

Epidemiological Studies of Rice Blast Disease Caused by *Pyricularia oryzae* Cavara

II. Sporulation and Conidia Release from Naturally Infected Lesions

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金章圭·吉野嶺一·李銀鍾：벼稻熱病의 疫學의 研究. II. 自然感染病斑의 孢子
形成과 離脫

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ABSTRACT Sporulation potential and conidia release phase of *Pyricularia oryzae* on lesions under the natural conditions were measured in 1985 and 1986 leaf blast seasons. The amount of conidia produced in lesions on detached leaves and conidia released under the field condition were reached peak at 5~7 days after lesion appearance. The maximum numbers of conidia produced and released were 16,200 and 15,900, respectively. Conidia release under the natural conditions lasted for 30 days.

INTRODUCTION

A series of activities of rice blast fungus, *Pyricularia oryzae*, has its importance in blast epidemics. Sporulation potential and conidia release phase under natural conditions are especially important to forecast blast outbreak. Concerning sporulation potential, Kato et al. (4) extensively studied the potential of sporulation at different temperature regimes with artificially inoculated rice seedlings. Barksdale et al. (1) and Iwano (2,3) directed their attention to spore release with artificially inoculated seedlings and Yoshino (7) researched on the lesions developed under the natural conditions. On the contrary, Suzuki (6) studied the release and dispersal phases of *P. oryzae* in detail.

The main objective of this study is to measure the amount of conidia produced and released from naturally infected lesion.

MATERIALS AND METHODS

Cultural practices. Japonica type cultivars Jinheung and Jinju were transplanted by 27×

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15cm spacing (80 hills/3.3m²) on May 23 in 1985 and 1986. Standard fertilizer level of N:P:K was 110, 60 and 70kg per hectare, but 30% of nitrogen was overdressed to induce leaf blast outbreak.

Determination of sporulation potential.

Fifteen to twenty leaves bearing naturally infected fresh lesions with more or less 2.5mm long were preliminarily labelled by one week interval and three lesions were collected at 2-days interval. Both sides of lesions were cleaned with tap water to eliminate conidiophores and conidia already formed and then the leaf was cut into 3cm long with a discrete lesion centred. The lesion bearing leaf piece was put into a small vial with 0.4ml of sterilized water and kept in a 28°C incubator for 15 hours under dark condition (4). Newly formed conidia were removed using syringe and hemacytometer was used for spore counting. Lesion size was also measured on each sampling date using a Vernier caliper.

Number of conidia released from lesions.

In 1985, three leaves which respectively bore a fresh discrete lesion were labelled and a plastic capsule (25×8×10mm) which is used as an internal pill wrapper was placed beneath each lesion for 24 hours starting 17p.m. Spores

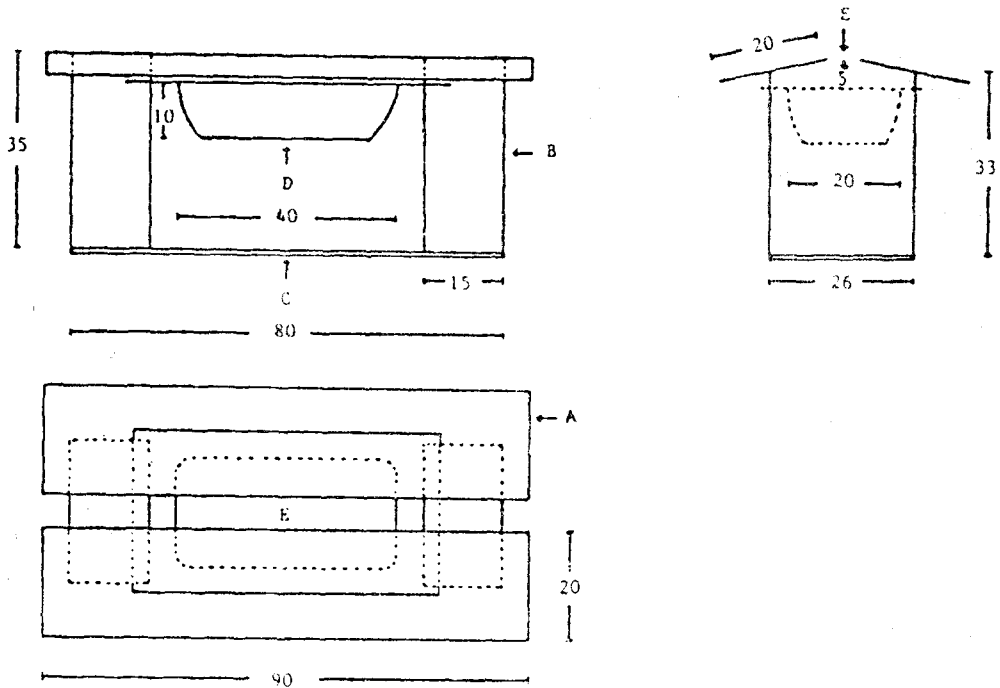


Fig. 1. Scratch map of a new type of spore trap (Length in mm)

A : Rain protector (Acryl) B : Protector holder C : Basal plate (Acryl)
D : Capsule E : Lesion position

were counted with hemacytometer after preparing spore suspension by adding 0.1ml of sterilized water into the capsule. As a result of 1985, the foresaid capsule was considered not proper as a spore trap due to the small size and no space available between the capsule and lesion which in turn might disturb free sporulation of the lesion. Thus, a new type of spore trap (5) was devised and used in 1986 to pursue the conidia release phase under the

natural conditions (Fig. 1).

RESULTS

Lesion enlargement in general was more obvious immediately after lesion appearance and daily mean enlargement was more or less 1.5mm (Table 1 and 2). As shown in Table 1, mean number of conidia produced per lesion in 1985 leaf blast season ranged from 1,050 to 7,500 and the maximum number of conidia

Table 1. Lesion length and number of conidia of *Pyricularia oryzae* produced from lesions naturally infected on a cultivar, Jinheung at different stage in 1985 leaf blast season

Date	Lesions appeared on July 8~10		Lesions appeared on July 15~16	
	Length(mm)	No. of spores ^a	Length(mm)	No. of spores ^a
July 18	14.7	1,300	5.8	7,500
20	16.4	1,800	10.7	4,900
22	19.6	1,700	12.0	7,500
24	21.2	4,100	17.1	1,050
26	21.4	1,300	18.7	4,200

^a Average of 3 lesions and the number indicates the amounts of conidia produced at 28°C for 15 hours under humid condition (>95% RH).

Table 2. Lesion length and number of conidia of *Pyricularia oryzae* produced from lesions naturally infected on cultivars Jinheung and Jinju at different stage in 1986 leaf blast season

Date	Lesions appeared on July 6~7 (Jinheung)		Lesions appeared on July 12~13			
	Length(mm)	No. of spores ^a	Length(mm)		No. of spores ^a	
			Jinheung	Jinju	Jinheung	Jinju
July 8	3.3	3,200	—	—	—	—
10	7.8	8,900	—	—	—	—
12	11.3	1,930	—	—	—	—
14	15.0	4,220	2.8	2.5	2,000	1,600
16	18.1	1,700	7.1	6.9	2,770	3,000
18	—	—	9.2	10.1	2,730	4,300
20	—	—	11.5	14.5	1,370	1,400
22	—	—	15.5	17.5	700	1,800
25	—	—	20.0	20.2	530	1,470

^a Average of 3 lesions and the number indicates the amount of conidia produced at 28°C for 15 hours under humid condition (>95% RH).

production was 16,200 from a lesion sampled on July 22. There was a big difference in sporulation potential between old and new lesions exhibiting high sporulation potential on newly developed lesions except July 24. At the same time, there was a tendency that sporulation potential was greater on the lesions within one week from their appearance despite the smaller lesion size. This series of same relationship was also found during 1986 leaf blast season (Table 2).

Number of conidia released from lesions under the natural conditions in 1985 and 1986 is shown in Tables 3 and 4. In 1985, each set of lesions was labelled at one week interval to compare the amount of conidia released based on the age of lesion after appearance. On the lesions appeared during July 8~10, conidia release was checked from July 11 to August 10 and for those appeared during July 15~16, it was done from July 19 to August 18. Table

3 shows that average range of conidia released from lesions appeared during July 8~10 was 100~3,100 and that of July 15~16 was 100~5,830 indicating the abundance of conidia release from fresh lesions. At the same time, the maximum numbers of conidia released were 5,400 and 15,900, respectively, and the lesions were capable to release conidia up to 27~30 days.

During 1986 leaf blast season, the amount of conidia released was measured with a new type of spore trap. The mean number ranged 40~2,440 and 40~2,300 indicating not much difference by the date of lesion appearance (Table 4). However, there was a big difference in daily number of conidia released and it seemed that more conidia were released on cloudy days followed by rainy days.

DISCUSSION

In relation to lesion enlargement, Kato *et al.*

Table 3. Number of conidia of *Pyricularia oryzae* released from lesions under the natural conditions during 1985 leaf blast season

Date lesion appeared	No. of conidia released ^a		Duration for successive conidia release (days)
	Average range	Max.	
July 8~10	100~3,100	5,400	30
July 15~16	100~5,830	15,900	27

^a Average of 3 lesions during July 11-August 18.

Table 4. Number of conidia of *Pyricularia oryzae* released from lesions under the natural conditions during 1986 leaf blast season

Date	Lesions appeared on July 6~7 (Jinheung)	Lesions appeared on July 12~13		Weather conditions
		Jinheung	Jinju	
July 9	320 ^a	—	—	Slight rain, cloudy
10	40	—	—	Fine
11	40	—	—	Rain
12	280	—	—	Cloudy, later fine
13	1,670	—	—	Cloudy
14	2,440	—	—	Overcast
15	2,410	540	200	Slight rain, cloudy
16	130	1,040	133	Rain
17	80	460	240	Cloudy, later fine
18	730	2,309	1,133	Cloudy, slight rain
19	—	40	220	Rain, later cloudy
20	—	700	893	Cloudy
21	—	640	640	Rain
22	—	1,680	1,720	Cloudy
23	—	2,040	220	Cloudy, later rain
24	—	—	—	Rain
25	—	1,920	300	Cloudy
26	—	1,320	720	Slight rain, fine
27	—	480	80	Cloudy

^a Average of 3 lesions.

(4) reported that lesions under the maximum temperature over 30°C almost stopped enlargement since 12 days after appearance, but those below 25°C continuously enlarged until 25 days after appearance. However, the lesions in the present study when the maximum temperature exceeded 30°C continued enlargement up to 17 days after appearance which might be the attributes of high nitrogen cultivation. There were cases that the lesion length immediately after appearance was the same but the enlargement in later stage showed a big difference and the reason is still under question which is the future target to be solved. To measure the lesion length is a very important factor for forecasting the blast disease spread and it is also possible to imagine the inoculum potential of *Pyricularia oryzae* by knowing the enlargement speed of lesions.

Kato *et al.* (4) defined the sporulation potential as a fungal capacity in host tissues to produce conidia under a decided condition per

unit time especially with the optimum at 28°C under more than 95% of relative humidity for 15 hours at dark condition. This fact in turn suggests that sporulation in lesions on detached leaves was the best at 28°C. However, Iwano (2,3) reported that conidia release was more favored at 25°C than 21°C or 28°C. He also pointed out that reaction of *P. oryzae* against temperature in terms of conidia release was greatly different between isolates. In the present study, number of conidia produced in lesions on detached leaves ranged 1,050~7,500 in 1985 and 530~8,900 in 1986. In general, the sporulation potential increased up to 5~7 days after lesion appearance and decreased since thereafter exhibiting coincidence with the results by Kato *et al.* But there were some exceptions like 4,100 conidia on July 24 (two weeks after lesion appearance) in 1985 and less in lesions developed one week later. This was considered mainly due to the small sampling size originating the difference of sporula-

tion potential among lesions and thus a sufficient number of lesions is required to measure sporulation potential as indicated by Kato *et al.* (4).

The maximum number of conidia released from lesions under the natural conditions was 15,900 in 1985 and 4,640 in 1986. This amount was equivalent to only one third-one tenth compared with 48,000 by Iwano(3) and 53,000 conidia by Yoshino(7). It is natural that blast fungus activity in the nature is greatly influenced by minute change of microclimate in the canopy. From this point of view, it is considered that there might be some difference in microclimate for each situation, but what does make such a big difference is still under the question and problems to be solved in the future.

摘 要

自然狀態에서 發現된 病斑을 利用, 稻熱病菌의 孢子形成量과 離脫量을 1985~1986년에 調査하였다. 切取한 病斑의 孢子形成量과 自然狀態下에서의 孢子離脫量은 病斑發現後 5~7日 사이에 最高値에 達했으며 그 數는 各各 16,200個와 15,900個였다. 自然狀態下에서의 孢子離脫은 30日間 持續되었다.

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