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

I. Measurement of the Amount of Spores Released from a Single Lesion

Chang Kyu Kim and Reiichi Yoshino*

Department of Plant Pathology, Institute of Agricultural sciences, Suweon, Korea
**Hokuriku Agricultural Experiment Station, Joetsu, Niigata, Japan*

벼 稻熱病의 疫學的 硏究

I. 單一病斑으로부터의 胞子離脫量 調査

金章圭 · 吉野嶺一*

農業技術硏究所 病理科, 水原, 大韓民國
*北陸農業試驗場 病害第 2 硏究室, 上越, 新潟, 日本國

ABSTRACT

Four types of spore trap (Kim's original, improved Kim's original, Yoshino's original and mixed type of Kim's and Yoshino's original) were evaluated for their efficacy to estimate the amount of spores released from leaf blast lesions under the natural conditions. It was found that all four types had one or two defects in allowance for adequate sporulation/release, spore catch or spore counting. Thus, an improved type of spore trap was devised considering that it could cover the defects mentioned above. As a result, newly developed spore trap was quite satisfactory in above mentioned aspects and it could be used for pursuit of spore release phase under the natural conditions.

Key words: Pyricularia oryzae, spore release, spore trap.

要 約

自然狀態下에서 잎稻熱病 病斑으로부터 離脫되는 胞子의 量을 測定하기 爲하여 4 種의 胞子採集器 (金의 原型, 金의 改良型, 吉野의 原型, 金 · 吉野의 改良型)를 檢討한 結果, 圓滑한 胞子形成 및 離脫, 胞子採集, 檢鏡에 있어서 한 두가지의 缺點이 發見되었다. 따라서 위의 缺點을 補完할 수 있는 새로운 胞子採集器를 考案, 性能을 檢討하여 上記 4種의 採集器의 缺點을 補充할 수 있는 結果를 얻었으므로 今後 自然狀態下에서 胞子離脫量 調査에 有用한 것으로 생각된다.

INTRODUCTION

Sporulation, spore release and dispersal phases of the rice blast fungus, *Pyricularia oryzae*, are all important processes in the disease cycle and epidemics of leaf blast. Among those, spore release phase was the one interested by many workers (1, 2, 3,

4, 5). Barksdale (1), Iwano (2) and Yoshino(5) estimated the amount of spores produced from leaf blast lesions using different methods without leteching rice leaves. However, the methods had some difficulties, especially in time consuming spore counting procedure. In the present study, a new simple method for spore trapping was devised and its applicability was tested.

MATERIALS AND METHODS

In 1985, four types of spore trap were tested to examine the efficacy of estimating the amount of spores released from leaf blast lesions under the natural conditions.

Type A (Kim's original): A plastic capsule (25x8x10mm) which is an internal medicine pill wrapper was placed beneath a leaf blast lesion. No space was allowed between the capsule and the lesion. Spores were counted with a hemacytometer after adding 0.1ml of sterilized water into the capsule.

Type B (Improved Type A): The same capsules as Type A were used, but inflow of air was possible by allowing a few millimeter spaces at both sides of lesion and capsule. Methods of spore counting was the same as Type A.

Type C (Yoshino's original): A slide glass with lines which is used for plankton measurement was pasted with glycerin jelly inside specially attached frame (50x20mm) and placed 1cm below a lesion. Spores were directly counted under a compound microscope.

Type D (Mixture of Type A and Type C): The same capsule as Type A was installed 1cm under a leaf blast lesion. Spores were counted by the same method as Type A.

In each type, translucent acryl plates were attached to prevent inflow of rain into the capsule or slide glass, and a rice leaf blade containing one discrete lesion was fixed on the acryl rain protector with Shurtape (both side adhesive). The lesion was centred allowing more enough space toward basal part of leaf. This was due to the attribute of lesions to elongate more fastly toward basal part. Capsules and slide glasses were allowed to catch spores for 24 hours daily starting 17:00 hour during July 29-August 7.

RESULTS

Among four types of spore trap tested, Type C was the most effective in terms of the number of spores caught during July 30 to August 8 (Table 1), and followed by Type B, Type D and Type A. However, it is important to know whether a series of events from sporulation to spore counting is properly occurred and conducted or not. Thus, the following points were checked based on the result of Table 1. Firstly, whether or not the sporulation and release of spores were allowed to occur naturally without disturbance by installation of spore traps. Secondly, whether the releasing spores could be caught effectively or not. Thirdly, easiness in spore counting. Table 2 shows the advantages and disadvantages of each trap. Type A was considered to catch most of the spores released, but the amount of spore catched was the least due to the air-tight nature between the lesion and the capsule which might hinder sporulation. Type C was able to

Table 1. Number of conidia of *Pyricularia oryzae* released from leaf blast lesions collected by four types of spore trap

Collection date		No. of conidia trapped by[a]			
		A	B	C	D
July	30	1,430	1,830	4,450	1,100
	31	670	500	4,500	300
Aug.	1	330	130	7,450	100
	2	1,200	3,630	11,750	2,700
	3	2,530	2,500	10,500	830
	4	100	630	2,250	100
	5	100	100	1,700	100
	6	330	250	1,200	870
	7	270	630	450	530
	8	200	2,170	400	1,100
Mean		716	1,237	4,465	773

[a]Values are the average of 3 lesions. A: Kim's original, B: Improved Type A, C: Yoshino's original, D: Mixture of Type A and Type C.

Table 2. Comparison of efficacy of four types of spore trap

Type of spore trap[a]	Sporulation and spore release	Spore catch	Spore counting	Defect
A	X[b]	0	0	Limitted number of sporulation
B	Δ	Δ	0	Little number of spore catch
C	0	0	X	Time consuming in spore counting
D	0	X	0	Small size of capsu for spore catch

[a]A: Kim's original, B: Improved Type A, C: Yoshino's original, D: Mixture of Type A and Type C
[b]O: Good, Δ: Intermediate, X: Poor

catch most spores. This is thought to be due to the open space between the lesion and slide glass which might allow the lesion to sporulate and release without disturbance. However, this method had one difficulty in time consuming process for direct counting of spores. Thus, Type C was considered not reasonable for actual use. Type B was applicable in terms of sporulation and counting, but the number of trapped spores showed much variation between lesions. On the contrary, Type D was unable to catch spores efficiently due to the small size of capsule. Based upon above mentioned reasons, four types of spore trap tested in thi experiment were all considered not suitable fo pursuit of spore release phase under the natura conditions. This leads to the development of a nev spore trap which could cover the defects of abov spore traps.

As shown in Figure 1, a new type of spore trap was devised considering the following points. A: Enhancement of the capacity of spore catch using larger size of capsules, B: Permission of natural sporulation and release of spores by keeping 5mm distance between lesion and capsule, and C:

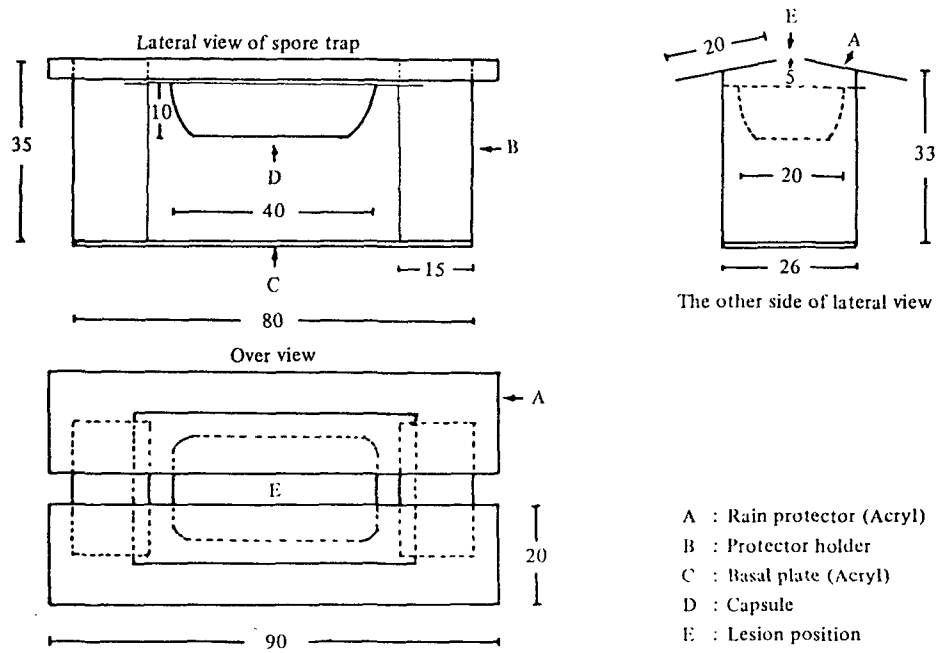

Fig. 1. Scatch map of a new type of spore trap (Scale in millimetre).

Use of protector cover to prevent inflow of rain or water drops. Considering lesion size (sometimes more or less 40mm), a basin type acryl capsule (40x20x10mm) was used to capture releasing spores. This new spore trap was tested during 1986 blast season and found that it was quite simple and convenient to manipulate. It took only less than 10 minutes to complete spore counting of one capsule, but the number of trapped spores per day was rather small ranging 40 to 4,640 spores per capsule.

DISCUSSION

Spore release phase throughout the life cycle of *Pyricularia oryzae* plays an important role for blast epidemics, and it is very important to know the amount of spores released from a lesion. To measure the number of spores, Barksdale (1) and Iwano(2) devised spore traps using petri dish method without detaching rice leaves. They maintained moist condition by placing a piece of moist filter paper inside the petri dish and a lesion bearing leaf blade was centred inside the dish. On the other hand, Yoshino(5) devised an open spaced spore trap which allows free sporulation and release of spores just like under the natural conditions. Some kind of apparatus that should not disturb the nature is necessary to observe a natural phenomenon. Four types of spore trap including Yoshino's original type tested in this experiment had unfortunately one or two kinds of defects.

To meet demands for observations of natural sporulation and spore release with effective spore catch and easiness in spore counting, the authors devised a new type of spore trap which was modified from Yoshino's original and Kim's original. This trap was thought to be quite satisfactory to cover the defects of four spore traps tested in 1985, but it was revealed that the number of spores trapped varied with lesions which might be the future target to study.

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