Seedborne Infection of Rice by *Pyricularia oryzae* and Its Transmission to Seedlings

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ABSTRACT

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Seedborne infection of rice by *Pyricularia oryzae* and its transmission to seedlings were studied quantitatively with naturally infected seeds of three rice cultivars collected from three locations in Nepal. A linear relationship on a logistic scale was found between panicle symptoms and seed infection, i.e., the more symptoms the higher seed infection. However, healthy-looking panicles and branches of panicles could also yield infected seeds. Postharvest measures such as winnowing and sun-drying significantly reduced seed infection by *P. oryzae* and filled grains had a lower degree of infection than unfilled grains. Sporulation of *P. oryzae* was most often confined to the embryonal end of germinating seeds. In contrast, most of the nongerminating seeds had sporulation all over the seed surface. Transmission of *P. oryzae* from seeds to seedlings, studied under various seeding conditions, showed that the transmission rate was always low. Thus, a seed sample with 21% seed infection resulted in less than 4% seedlings with blast lesions. Seed transmission was found for light covering of the seeds with soil or for moist seeding without covering. Transmission was rarely found when seeds were completely covered, and never in seedlings raised under water seeding conditions. Lower infection frequency was observed in seedlings raised in unsterilized soil than in seedlings raised in sterilized soil. Also, percent recovery of *P. oryzae* from infected seeds was higher in sterilized soil than in unsterilized soil and declined with time. Seedlings grown under low temperature (15 to 20°C) conditions did not develop blast lesions but when the same plants were transferred to high temperature (25 to 30°C) conditions, blast lesions were detected. This confirmed the latent infection in seedlings by *P. oryzae* grown under low temperature conditions.

Additional keywords: rice blast

Pyricularia oryzae Cavara [synonym *Pyricularia grisea* (Cooke) Sacc., anamorph of *Magnaporthe grisea* (T. T. Herbert) Yaegashi & Udagawa], the cause of rice blast, is one of the most important fungal pathogens of rice (*Oryza sativa* L.) because of its widespread occurrence and destructive nature (25). The fungus can attack any aerial part of the rice plant, including seeds, in which the fungus may overwinter for several years (9,19). Recent investigations (19) suggested systemic transmission of the fungus from seeds to seedlings. Seed transmission of *P. oryzae* was first reported from Japan (13,31) and later from other parts of the world (4,14,22,27). However, most of the earlier studies were based on the growth of *P. oryzae* on seeds or seedlings incubated under labo-

ratory conditions (4,13,14,31), or were based on brief observations with few details given (22,27).

In the tropics, where airborne inoculum is present throughout the year, overwintering of *P. oryzae* is not important in the disease cycle (25). But in other regions, where overwintering is necessary for the fungus, infected seeds may play an important role in the disease cycle (15). Infected seeds can also be the primary source of inoculum when seeds are densely sown in seedling boxes for mechanical transplanting, as has been practiced in Japan (8). Although it has been known for a long time that the fungus is also seedborne, the significance of seed transmission has not been adequately studied since inoculum was mainly considered to be airborne.

The aim of this research was to examine the importance of seedborne infection by *P. oryzae* in rice seeds and its transmission to seedlings. First, attempts were made to study the nature of seedborne infection. Secondly, seed transmission was investigated for various seeding methods under greenhouse or growth room conditions.

MATERIALS AND METHODS

Some experiments on seed infection were conducted in Nepal, using the facilities at the Plant Pathology Division of the Nepal Agricultural Research Council, Khumaltar, Nepal. Other experiments were conducted in Denmark either in greenhouses or growth rooms belonging to the Royal Veterinary and Agricultural University or in growth rooms of the Danish Government Institute of Seed Pathology for Developing Countries.

Seed infection: Collection of seed samples. Seed samples of rice cultivars Taichung-176 (Tsai-Yuan-Chung × degeo-woo-gen, from Taiwan), Masuli (Mayang Ebos 80*2 × Taichung 65, from Malaysia), and Radha-17 (Mallika × Masuli, from Nepal) were collected in the Lalitpur, Chitwan, and Makawanpur districts of Nepal, respectively, from rice showing neck blast symptoms. Each cultivar was grown in only one location. All three cultivars are susceptible to *P. oryzae* under field conditions in Nepal (18; H. K. Manandhar, unpublished results). This collection provided seed samples of different genotypes and geographical origin, representing different natural infection levels by *P. oryzae*. From each site, three samples were collected from three different plots. Seeds were harvested from 12 m² of each plot. Before seed harvest, 24 hills from each sample area were examined for neck blast symptoms and the incidence was determined by the method adopted by the Agricultural Production Bureau, Japan (2), in which percent neck blast incidence = $(4A + 3B + 2C + D + 0E) / (4 \times \text{number of hills examined})$, where A = hills for which 61% or more panicles are showing neck blast symptoms, B = hills for which 31 to 60% panicles are showing neck blast symptoms, C = hills for which 11 to 30% panicles are showing neck blast symptoms, D = hills for which 10% or less panicles are showing neck blast symptoms, and E = hills for which 0% panicles are showing neck blast symptoms.

Panicles and seed samples were harvested during November and December 1994 and were stored at 15°C for approximately 4 to 6 weeks before any postharvest treatment took place.

Effect of postharvest measures on seed infection by *P. oryzae*. Seed samples

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of cvs. Radha-17, Taichung-176, and Masuli collected from each plot at the three sites (three plots per site) were divided into three equal parts to examine the influence of postharvest measures on the seed infection. The first part, unwinnowed, was tested directly for infection by *P. oryzae*. The second part was tested for infection after winnowing. The third part was tested after winnowing and sun-drying for 2 days (approximately 6 h daily). Winnowing was done manually with a very common local winnower called a *nanglo*. It consists of a round mat (approximately 0.65 m in diameter) made of longitudinal bamboo slices supported by a frame of bamboo sticks. After the treatments, the seed samples were stored at 5°C and seed health testing was started within a week.

Seed health testing. Seed samples were tested for the presence of *P. oryzae* by the standard blotter method modified from Mathur (21). Four hundred seeds were plated in replicates of 25 seeds per 9-cm-diameter plastic petri dish (Sterilin, Bibby Sterilin Ltd., Stone, Staffordshire, UK) lined with three layers of water-soaked blotter papers. The dishes were incubated at $20 \pm 2^\circ\text{C}$ for 4 days in alternating cycles of 12 h darkness and 12 h near-UV light (Philips TLD 36W/08, Philips Lighting B.V., Roosendaal, The Netherlands). Observations were made on whether the seeds were infected by *P. oryzae* or not. Occurrence of other organisms on the seeds was also recorded. Seed health testing of all seed samples lasted for approximately two and a half months.

For determining the preferred site of sporulation, observations were made on whether the seeds germinated, whether they were infected, and, if infected, from which part of the seed the fungus was sporulating, i.e., from the embryonal end alone or from the entire seed surface, including the embryonal end.

Relationship between panicle symptoms and seed infection by *P. oryzae*. Hundred and fifty panicles with neck blast symptoms and 150 healthy-looking panicles were collected from each plot at the three sites (nine samples) before harvest in order to examine the correlation between panicle symptoms and seed infection. Filled and unfilled grains were separated from each 100 panicles with or without neck blast symptoms and tested separately for infection by *P. oryzae*. Seeds that went off the winnower during winnowing were considered to be unfilled grains (included both empty and partly filled grains).

The remaining 50 panicles with neck blast symptoms from each sample were separated into secondary grain-bearing branches with symptoms and healthy-looking, secondary grain-bearing branches. Four hundred seeds from each of these two groups for each cultivar were examined for the presence of *P. oryzae* as described before.

Seed transmission. Three different types of experiments were conducted with seed samples of Radha-17, Taichung-176, and Masuli having 21, 13, and 8% natural infection by *P. oryzae*, respectively. The seed infection percentage was determined by seed health testing 1 week prior to the start of the seed transmission studies. These three samples, stored at 5°C after seed health testing, were used throughout the seed transmission studies. The time interval between the first and last seeding was about 3 months.

Recovery of *P. oryzae* from blast-infected seeds after sowing in soil. One hundred seeds of each cultivar were sown in 10×12 cm plastic pots in rows approximately 12 mm apart. Within the rows, the seeds were placed approximately every 10 mm. The pots were filled with either sterilized or unsterilized greenhouse peat soil 'Weibulls Enhetsjord' (Svalöf Weibull,

Hammenhög, Sweden) (10). Two different seeding methods were employed. Water seeding was done by placing seeds on the soil without covering, and subsequently applying water until the seeds were completely immersed. Moist seeding with complete covering was done by covering the seeds with a 1 to 1.5 cm layer of soil, compressed by hand. The soil was kept moist throughout the growing period, but not flooded. The pots were arranged in a randomized complete block design with four replications and placed in a growth chamber at 25 to 28°C under 12 h cycles of light (approximately $200 \mu\text{E s}^{-1} \text{m}^{-2}$) supplied by fluorescent white tubes (Philips TLD 36W/83) and darkness. Fertilization of the plants was done as described before (20). Seeds or seedlings were removed from the soil 3, 5, 8, or 15 days after seeding and the seeds separated from shoots. The seeds were washed thoroughly under running tap water and tested for the presence of *P. oryzae* by the blotter method.

Development of blast lesions on rice leaves from seed samples varying in infection percentage. One hundred seeds each of Radha-17, Taichung-176, and Masuli were sown in 10×12 cm plastic pots by four seeding methods. Water seeding and moist seeding with complete covering were performed as before. The last two methods were moist seeding without covering and moist seeding with light covering (seeds were covered with a <0.5 cm layer of soil without compression so that no part of any seed was visible). The pots were arranged in a completely randomized block design with four replications and kept either in a greenhouse at 25 to 30°C under natural daylight conditions or in growth rooms at 25 to 30°C or 15 to 20°C under 12-h cycles of light and darkness as described for seed health testing.

To avoid cross contamination of *P. oryzae* between treatments and to maintain humidity for disease development, plants were grown in clear plastic bags of appropriate size supported by rectangular steel frames.

Plants were thoroughly examined for the presence of blast lesions every second or third day from emergence until they were 4 weeks old, and the number of seedlings with blast lesions was recorded.

Detection of latent infection. Rice plants were raised from blast-infected seeds of Radha-17, Taichung-176, and Masuli by the seeding methods described previously. The aerial parts of 200 seedlings were separated from the seeds 8 or 15 days after seeding and cut in pieces approximately 4 cm long. The tissue pieces were washed thoroughly under running tap water, and tested for the presence of *P. oryzae* by the deep-freeze blotter method (21) in order to enhance sporulation of the fungus that might be present as latent infections. The plant material was plated on

Table 1. Percent infection of *Pyricularia oryzae* in rice seeds with and without post-harvest treatments^a

Cultivar/location	Plot no.	Percent neck blast infection	Percent seed infection ^b		
			Unwinnowed	Winnowed	Winnowed + sun-dried
Taichung-176/Khumaltar	1	10.4	7.8	5.3	5.0
	2	14.6	11.0	8.3	6.5
	3	24.0	14.3	9.5	8.0
Masuli/Rampur	1	29.2	12.3	7.3	5.8
	2	46.9	15.8	8.8	7.3
	3	52.1	17.0	11.3	8.8
Radha-17/Hetaunda	1	59.4	28.3	17.5	15.3
	2	62.5	26.0	18.8	18.5
	3	76.0	31.3	23.5	20.5
Odds ratio ^c			1.00	0.61***	0.52***
β		0.020***			
Odds ratio ^d		1.22			

^a Harvested from field plots with varying degrees of neck blast symptoms.

^b Based on 400 seeds for each sample.

^c Odds ratio for comparison of treatments to unwinnowed sample (odds ratio = 1.00 used as a reference). *** = significance at $P \leq 0.001$.

^d Odds ratio for slope ($\beta = 0.020$) from regression corresponding to a 10% increase in neck blast. *** = significance at $P \leq 0.001$.

moist blotter paper, placed at -20°C for 6 h, and then transferred to an incubation room at $20 \pm 2^{\circ}\text{C}$ for 5 days in cycles of 12 h darkness and 12 h near UV-light as described for seed health testing.

Statistical analysis. All parameters recorded were discrete variables since it was recorded whether or not a certain event had taken place, e.g., whether or not a seed was infected. These results were, therefore, analyzed by logistic regression, corrected for over dispersion (5), when present, by procedure GENMOD in PC-SAS, release 6.11 (SAS Institute, Cary, NC). For comparison of the variables (percentages), odds ratios (5) were calculated. For example, the odds ratio for the percent seed infection for winnowed and sun-dried seeds is 0.52^{***} (Table 1). This means that odds (that is, $P/[1 - P]$, where P is the probability of infected seeds) are approximately 2 times higher for unwinnowed seeds than odds for winnowed and sun-dried seeds. Three asterisks indicate that the difference is significant at $P \leq 0.001$. Hypotheses were rejected for $P > 0.05$.

All experiments were performed three times with similar results. Representative data are presented here. Since the three rice cultivars were grown at different locations, no direct comparisons were attempted between them.

RESULTS

Seed infection. There was a linear relationship on a logistic scale between neck blast symptoms and seed infection by *P. oryzae* (Table 1). The estimated slope was $\beta = 0.020$ (significant at $P \leq 0.001$ for the hypothesis $\beta = 0$) and the odds ratio corresponding to a 10% increase in neck blast was 1.22. This means that a 10% increase in neck blast resulted in a 22% increase in odds for seed infection. Similarly, seeds collected from panicles with neck blast symptoms had a significantly higher degree of infection than seeds collected from healthy-looking panicles (Table 2). Filled grains had a significantly lower degree of infection than unfilled grains for all cultivars (Table 2). Furthermore, seeds from secondary grain-bearing branches of panicles with symptoms had a significantly higher degree of infection by *P. oryzae* than healthy-looking branches (Table 3). The postharvest measures of winnowing and winnowing + sun-drying significantly reduced the seed infection by *P. oryzae*, compared with unwinnowed seeds (Table 1). Winnowing + sun-drying also gave significantly lower seed infection than winnowing alone. For this comparison, odds ratio were as follows: winnowing (used as a reference) = 1.00 and winnowing + sun-drying = 0.85 ($P \leq 0.05$).

Sporulation of *P. oryzae* at the embryonal end of seeds alone was seen significantly more often in germinating than in nongerminating seeds (Table 4). Thus, sporulation was confined to the embryonal

end of about 80% of the germinating seeds whereas, in more than 80% of the nongerminating seeds, sporulation of *P. oryzae* occurred all over the seeds.

Seed transmission. When tested 3 or more days after seeding, *P. oryzae* was never recovered from seeds sown in sterilized or unsterilized greenhouse peat soil under water seeding conditions (data not shown). With unsterilized soil and moist seeding conditions (complete covering of seeds with a 1 to 1.5 cm layer of soil) at 25 to 28°C , *P. oryzae* was recovered up to 5 days after seeding (Table 5). For sterilized soil, the fungus was recovered up to 8 days after seeding. Percent recovery was higher in sterilized than in unsterilized soil, and significantly declined with

time for both sterilized and unsterilized soil.

Blast lesions first appeared on the second or third leaf of seedlings raised from infected seeds about 3 weeks after seeding at 25 to 28°C (Table 6). The time of appearance for blast lesions was always the same under the experimental conditions employed. Lesions were never found on seedlings raised under water seeding conditions in either unsterilized or sterilized greenhouse peat soil (data not shown). Seedlings raised in unsterilized soil had a significantly lower infection frequency than seedlings raised in sterilized soil. In unsterilized soil under moist seeding, the transmission of *P. oryzae* from infected seeds to seedlings depended on the degree

Table 2. Percent infection of filled and unfilled rice seeds by *Pyricularia oryzae* collected from healthy-looking panicles and panicles with neck blast symptoms

Cultivar/location	Percent seed infection ^a					
	Healthy-looking panicles		Panicles with neck blast		Odds ratio ^b	
	Unfilled	Filled	Unfilled	Filled	Unfilled	Filled
Taichung-176/Khumaltar	5.9	2.6	27.4	15.1	1.00	0.44***
Masuli/Rampur	4.5	2.3	23.4	14.4	1.00	0.54***
Radha-17/Hetaunda	9.5	4.9	32.2	25.4	1.00	0.65***
Odds ratio ^c	Healthy = 1.00		Infected = 6.05***			

^a Based on 1,200 seeds of filled or unfilled grains (400 seeds per sample; three samples per location).

^b Odds ratio for comparison of filled and unfilled grains. There was a significant interaction between cultivar and filling of grains. Hence, odds ratio for each cultivar are presented separately. Unfilled grains (odds ratio = 1.00) were used as a reference. *** = significance at $P \leq 0.001$.

^c Odds ratio for comparing healthy and infected panicles; Healthy panicles (odds ratio = 1.00) were used as a reference. *** = significance at $P \leq 0.001$.

Table 3. Percent infection of rice seeds by *Pyricularia oryzae* obtained from healthy-looking and diseased secondary grain-bearing branches of the same panicles

Rice cultivar/location	Percent seed infection ^a	
	Healthy-looking branches	Diseased branches
Taichung-176/Khumaltar	3.3	67.8
Masuli/Chitwan	4.3	47.3
Radha-17/Hetaunda	5.8	78.8
Odds ratio ^b	1.00	40.74***

^a Based on 400 seeds of each cultivar. Fifty panicles with neck blast symptoms of each sample were separated into secondary grain-bearing branches with symptoms and healthy-looking secondary grain-bearing branches. Seeds from each of these two groups were examined for the presence of *P. oryzae*.

^b Odds ratio for comparing healthy and infected branches of panicles; Healthy panicles (odds ratio = 1.00) were used as a reference. *** = significance at $P \leq 0.001$.

Table 4. Site of sporulation of *Pyricularia oryzae* on nongerminating and germinating filled rice seeds

Cultivar	Percent seeds having sporulation of <i>P. oryzae</i>			
	Confined to the embryonal end		All over the seed	
	Nongerminating seeds ^a	Germinating seeds ^a	Nongerminating seeds	Germinating seeds
Taichung-176/Khumaltar	11.0	83.0	89.0	17.0
Masuli/Rampur	8.0	78.5	92.0	21.5
Radha-17/Hetaunda	17.0	86.0	83.0	14.0
Odds ratio ^b	Nongerminating seeds 1.00		Germinating seeds 36.14***	

^a Based on 100 nongerminating seeds and 400 germinating seeds with *P. oryzae* of each cultivar. A total of more than 6,000 seeds of each cultivar were examined to get this number of seeds.

^b Odds ratio comparing nongerminating (odds ratio = 1.00 as a reference) and germinating seeds regarding sporulation confined to embryonal end of the seeds. *** = significance at $P \leq 0.001$.

Table 5. Percent recovery of *Pyricularia oryzae* from naturally infected rice seeds 3, 5, 8, and 15 days after sowing in unsterilized or sterilized greenhouse peat soil^a

Cultivar/location	Percent recovery of <i>P. oryzae</i> after sowing ^b				
	0 days ^c	3 days	5 days	8 days	15 days
Unsterilized soil					
Taichung-176/Khumaltar	12.8 (1.00) ^d	9.8 (0.74) NS	6.8 (0.50)**	0.0 ND ^e	0.0 ND
Masuli/Rampur	7.8 (1.00)	2.3 (0.27)***	1.5 (0.18)***	0.0 ND	0.0 ND
Radha-17/Hetaunda	21.3 (1.00)	13.5 (0.57)***	9.0 (0.36)***	0.0 ND	0.0 ND
Sterilized soil					
Taichung-176/Khumaltar	12.8 (1.00)	11.8 (0.91) NS	9.3 (0.70) NS	7.5 (0.55)**	0.0 ND
Masuli/Rampur	7.8 (1.00)	3.5 (0.43)**	2.3 (0.27)***	0.0 ND	0.0 ND
Radha-17/Hetaunda	21.3 (1.00)	18.5 (0.83) NS	13.8 (0.58)**	5.5 (0.21)***	0.0 ND

^a Seeds were planted 1.0 to 1.5 cm deep in moist soil at 25 to 30°C.

^b Based on 400 seeds.

^c Percent seed infection before sowing.

^d Odds ratio for comparison at days after sowing, with 0 days (odds ratio = 1.00) used as reference. There was a significant interaction between cultivar, time, and soil treatment (unsterilized vs sterilized soil). Therefore, data were analyzed and odds ratios are presented separately for each cultivar and soil treatment, making it possible to compare the percentages of recovery as a function of time. In each case, odds ratios are presented in parentheses under the percentage of seed infection and should only be compared horizontally. NS = nonsignificant difference, ** and *** = significance at $P \leq 0.01$ and $P \leq 0.001$, respectively.

^e Not determined.

Table 6. Development of blast lesions 3 weeks after seeding on the second or third leaf of rice seedlings raised from seeds infected by *Pyricularia oryzae* under different moist seeding conditions at 25 to 30°C, with unsterilized or sterilized greenhouse peat soil

Cultivar/location	Percent seed infection	Percent seedlings with typical blast lesions			Odds ratio ^a
		Uncovered	Lightly covered	Completely covered	
Unsterilized soil					
Taichung-176/Khumaltar	12.8	1.8	1.4	0.0	1.00
Masuli/Rampur	7.8	0.2	0.0	0.0	
Radha-17/Hetaunda	21.3	2.3	1.2	0.1	
Odds ratio ^b		1.00	0.57*	0.02***	
Sterilized soil					
Taichung-176/Khumaltar	12.8	2.9	1.5	0.0	1.47*
Masuli/Rampur	7.8	0.0	0.4	0.0	
Radha-17/Hetaunda	21.3	3.6	1.8	0.1	
Odds ratio ^b		1.00	0.46***	0.02***	

^a Odds ratio comparing the soil treatments. Sterilized soil (odds ratio = 1.00) was used as a reference. * = significance at $P \leq 0.05$.

^b Odds ratio comparing the covering of seeds. Uncovered seeds (odds ratio = 1.00) were used as a reference. * and *** = significance at $P \leq 0.05$ and $P \leq 0.001$, respectively.

Table 7. Development of blast lesions 4 weeks after seeding on the third or fourth leaf of rice seedlings raised from seeds infected by *Pyricularia oryzae* under different moist seeding conditions at low temperature (LT) and at LT followed by high temperature (HT), with unsterilized greenhouse peat soil

Cultivar/location	Percent seed infection	Percent seedlings with blast lesions ^a					
		Uncovered		Lightly covered		Completely covered	
		LT ^b	LT+HT ^c	LT	LT+HT	LT	LT+HT
Taichung-176/Khumaltar	12.8	0.0	2.5	0.0	1.8	0.0	0.3
Masuli/Rampur	7.8	0.0	0.0	0.0	0.0	0.0	0.0
Radha-17/Hetaunda	21.3	0.0	3.8	0.0	1.3	0.0	0.0
Odds ratio ^d			1.00		0.47*		0.04**

^a Based on seedlings raised from 400 seeds.

^b Seedlings grown at LT (15 to 20°C) for 4 weeks.

^c Seedlings grown at LT for the first 3 weeks followed by 1 week at HT (25 to 30°C).

^d Odds ratio for comparison of covering of seeds. Uncovered seeds (odds ratio = 1.00) were used as a reference. * and ** = significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

of covering of the seeds (no covering vs light covering vs complete covering). Light and complete covering gave a significantly lower seedling infection than no seed covering (Table 6). There were also significant differences between complete covering and light covering, light covering giving significantly higher seedling infection than complete covering (odds ratio for lightly covered [used as a reference] = 1.00, completely covered = 0.03, $P \leq 0.001$). Similar results were obtained for sterilized soil (Table 6). For sterilized soil, odds ratios were 1.00 for lightly covered seeds (used as a reference) and 0.04 for completely covered seeds ($P \leq 0.01$).

Seedlings raised under different moist seeding conditions (no covering, light covering, or complete covering) at 15 to 20°C did not develop blast lesions within a growth period of 4 weeks (Table 7). When 3-week-old seedlings raised at 15 to 20°C were transferred to a higher temperature of 25 to 30°C, blast lesions were detected within a week in Taichung-176 and Radha-17. No lesions at all were observed on Masuli for any treatment and, therefore, data for this cultivar were not included in the analysis. The degree of covering (no covering vs light covering vs complete covering) had a significant effect on the percentage of seedlings with lesions; lightly covered and completely covered seeds resulted in significantly lower disease incidence than uncovered seeds. There was also a significant difference between light covering and complete covering; complete covering yielded significantly lower disease incidence than light covering (odds ratio for lightly covered = 1.00 [used as a reference], and completely covered = 0.08, $P \leq 0.05$).

Latent infection was confirmed when 2-week-old seedlings, raised at low or high temperature, were examined for *P. oryzae* by the deep-freeze blotter method (Table 8). Growth of the fungus was found on shoots that had no visible necrotic symptoms. No growth was found on Masuli at all and, therefore, this cultivar was not included in the analysis. For both Taichung-176 and Radha-17, completely and lightly covered seeds had a significantly lower incidence of growth of *P. oryzae* than uncovered seeds whereas there was no significant difference between lightly covered and completely covered seeds. No significant difference was found between low and high temperature treatments.

DISCUSSION

A significant linear relationship on a logistic scale was found between neck blast symptoms and seed infection by *P. oryzae*. The relationship between plant symptoms and seed infection was further demonstrated when seeds collected from diseased secondary branches of panicles were found to give a high proportion of infected seeds. To our knowledge, these are the first quan-

titative studies on the relationship between panicle and seed infection. Previously, Aulakh et al. (3) briefly mentioned a positive correlation between plant infection in the field and seed infection by *P. oryzae*. A relationship between plant and seed infection has also been reported for other plant diseases. Schilder and Bergstrom (28) found a positive correlation between the severity of tan spot caused by *Pyrenophora tritici-repentis* on the flag leaves of wheat immediately after anthesis and seed infection by the same fungus.

Although there is a strong correlation between panicle symptoms and seed infection, it is not clear whether seeds (spikelets) become infected from airborne inoculum or by systemic spread from the panicle branches. According to Kato et al. (12), rice spikelets may become infected soon after heading, and conidia produced on spikelets serve as secondary inoculum for further infection of the panicle. Hirano and Goto (7) found that primary infection occurs frequently in empty glumes, pedicels, and the distal ends of hulls. Further studies are needed to investigate whether seeds from infected panicles become infected via systemic growth of the fungus from the panicle branches to the seeds or from spread of conidia from sporulating lesions on other plant parts.

Another important observation made from these field collections was that healthy-looking panicles and secondary branches of panicles may also yield infected seeds. Thus, absence of foliar symptoms under field conditions does not necessarily mean that seeds are healthy. Suzuki (32) found similar results by artificial inoculation. This finding is important, especially concerning seed certification programs in which seed lots are certified on the basis of field inspection.

We found that unfilled grains had a higher degree of infection by *P. oryzae* than filled grains did. This was also observed by Singh and Mathur (30) for the infection of rice by *Sarocladium oryzae*. Early and severe infection of panicles by the fungus may have been the cause of most of these unfilled grains. Also, Suzuki (32) reported that severe infection by *P. oryzae* may prevent rice seeds from developing completely.

Less infection by *P. oryzae* was found in samples that were subjected to winnowing. The reason is that the unfilled grains, which are more likely to be infected, are easily removed by winnowing. Furthermore, seed infection was lower in samples that were sun-dried. Sun-drying may reduce the inoculum present on the seed surface or even reduce the initial level of infection. This may explain why rice seed samples submitted for seed health testing usually have low seed infection (23). The samples have usually already gone through postharvest measures such as winnowing and sun-drying. Low seed infection levels

have been observed regularly at the Danish Government Institute of Seed Pathology for Developing Countries, Denmark, where hundreds of rice seed samples from different parts of the world have been examined each year since 1967 (S. B. Mathur, unpublished results). Postharvest measures such as winnowing and sun-drying are very commonly practiced in rice-growing areas and therefore help farmers to reduce the level of seed infection.

Earlier work (4,6,11) reported that sporulation of *P. oryzae* is confined to the embryonal end of rice seeds. In the present investigation, we found that sporulation occurred either from the embryonal end alone or from the entire seed surface, including the embryonal end, in both germinating and nongerminating seeds. However, the percentage of germinating seeds having sporulation of *P. oryzae* confined to the embryonal end was much higher than that of the nongerminating seeds. Similar results were found when rice seeds were artificially inoculated with *P. oryzae* (data not shown). Collectively, these observations clearly suggest that the embryonal end of the living seed is the most susceptible to infection by *P. oryzae*. It is well known that the embryonal end of rice seeds has a high protein content and this may possibly enhance the sporulation of *P. oryzae*. When the seeds are dead, the fungus can utilize the whole seed as a substrate, as suggested by Limonard (16), who observed abundant sporulation of *Fusarium*, *Helminthosporium*, and *Septoria* spp. on frozen (dead) seeds of different cereals. Similar observations have also been made for other plant-pathogen systems. Sporulation of *P. grisea* and *Bipolaris nodulosa* was higher on incubated finger millet (*Eleusine coracana*) seeds tested by the deep-freeze blotter method than on those tested by the blotter method because the seeds were killed by deep-freezing (26). The embryonal end of rice seeds as a preferred site for *P. oryzae* is important in nature since the fungus gets

easy and quick access to the shoot coming out from the seed.

Since the experiments on seed transmission were conducted in a non-rice-growing country, Denmark, infection of seedlings must have come from the infected seeds. The present investigation shows that the extent of transmission of *P. oryzae* from seed to plant is very low. Even the seed samples of Radha-17, with 21% infection, and Taichung-176, with 13% infection, only gave less than 4 and 3% seedlings with blast lesions on the leaves, respectively. Masuli often failed to produce blast-infected seedlings. This could be because Masuli had a low percentage of seed infection by *P. oryzae* (8%) and relatively high incidences of other pathogens, including *Alternaria padwickii*, *Bipolaris oryzae*, and a *Phoma* sp., compared with the other two cultivars (data not shown). Low seed transmission rates were also observed by Chung and Lee (4) who found that only 7 to 8% of seedlings on water agar in test tubes developed blast lesions even though the seedlings were raised from a heavily infected seed sample having 65% infection. Likewise, Lamey (14) reported that 7 to 13% of seedlings became diseased in the greenhouse from a seed sample having 40% infection. Lamey (14) did not observe blast lesions on leaves but the transmission percentage was calculated on the basis of the observation of *P. oryzae* growth on seedlings that were incubated in a moist chamber after emergence. Our results on seed transmission were based on blast lesions recorded on seedlings that were raised under different seeding conditions. Seeding methods corresponded to those practiced for raising seedlings under field conditions. Water seeding corresponds to a wet seed bed, seeding without covering corresponds to a seedling box (dapog), and seeding with complete or light covering is equivalent to a dry seed bed.

Blast lesions were detected for moist seeding without covering or with light covering of the seeds with soil. Lesions

Table 8. Detection of latent infection of *Pyricularia oryzae*^a 2 weeks after seeding on the shoots of rice seedlings raised from naturally infected rice seeds under different moist seeding conditions at two temperature regimes, with unsterilized greenhouse peat soil

Cultivar/location	Percent seed infection	Percent seedlings with latent infection ^b					
		Uncovered		Lightly covered		Completely covered	
		LT ^c	HT ^d	LT	HT	LT	HT
Taichung-176/Khumaltar	12.8	1.5	1.0	1.0	0.5	0.0	0.0
Masuli/Rampur	7.8	0.0	0.0	0.0	0.0	0.0	0.0
Radha-17/Hetaunda	21.3	2.0	1.5	1.5	1.0	0.0	0.5
Odds ratio ^e			1.00		0.66 NS		0.08*

^a Aerial parts of the seedlings were tested for the presence of *P. oryzae* by the deep-freeze blotter method.

^b Based on 200 seedlings.

^c Seedlings grown at low temperature (LT: 15 to 20°C) for 2 weeks.

^d Seedlings grown at high temperature (HT: 25 to 30°C) for 2 weeks.

^e Odds ratios for comparison of covering of seeds. Uncovered seeds (odds ratio = 1.00) were used as a reference. Comparison of low and high temperature: Odds ratio for LT = 1.00 (used as a reference) and HT = 0.75 NS. NS = nonsignificant difference; * = significance at $P \leq 0.05$.

were rarely found for complete covering, and were never detected in seedlings raised under water seeding conditions. Lamey (14) also reported that soaking of rice seeds in water for 72 h controlled seed-borne infection by *P. oryzae* completely. From these results, it seems that the fungus needs free atmosphere to survive, sporulate, and infect the seedlings. The results are consistent with the observations made in Brazil under upland conditions in an isolated, virgin land in Goiania, Goias (1). Blast was first observed in seedlings that developed from seeds that fell on the ground and were not covered by soil. Not only the covering of seeds, but also soil moisture may influence transmission of pathogen. Usually, high soil moisture reduces the seed transmission rate, as demonstrated by Youcef-Benkada et al. (35) for *Drechslera teres* in barley. In addition, antagonistic soil microorganisms may inhibit seed transmission of pathogens. This is apparent from the present investigations in which the recovery of *P. oryzae* was lower and declined faster from seeds sown in unsterilized soil than from seeds sown in sterilized soil. Also, seedlings raised in unsterilized soil had a lower infection frequency than seedlings raised in sterilized soil. Collectively, these findings suggest that under field conditions wet seed bed or dry seed bed with proper covering may effectively reduce infection of seedlings from seedborne inoculum.

The present investigation demonstrates that latent infections by *P. oryzae* may occur in rice seedlings. This was evident when plants grown under low temperature conditions did not develop blast lesions, but produced blast lesions in a week after they were transferred to high temperature conditions. Latent infection was further evident when growth of *P. oryzae* was observed on 2-week-old seedlings, grown at low temperature and without symptoms, after incubation on wet blotters. This observation clearly shows that such infected seedlings could be a potential source of disease in the field. This may be important in rice-growing areas where cool weather is prevalent, especially during the seedling stage. In several countries, including Nepal, rice is grown in sub-temperate regions with a short summer period. Seedlings are therefore raised in seed beds during the period when the weather is still cold and not favorable for disease development. Once the weather becomes warm and wet, transplanting of the rice takes place. Thus, seedlings carrying the fungus without showing symptoms will develop the disease and act as the source of inoculum in the field. Latent infections in plants raised from infected seeds have also been reported in several other plant-pathogen systems such as maize (*Zea mays*)/*Rhizoctonia solani* (24), maize/*Fusarium moniliforme* (24), rice/*Xanthomonas campestris* pv. *oryzae* (33), rice/*Pseudomonas avenae*

(29), and *Plantago lanceolata*/*Diaporthe adunca* (17).

Whether the infection is from seed or other sources, seedling blast is very important in blast epidemics. Once the inoculum is present, rapid development of the disease, especially in seed beds, is very likely to occur. Disease spread is rapid because the plants are concentrated in a very small area and the micro-environment is usually favorable for disease development. The usual seeding rate is 35 to 40 kg/ha for transplanting rice (34); this gives about 1 to 1.5 million seeds in 1 ha of land. In Nepal, the average transplanting density is 100 seedlings per square meter, i.e., one million plants per hectare. This means that if the seedling infection rate is, e.g., 1%, there will be an average of one infected seedling per square meter or in total about 10,000 infected seedlings per hectare. This high number is enough to supply sufficient inoculum to the emerging new leaves/tillers in the plant population since conidia of *P. oryzae* spread horizontally up to 1 m from the inoculum source (25).

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