survival because of the low occurrence rate of these pathogen-inducing diseases, it is plausible to assume that stubble is rather more important than surface soil for *R. oryzae* survival in paddy fields because of its sclerotial formation mode.

S. hydrophilum and *R. fumigata* in 3 paddy fields were found not in clusters but widely dispersed regardless of the difference in isolation sources and, in the TW field, the places where fungi were detected differed considerably between 1991 and 1992 and 1992 and 1993. Since plant debris and the sclerotia of *S. hydrophilum* and *R. fumigata* play an important role in the dispersal of pathogens in paddy fields, we think the present results are due to the displacement of these residues within paddy fields due to rainfall as well as plowing after a harvest. In addition, mechanized harvesting and plowing operations recently developed for rice cultivation may be particularly effective in dispersing these residues in paddy fields.

At the maturation stage of rice plants in both TW and TM fields, gray sclerotium disease was considered to be the most prevalent out of the 4 sclerotial diseases throughout our test periods, although no survey was conducted for its occurrence in the 1991-TW field, whereas the other sclerotial diseases, especially bordered sheath spot, occurred seldom, if ever. Around 5 months after the sclerotial disease season in paddy fields, i.e., March of the following year, *R. fumigata* was widely detected in 20 to 80% of the area within a field, whereas *R. oryzae, R. oryzae-sativae* and *R. solani* AG-2-2 (IIIB) were, with a few exceptions, detected in less than 20%. These results show that, within a field from the harvest until the following spring, these sclerotial disease fungi undergo no major fluctuations in quantity based on the egress / ingress of water between bordering fields[16], although fungal intrafield-dispersal occurs often as mentioned above. Actually, paddy fields in central to southern Japan during noncultivating seasons, i.e., generally from October / November to April / May of the following year, experience drier conditions and less overflow than during the rice growing seasons. Consequently, the fungi causing widespread sclerotial diseases overwinter until the following spring at a higher rate compared to those that cause diseases to a far lesser degree.

The present study is the first to show the actual states of survival and distribution of 5 rice sclerotial disease fungi in paddy fields before the transplanting of rice seedlings and also to demonstrate the relationships between occurrences of sclerotial diseases at the late growth stage of rice plants and the survivals of these disease-inducing pathogens at the pretransplanting season of the following year. Many species in the genera *Rhizoctonia, Sclerotium, Magnaporthe* and *Helmintosporium* have been shown to survive using artificially-produced sclerotia under both natural[17,18] and experimental[19] conditions and these reports reveal that sclerotial disease fungi survive in the form of sclerotia at rates of around 50 to 100% for at least 5 to 6 months, which is the equivalent period from harvest to the following spring in Japan. However, the relationship between overwintered sclerotial disease fungi and subsequent disease development in paddy fields has yet to be quantitatively determined.

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