Distribution of Pythium Snow Rot Fungi in Paddy Fields and Upland Fields

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Cultivation of wheat and barley has been increasing in Japan since 1978. There were very heavy snow falls in 1981, 1984, 1985 and 1986 in the Hokuriku district, and control of snow mold became an important problem for wheat and barley cultivation. Typhula snow blight caused by *Typhula incarnata*, pink snow mold by *Fusarium nivale* and Pythium snow rot by *Pythium* spp. were found to be common in the Hokuriku district[8]. Pythium snow rot has been increasing in recent years in this district, probably because of wheat and barley cultivation in ill-drained paddy fields.

There are several fungicides for controlling snow mold, but none of them are very effective against Pythium snow rot. Further, Pythium snow rot can not be adequately controlled by mere application of fungicides because the pathogens are typical soil-inhabiting fungi. Thus, ecological control measures, based on adequate knowledge on the ecology of the pathogens, must be employed for controlling the disease.

From the above point of view, this study was conducted to identify and classify the causal fungus of Pythium snow rot, the most severe snow mold of wheat and barley in the Hokuriku district, and to investigate the ecology of the disease.

Pythium snow rot fungi and their pathogenicity

Pythium snow rot was first reported in 1933 in Toyama Prefecture, Japan[6]. A Latin description of the fungus was then provided and it was named *P. iwayamai* S. Ito[5]. Besides this fungus, three additional *Pythium* spp. (*P. graminicola* Subr., *P. horinouchiense* Hirane, *P. paddicum* Hirane) were described as causal organisms, but two other species remained unidentified as minor pathogens[2].

The author obtained 358 isolates of *Pythium* spp. from the rotted leaves of wheat and barley just after snow melt during the period between 1982 and 1984, and 343 isolates were identified as belonging to six different species. The remaining 15 isolates were several other species isolated with very low frequency. *Pythium okanoganense* Lipps, *P. vanterpoolii* V. Kouyeas & H. Kouyeas, and *P. volutum* Vanterpool & Truscott were newly identified as pathogens in Japan. The former two species were also the first to be recorded in Japan. *Pythium iwayamai* was isolated most frequently (43%), followed by *P. paddicum* (29%), *P. graminicola* (7%), *P. okanoganense* (7%), *P. volutum* (6%), *P. vanterpoolii* (4%) and unidentified *Pythium* spp. (4%) (Table 1). *Pythium paddicum* was prevalent in paddy fields where wheat or barley was first planted after continuous rice cropping. On the other hand, *P. iwayamai* was prevalent in fields where wheat or barley had been planted for years.

Pathogenicity tests were performed by using essentially the same method as described previously[4,7]. Seeds of the snow endurance wheat cultivar, Nanbu komugi, and the susceptible, Norin No. 61, were grown in a plastic box (32×25×7 cm) under glasshouse conditions until the 3 leaf stage. *Pythium* isolates grown on a wheat bran-sand mixture at 15°C for one month were

Table 1. Frequency of isolation of *Pythium* spp. from diseased wheat and barley leaves just after snow melt[a)]

Pythium spp.	Wheat			Barley			Sum total
	1 st cropping	Continuous cropping	Total	1 st cropping	Continuous cropping	Total	
No. of field surveyed	37	10	47	85	25	110	157
No. of leaf pieces used	254	209	454	621	210	831	1285
P. graminicola	2	0	2	2	20	22	24
P. iwayamai	12	70	82	33	39	72	154
P. okanoganense	2	0	2	21	2	23	25
P. paddicum	27	7	34	60	11	71	105
P. vanterpoolii	1	1	2	4	8	12	14
P. volutum	2	13	15	1	5	6	21
Pythium spp.	7	1	8	3	4	7	15
Total	53	92	145	124	89	213	358

a) : Isolated between 1982 and 1984.

Table 2. Pathogenicity of *Pythium* spp. to different cultivars of wheat

Pythium spp. (Isolate no.)		Nanbu komugi	Norin No. 61
P. graminicola	(H-82-35)	16/29[a)]	1/29
〃	(H-82-36)	21/30	3/30
P. iwayamai	(W-82-24)	0/29	0/30
〃	(W-82-50)	0/30	0/29
P. okanoganense	(H-82-69)	4/30	0/30
〃	(H-82-72)	2/30	0/30
P. paddicum	(W-82-15)	2/29	0/30
〃	(H-82-73)	29/29	12/30
P. vanterpoolii	(H-82-13)	30/30	24/30
〃	(H-82-18)	24/30	13/30
P. volutum	(H-82-60)	27/30	14/30
〃	(H-82-82)	21/31	0/30
Uninoculated	1	30/30	30/30
〃	2	30/30	30/30

a) : Ratio of seedlings surviving to those inoculated.

applied onto the soil surface (100 g/800 cm^2) in the plastic box, then covered with a pad of wet absorbent cotton to simulate snow cover. After placing the lid, the box, with inoculated seedlings, was transferred into a cold storage room (0–1°C) and kept for 40 days in the dark, then returned to the glass-house. The number of surviving plants was recorded after the 10-day recovery period.

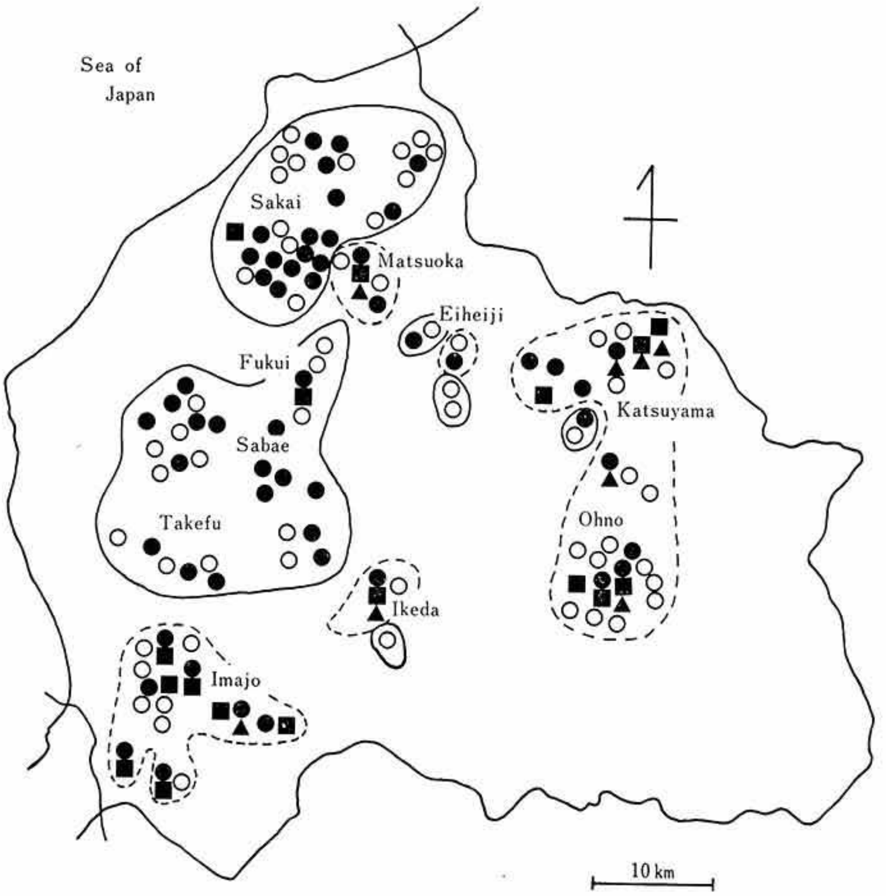
All of the tested isolates caused Pythium snow rot with the artificial inoculation (Table 2). *Pythium iwayamai*, *P. okanoganense* and *P. paddicum* caused severe damage on wheat. The virulence of the other three species was low and seemed to be minor pathogens of snow rot. Further examinations revealed that *P. iwayamai* had higher virulence than *P. okanoganense* and *P. paddicum*.

These results show that *P. iwayamai*, *P. okanoganense* and *P. paddicum* are the main pathogens of Pythium snow rot in Japan.

Detection of the main pathogens in diseased leaves of wheat and barley grown in paddy fields and upland fields

Out of 157 paddy fields surveyed (Table 1), 122 fields were planted to wheat or barley for the first time after continuous rice cropping. Those fields were classified according to the degrees of drainage, and kinds of pathogens isolated from the respective fields were investigated (Fig. 1)[9)].

Pythium snow rot occurred at 37 fields out of 69 ill-drained paddy fields, and *P. paddicum* was isolated from 36 fields (97%). *Pythium iwayamai* was isolated from only two fields (5%), and no *P. okanoganense* was isolated. The disease occurred at 27 fields out of 53 well-drained paddy fields. *Pythium paddicum*,



■, ▲, ●: The fields where *P. iwayamai*, *P. okanoganense* and *P. paddicum* were isolated, respectively.

○: The fields where none of these pathogens was isolated.

●▲, ●■, ■▲, ●■▲: More than 2 pathogens were isolated from the same field, simultaneously.

(dashed outline): The regions of well-drained paddy fields.

(solid outline): The regions of ill-drained paddy fields.

Fig. 1. Survey points and detection of Pythium snow rot pathogens in paddy fields in Reihoku district of Fukui Prefecture, Japan, between 1982 and 1984

P. iwayamai and *P. okanoganense* were isolated from 19 (70%), 15 (56%) and 8 (30%) fields, respectively. On the other hand, *P. iwayamai* was prevalent in upland fields (Table 3). From these results the relationships between kinds of Pythium snow rot fungi and the degrees of drainage in the fields can be illustrated as Fig. 2.

Detection of the pathogens in the soils having no cultivation history of wheat and barley

The presence, or absence, and kinds of main Pythium snow rot fungi in the soils where wheat and barley had not been planted were investigated. A survey was performed

Table 3. Frequency of isolation of *Pythium* spp. from diseased wheat and barley in upland fields just after snow melt

Fields surveyed	Year	Plant (cv.)	Number of leaf pieces used	Number of isolations: *P. iwayamai*	*P. okanoganense*	*P. paddicum*	*Pythium* spp.	Remarks
A	1984	Wheat (Nanbu komugi)	6	1	0	0	2	Summer cropping with soybean for more than 10 years
	1985	Barley (Minori mugi)	12	9	0	0	0	
	1986	Barley (Minori mugi)	12	8	0	0	3	
B	1984	Wheat (Nanbu komugi)	6	1	0	0	0	1st cropping after cultivating water melon for several years
	1985	Barley (Minori mugi)	12	9	0	0	0	2nd cropping
	1986	Barley (Minori mugi)	12	6	0	0	0	3rd cropping
C	1985	Barley (Benkei mugi)	12	11	0	0	0	1st cropping after cultivating soybean for several years
		Wheat (Nanbu komugi)	12	6	0	1	0	
D	1986	Wheat (Nanbu komugi)	12	12	0	0	0	1st cropping in nursery field
E	1986	Wheat (Nanbu komugi)	12	5	0	0	0	2nd cropping in mulberry field
F	1986	Barley (Minori mugi)	12	7	0	1	1	〃
G	1986	Barley (Minori mugi)	12	8	0	2	0	〃
Total 7 fields	1984–1986	Wheat and barley	132	83	0	4	6	

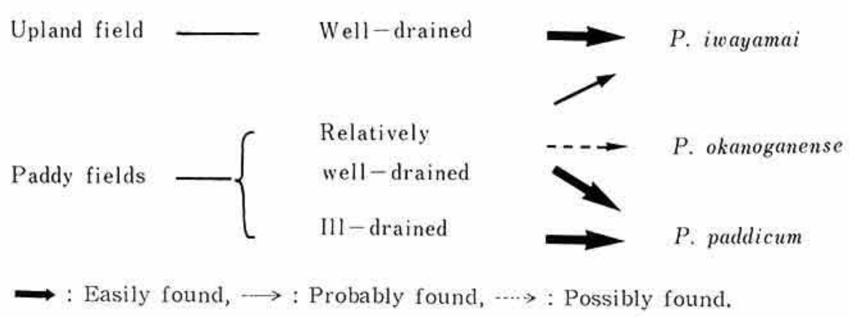
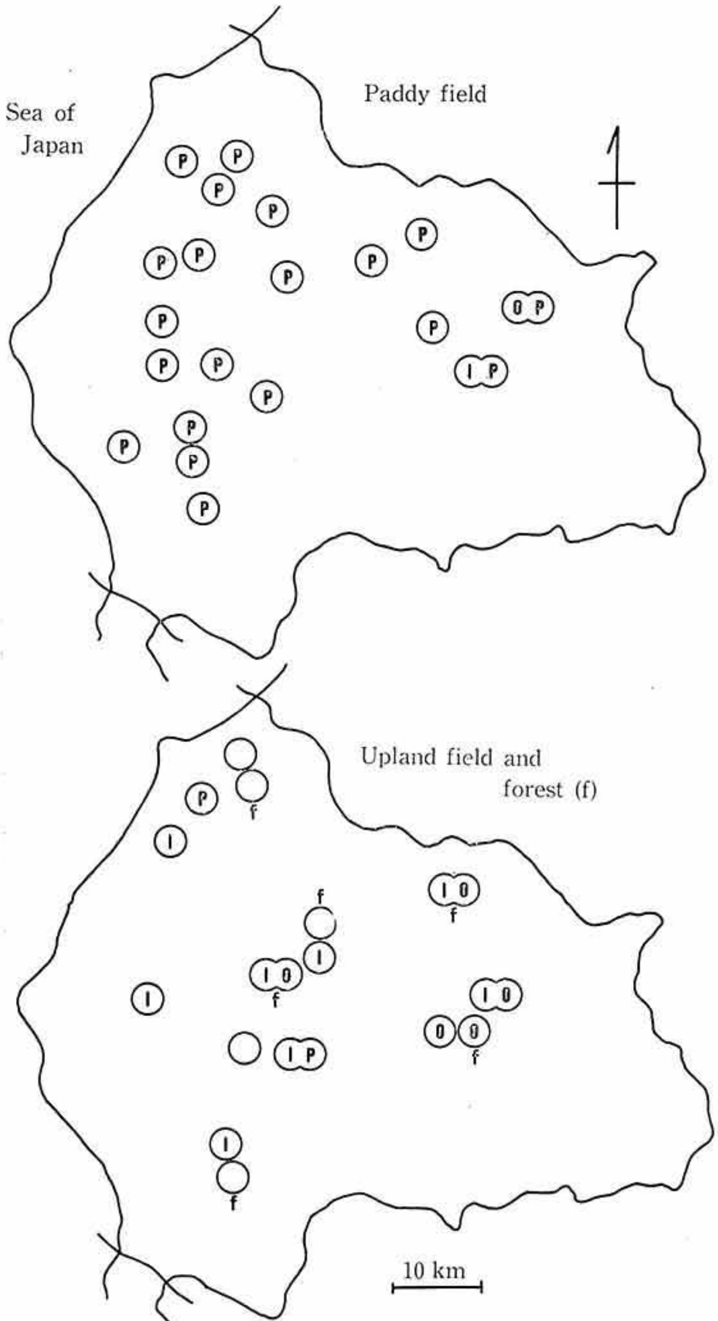


Fig. 2. Relationship between kinds of Pythium snow rot fungi and the degrees of drainage in the fields



I, O, P : The places of isolation of *Pythium iwayamai*, *P. okanoganense* and *P. paddicum*, respectively.

IO, IP, OP : The places where the respective two fungi were isolated together.

○ : The places of non isolation of these species.

Fig. 3. Differences in distribution of three *Pythium* species in the soils having no cultivation history of wheat and barley surveyed during early winter of 1985 and 1986

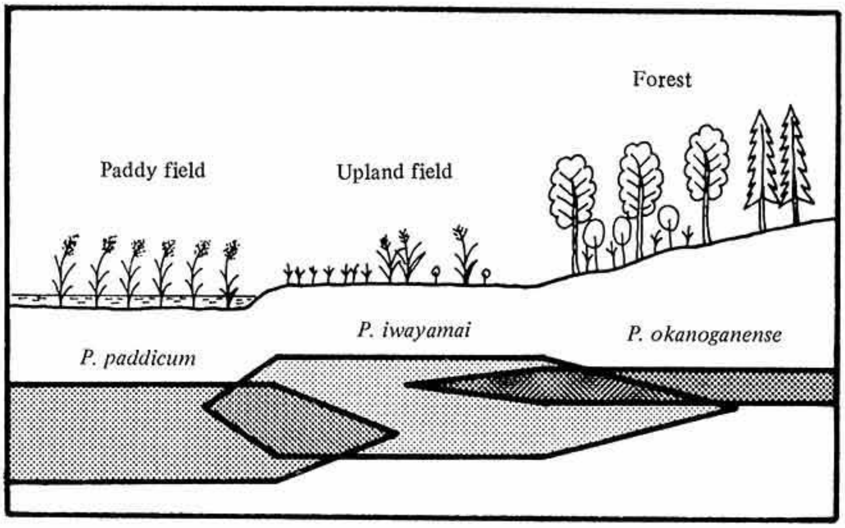


Fig. 4. Distribution of the main Pythium snow rot fungi in the fields

in 1985 and 1986. Twenty paddy fields, ten upland fields and six forests were selected at random as survey points in the north-eastern part of Fukui Prefecture. In early December, about 500 g of soils were collected from 0–3 cm depth of 20 sites of each survey point since the pathogens can easily be detected during this season. All samples were air-dried for 2–3 days and filtered through a 16 or 9 mesh-sieve. A direct isolation method[3], a baiting method[10] with young wheat leaf pieces and an indexing method[10] with wheat seedlings were employed to detect *Pythium* in soil. As shown in Fig. 3, *P. paddicum* was detected in all survey points of the paddy field. *Pythium iwayamai* was detected from one paddy field by the baiting method and *P. okanoganense* was detected with low frequency in another field by the indexing method. Paddy field soils where *P. iwayamai* and *P. okanoganense* had been detected were well-drained volcanic ash. *Pythium iwayamai* was detected in 6 of 10 upland fields in 1985 and 1986. *Pythium okanoganense* was detected in one field for 2 years successively, but detected in another field only in 1986. *Pythium iwayamai* and *P. okanoganense* were detected from 2 and 3 of the forest soils, respectively. Two places out of 3 where *P. okanoganense* had been detected were chestnut groves. No *P. paddicum* was detected in forest soils. Three species of *Pythium* isolated in this survey were pathogenic on wheat by artificial inoculation.

From the present survey, *Pythium* spp. which are pathogenic on wheat apparently survive in paddy fields, upland fields and forest soils. Since these fungi are known to attack many plants under snow[1], their growth and survival in soils may be possible without cultivation of wheat and barley. *Pythium paddicum* can also be detected in both paddy field and wetland soils although both wheat cultivation and deep snow are not found. Moreover, this fungus proved to be pathogenic on wheat by using the same method as described previously[4,7] (Ichitani, unpublished data). This may indicate that this fungus is able to become a snow rot fungus. The present results show that *P. paddicum* is wide-spread in paddy fields, whereas *P. iwayamai* is considered as an upland fungus. These differences in distribution in the soils without cultivation history of wheat and barley may result in each Pythium snow rot fungus where the hosts are growing.

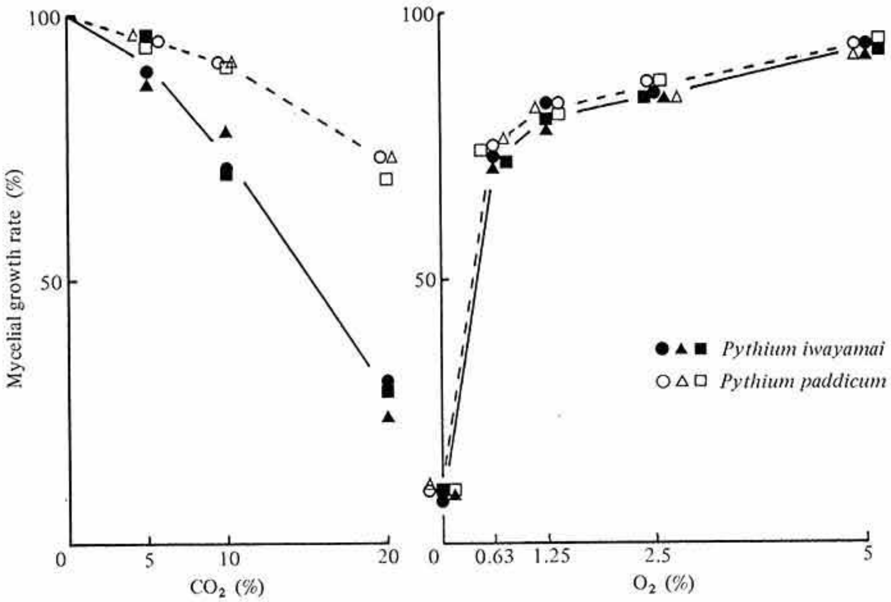


Fig. 5. Mycelial growth of three isolates of each of *Pythium iwayamai* and *P. paddicum* on cornmeal agar at various concentrations of CO_2 and O_2, expressed as percentage of the growth rate in the air

Both *P. iwayamai* and *P. paddicum* can be isolated from diseased leaves of wheat and barley in well-drained paddy fields. *Pythium paddicum* was, however, detected only in paddy field soils, but *P. iwayamai* was rarely found in such soils. Then, it is possible that *P. iwayamai* was dispersed from upland fields into paddy fields after paddy-upland rotation.

From these results, the distribution pattern of the main Pythium snow rot fungi can be illustlated as Fig. 4.

Factors influencing the distribution of the pathogens

Factors involved in the different distributions of *P. iwayamai* and *P. paddicum* in field soils were investigated from the aspects of differences in endurance of the fungi to osmotic potential and gas environment. But no significant difference was observed in endurance to osmotic potential between the two fungi.

The two fungi were then grown on cornmeal agar in atmospheres containing various concentrations of CO_2 and O_2 (Fig. 5). The mycelial growth of these fungi was retarded as CO_2 concentration increased, and above 5% CO_2, the growth of *P. iwayamai* was more rapidly retarded than that of *P. paddicum*. The percentage inhibition of growth at 20% CO_2 was 70% in *P. iwayamai* and 30% in *P. paddicum*. This result indicates that the paddy field inhabitant fungus, *P. paddicum*, is more tolerant to high CO_2 concentration than the upland field inhabitant fungus, *P. iwayamai*. On the other hand, no significant difference in tolerance for O_2-deficiency was found between them. The percentage inhibition of growth of both fungi was only 20–30% at 0.63% O_2, which may indicate that these fungi are tolerant to O_2-deficiency.

Concentrations of CO_2 and O_2 in paddy field and upland field soils, and kinds of Pythium snow rot fungi surviving in the soils were investigated. Two sites (2×2 m) in each paddy field and upland field having no cultivation history of wheat and barley were selected as survey points. A plastic funnel having a brazen net in its cavern was buried upside down at 3, 6 and 9 cm below

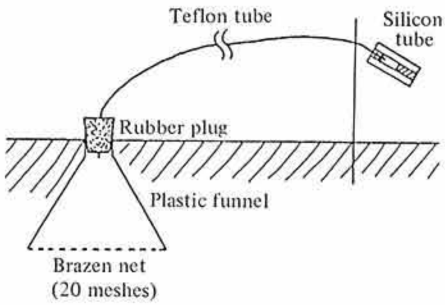


Fig. 6. A device for sampling gas in soil

Table 4. Concentrations of CO_2 and O_2 in upland field and paddy field soils

Kind of field	Soil depth (cm)	CO_2 (%)			O_2 (%)		
		Time of gas sampling					
		9–10	13–14	17–18	9–10	13–14	17–18
Upland field	3	0.1[a]	0.1	0.1	20.5	21.2	20.1
	6	0.1	0.1	0.2	20.9	21.0	20.5
	9	0.3	0.2	0.3	20.7	20.3	20.3
Paddy field	3	6.4	7.5	7.6	6.2	4.6	5.4
	6	20.5	22.0	23.6	5.3	3.3	4.5
	9	18.4	20.3	20.6	6.2	5.0	5.2

a) : Mean of two sites of each upland field and paddy field, with 2 replications at each site.

the soil surface, and was connected with a thin teflon tube which has a silicon tube in tip (Fig. 6). After seven days of burying, gas in the cavity of the funnel was collected through the teflon tube by a glass syringe, and CO_2 and O_2 concentrations in the gas sample were analyzed with a gas chromatography. As shown in Table 4, the concentrations of CO_2 were 6.4–7.6, 20.5–23.6 and 18.4–20.6% at 3, 6 and 9 cm below the soil surface of paddy field, respectively, which were quite high compared with 0.1–0.3% of upland field soils. From these results together with those shown in Fig. 5, it became evident that the mycelial growth of *P. iwayamai* may be significantly inhibited in the paddy field soil which has high concentrations of CO_2.

Baitings with wheat leaf pieces were carried out several times to detect the snow rot fungi from the field soils. *Pythium paddicum* had always been isolated from both sites of paddy field, whereas *P. iwayamai* had never been detected. On the other hand, *P. iwayamai* had always been isolated from both sites of upland field and *P. paddicum* had also been isolated from one of the sites.

These results suggest that high CO_2 concentrations in paddy field soils be an important factor in limiting survival of *P. iwayamai* in the fields. But this factor can not explain the reason why *P. iwayamai* prevails in upland field soils. It might be due to the differences in vilurence of the fungi on plants. Further studies are required to identify other factors which might influence the distribution of the fungi.

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