Plant Pathol. J. 16(3) : 147-150 (2000)

The Plant Pathology Journal

Sporulation of *Pyricularia grisea* at Different Growth Stages of Rice in the Field

Chang Kyu Kim* and Reiich Yoshino[1]

Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

[1]Research & Development Department, ZENECA K.K. Agrochemicals, 8-1-22 Akasaka, Tokyo, Japan

(Received on February 10, 2000)

Sporulation patterns of rice blast fungus were studied at relatively later stages of leaf blast and neck blast seasons in Icheon, Korea. This experiment was done by detaching lesion-bearing leaves and panicle bases. The number of conidia remaining on the leaf blast lesions of different cultivars from Jul 20 to Jul 23 ranged from 3,640 to 82,740 spores. More conidia were observed on the adaxial surface because they were released from abaxial surface. After heading, sporulation was observed from the lesions on the flag leaves but the number of spores was less than in the late July. Detached panicle bases or uppermost internodes infected by *Pyricularia grisea* produced abundant amount of conidia. Among these panicle bases, 30.1 mm size lesion recorded the highest count of 244,560 spores. When we compared the sporulation amount using the KY-type spore trap, more conidia were recorded from intact lesions than from the lesions which removed conidia and conidiophore. The ratio of conidia release against total sporulation ranged from 20.5%-25.0% for leaf blast and 8.2%-25.3% in the neck blast. Effective inoculum potential was also discussed.

Keywords : effective inoculum potential, *Pyricularia grisea*, sporulation.

Sporulation and conidial release phases of the rice blast fungus from leaf blast lesions were previously studied in field conditions under different weather conditions(Kim et al., 1989a; 1989b; 1990a). Conidial release pattern reached a peak at around 7:00 am under clear day conditions. Under cloudy conditions, the peaks were observed two to three times between 1:00 am to 8:00 am. Most of the works on sporulation and conidial release have been conducted during the leaf blast stage(Iwano, 1984; Kato, 1974; Kato and Sasaki, 1974). It was probably due to the importance of primary inoculum potential of leaf blast lesions to neck blast development. In the present study, sporulation and conidial release patterns from leaf blast lesions at booting stage and from panicle bases were examined to estimate the sporulation capacity.

*Corresponding author.
Phone) +82-31-290-0404, Fax) +82-31-290-0453
E-mail)ckkim@niast.go.kr

Materials and Methods

Selection of lesions. This experiment was conducted during late July (panicle initiation to booting stages) and late August (milk to dough stages), 1999 in Icheon experimental site, Korea, to measure number of conidia remaining on the lesions and sporulation capacity. Test cultivars were Jinheung, Ilpumbyeo, Jinmibyeo and Chucheongbyeo. One to three lesions cultivar^{-1} at different dates were sampled. Discrete lesion-bearing leaves and panicle bases were cut during daytime to measure the remaining conidia on the lesion. For sporulation measurement, the KY-type spore trap (Kim and Yoshino, 1987; 1988) was installed at 5:00 am and collected at 9:00 am on the following day.

Measurement of sporulation. Detached leaves were brought to the laboratory. To measure the number of conidia, lesions were thoroughly scraped to harvest them using 1 ml hypodermic syringe needle by adding 0.1 ml of 0.02% Tween 20 solution. Collected spore suspension was placed in an acrylic capsule and the conidia were immediately counted using a hemacytometer. For sporulation measurement from leaf blast lesions, a discrete lesion-bearing leaf was fixed on the KY-type spore trap using double-sided adhesive tape at 5:00 am. The acrylic capsule was collected at 9:00 am on the following day. The conidia were counted separately from the capsule and both sides of the lesion. For neck blast, a lesion bearing panicle base was fixed in the same way as for leaf blast. Conidia released from a lesion were counted from the capsule. The remaining conidia on the round surface of panicle bases were counted following the same procedure as described earlier.

Results

Number of *P. grisea* conidia remaining on the leaf blast lesion. From July 20 to 23, 1999, the number of conidia collected from detached lesions of three different cultivars was compared (Table 1). The lesion size ranged from 6.5 to 29.1 mm but most of them were around 20 mm of the chronic types. Considering lesion type and size, they were thought to be about 20 days after formed. In most cases, there were more conidia on the adaxial than on the abaxial leaf surfaces because lots of conidia were released from abaxial surface. The highest count of spores on the 20.5

Table 1. Number of *Pyricularia grisea* spores remaining on the leaf blast lesions of different cultivars in Icheon, Korea, 1999

Cultivar	Collection date	Collection time (h)	Lesion size (mm)	Number of spores on Adaxial	Abaxial	Total	Rainfall (mm)[z]
Jinheung	July 20	09:00	29.1	6,280	5,080	11,360	
		10:40	13.7	440	3,200	3,640	
	July 21	09:50	18.3	21,360	12,080	33,440	
		09:50	19.7	12,580	10,940	23,520	0.1
		09:50	13.0	18,740	6,240	24,980	
	July 23	11:00	6.5	3,660	3,380	7,040	
Ilpumbyeo	July 20	14:00	26.4	10,700	8,380	19,080	
	July 22	15:40	23.1	27,620	13,040	40,660	10.6
		15:40	20.5	11,060	71,680	82,740	
	July 23	08:30	27.2	800	24,100	24,900	5.9
Jinmibyeo	July 20	15:20	16.3	4,900	4,020	8,920	
	July 22	07:00	20.9	5,600	27,260	32,860	1.4
	July 23	08:30	22.0	11,420	6,400	17,820	5.9

[z]Amount of rainfall during 3 to 4 hr prior to sample collection.

mm size lesion of cultivar Ilpumbyeo was 82,740. More or less 20 mm lesions recorded $1.8\text{-}8.3 \times 10^4$ spores. It was considered that sampling time of lesions, in the morning or in the afternoon, did not affect the number of conidia remaining on the lesions.

The number of conidia remaining on the lesions of flag leaves was also counted on August 23-24 (Table 2). In general, lesions were shorter and spore counts were less than in July. The highest count of the 11.0 mm lesion was 11,900 spores.

Number of *P. grisea* conidia remaining on the panicle bases or uppermost internodes. During August 24 to 31, the lesion length and number of conidia remaining on the diseased panicle bases varied (Table 3). The highest count was 244,560 conidia on the diseased panicle base of 30.1 mm long. There was a trend that sporulation capacity in general seemed to be proportional with lesion length.

Sporulation of *P. grisea* on the lesions of leaf blast and neck blast. It was clear that sporulation amount was higher on the intact lesions than the ones from which conidia and conidiophore removed previously regardless of leaf and neck blast (Table 4). In case of leaf blast lesion, sporulation was 32,860 conidia on the intact lesion, but the lesion from which conidia were previously removed produced 8.8% conidia compared with the intact one. Conidia formed on the abaxial surface was 83%-90% of the total sporulation due to the released amount was included. On the neck blast lesions, sporulation amounts were 7,360-54,180 conidia on the intact lesions, while diseased panicle bases from which conidia were previously removed produced 680-2,960 conidia showing 5.5%-9.2% of the intact ones. The number of conidia released was 8.2%-25.3% of the total sporulation.

Discussion

In this study, the sporulation and release pattern of *P. grisea* was measured relatively late during the growth stages starting July 20. Thus, leaf blast lesions examined were mostly chronic types, because this time the rice plants have become resistant and new infection has almost been stopped. There were more conidia observed remaining on the leaf blast lesions at the end of July (panicle initiation to booting stage) than on the lesions of flag leaves at the end of August

Table 2. Number of *Pyricularia grisea* spores remaining on the lesions of flag leaves after heading in Icheon, Korea, 1999

Cultivar	Collection date	Collection time (h)	Lesion size (mm)	Number of spores on Adaxial	Abaxial	Total
Chu-cheong-byeo	Aug. 23	09:40	7.4	6,460	3.960	10,420
		09:40	9.7	6,060	2.680	8,740
	Aug. 24	11:00	11.0	3,760	8.140	11,900
Jinmibyeo	Aug. 23	12:00	16.5	680	1.020	1,700
		16:30	12.9	340	100	440
	Aug. 24	06:40	14.0	480	520	1,000
		11:00	10.5	460	280	740

Table 3. Number of *Pyricularia grisea* spores remaining on the panicle base / uppermost internode in Icheon, Korea, 1999

Cultivar	Collection date	Collection time (h)	Lesion size (mm)	No. of conidia
Jinmibyeo	Aug. 24	06:40	15.0	54,840
		06:40	18.0	33,680
	Aug. 30	10:00	8.5	9,040
		10:00	6.7	4,720
	Aug. 31*	15:00	28.2	1,740
Jinheung	Aug. 30	15:00	10.0	2,140
		15:00	30.1	244,560

*There was 3.0 mm rainfall from 03:00 am to 08:00 am.

Table 4. Sporulation of *Pyricularia grisea* on the lesions of leaf blast and neck blast in Icheon, Korea, 1999

Cultivar	Collection date	Lesion size (mm)	Rel[a]	Adax.[b]	Abax.[c]	Total[d]
Leaf blast						
Jinmibyeo						
Intact	July 22	20.9	6,720	5,600	20,540	32,860
Conidia removed		21.5	720	300	1,860	2,880
Neck blast						
Jinheung						
Intact	Aug. 31[e]	31.5	1,860		5,500	7,360
Conidia removed		33.4	80		600	680
Intact	Sept. 1	10.3	4,460		49,720	54,180
Conidia removed		12.9	320		2,640	2,960

[a] Total number of conidia collected by KY-type spore trap.
[b] Total number of conidia remained on the upper surface of lesion. Remained conidia were thoroughly removed with 0.02% Tween 20 solution and counted using a hemacytometer.
[c] Total number of conidia remained on the back surface of lesion. The numbers for neck blast indicate the total number of remained spores.
[d] Total sporulation amount derived from released and remained conidia from 05:00 pm to 09:00 am on the following day. Crop growth stage during the experiment for leaf blast was panicle initiation.
[e] There was 3.0 mm rainfall from 03:00 am to 08:00 am.

(milk to dough stage). Many factors such as host plant conditions, nitrogen level and weather components are affecting lesion formation and sporulation. But simply considering the host side, the difference in sporulation might have been due to the rice plant's age. Kato (1974) reported that leaf blast lesion continued sporulation more than 20 days, and we observed that it continued until 30 days after lesion appearance (Kim and Yoshino, 1987). However, the sporulation capacity was decreased when the lesion was getting old.

The number of conidia remaining on the surface was different in two ways: first, more conidia remained on the adaxial surface than on the abaxial surface when the intact lesions were randomly collected. This might be due to the uncounted released conidia from the lesion. Second, when the sporulation amount was measured using the KY-type spore trap, more conidia were counted from the abaxial surface exhibiting released conidia were captured by the acrylic capsule. The ratio of conidia formed on the abaxial surface including released ones ranged from 83%-90% of the total sporulation. The same trend was found in the previous study (Kim and Yoshino, 1995). The KY-type spore trap was devised to collect spores under similar natural conditions (Kim and Yoshino, 1987). The distance from the abaxial surface of lesion to top of capsule is 5 mm to allow free inflow of air. However, the setting of KY-type spore trap with a leaf blade might give more favorable conditions than the intact ones. The conidia released from the adaxial surface were uncountable and sometimes dew drops, which also include very few amount of conidia, were observed on the lesion because the leaf was set horizontally and this could have interfered with sporulation.

We observed the variability of conidia remaining on lesions from the panicle bases or uppermost internodes sampled randomly. In general, it seemed that the sporulation was in proportion with the length of infected parts. Duration after the appearance of lesion might have also influenced the sporulation capacity. Release of conidia from the intact neck blast lesions ranged from 8.2%-25.3% of the total sporulation while that of conidia and conidiophores removed lesions ranged from 10.8%-11.8%.

Trials to pursue the sporulation pattern under field condition are very few. One of the main objectives in studying sporulation and release patterns is to estimate the effective inoculum potential (EIP). It is practically very difficult to calculate EIP because so many factors are associated. For example, weather condition; plant age and growth status; size, type and age of lesion are all important components to be considered. Thus, each step of the pathogen's life cycle is influenced by the different factors. Temperature did not influence conidial release between 11°C-26°C. But, if the temperature became 30°C or 35°C, the number of conidia produced and released was suddenly decreased (Suzuki, 1969). Relative humidity (RH) over 90% accelerated conidia release (Hashioka, 1950; Kuribayashi and Ichikawa, 1953). Based on the results, we can recognize that low temperature and high RH in the field provide favorable conditions for release at nighttime except during heavy rainfall or strong wind. A lesion could start producing conidiophores 4 h after dew formation. After a conidiophore started sporulation, the first conidium matured in 40 min. A second conidium began to form 20 min after the first matured. Seven to nine conidia were produced from a single conidiophore in one night. Five hours after sporulation, conidia release began. Lesions reached their release peak 13 hr after dew formation (Toyota and Suzuki, 1952). This fact suggests that sporulation on the lesions and release from the lesions in the field occur quite differently. We experienced that mature conidia were not released all. Conidia release was mostly stopped after 1000 h but we observed little amount of release during rainfall or one hour later (Kim et al., 1990a; 1990b).

Based on the current results obtained, we can conclude that percent release of conidia from leaf blast lesion ranges from 4.0%-20.5%, percent landing on the rice plant's leaf is 0.5%-1.0% (Yoshino, personal communication) and percent penetration is 1%-20%. Spore landing on the leaf surface has been calculated by Yoshino at 0.5%-1% of the total sporulation, and Kato (1987) calculated it at 0.37%. It is quite desirable to make the spore landing phase clear in the nature. Let's suppose that sporulation amount is 10,000, then EIP will be as follows:

$10{,}000 \times (0.04\text{-}0.205) \times (0.005\text{-}0.01) \times (0.01\text{-}0.2) = 0.02\text{-}4.10$. This means that 0.0002%-0.041% of the total sporulation can take a role as EIP. However, EIP with the same sporulation amount might be different under fine, cloudy or rainy day conditions.

It has been reported that the tissues of panicle bases and branches are very weak immediately after heading. Then, they become temporarily resistant one week after heading and thereafter the tissues become weak until maturity (Tokunaga et al., 1965). In the present study, sporulation of panicle axis was observed until 20 days after heading and it was common that sporulation usually continued until harvest. If the temperature is high and relative humidity is low, released spores could not be functional as inoculum. Under frequent rainfall and/or abnormal weather conditions, they will cause secondary infection to parts of panicle axis. The mechanism of late infection on panicles is not clear, but it is reported that the secondary infection is due to the downward elongation of infected parts of kernels and branches, as well as the spores formed on the kernels (Hirano and Goto, 1963; Kato et al., 1970). In the 1999 crop season, we experienced lots of secondary infections on the panicle bases and branches.

Future studies would deal on the effective inoculum potential and the mechanism of secondary infection until harvest. At the same time, how long will the mature conidia be remained on the conidiophore as viable condition and what will be happened to them should be studied in the future.

References

Hashioka, Y. 1950. The microclimate of the paddy field in connection with prevalence of rice blast disease. *J. Agr. Met. Japan* 6:25-29.

Hirano, T. and Goto, K. 1963. Pathogenesis and ecology of panicle branch blast of rice plant. *Bull. Natl. Inst. Agric. Sci. Series C.* 16:1-56.

Iwano, M. 1984. Studies on the spore release of *Pyricularia oryzae* Cavara from lesions on rice leaves. *Bull. Hokuriku Natl. Agric. Exp. Stn.* 26:67-95.

Kato, H. 1974. Epidemiology of rice blast disease. *Rev. Plant Protec. Res. Japan* 7:1-20.

Kato, H. 1987. Epidemiology. In Rice Blast Disease. *Yokendo Co. Ltd. Japan.* 123-149.

Kato, H. and Sasaki, T. 1974. Epidemiological studies of rice blast disease, with special reference to reproductive process in lesions on rice plants and disease forecasting. *Bull. Natl. Inst. Agric. Sci. Series C.* 28:1-61.

Kato, H., Sasaki, T. and Koshimizu, Y. 1970. Role of kernel blast as secondary inoculum potential in the life cycle of rice blast disease. *Bull. Tohoku. Agr. Exp. Sta.* 39:33-54.

Kim, C. K., Min, H. S. and Yoshino, R. 1989a. Epidemiological studies of rice blast disease caused by *Pyricularia oryzae* Cavara. IV. Conidia release under rainy conditions. *Korean J. Plant Pathol.* 5:60-64.

Kim, C. K., Min, H. S. and Yoshino, R. 1989b. Conidia release and dispersal pattern of *Pyricularia oryzae* under cloudy and rainy conditions. *IRRN* 14(4):34.

Kim, C. K., Min, H. S. and Yoshino, R. 1990a. Epidemiological studies of rice blast disease caused by *Pyricularia oryzae* Cavara. V. Successive conidia release from different aged lesions in the nature. *Korean J. Plant Pathol.* 6:214-218.

Kim, C. K., Min, H. S. and Yoshino, R. 1990b. Epidemiological studies of rice blast disease caused by *Pyricularia oryzae* Cavara. III. Diurnal pattern of conidial release and dispersal under the natural conditions. *Ann. Phytopathol. Soc. Jpn.* 56:315-321.

Kim, C. K. and Yoshino, R. 1987. Epidemiological studies of rice blast disease caused by *Pyricularia oryzae* Cavara. I. Measurement of the amount of spores released from a single lesion. *Korean J. Plant Pathol.* 3:120-123.

Kim, C. K. and Yoshino, R. 1988. Device for field measurement of conidia release by a single leaf blast lesion. *IRRN* 13(3):29-30.

Kim, C. K. and Yoshino, R. 1995. Epidemiological studies of rice blast disease caused by *Pyricularia oryzae* Cavara. VI. Measurement of sporulation on blast lesions of naturally infected leaf. *Ann. Phytopathol. Soc. Jpn.* 61:89-92.

Kim, C. K., Yoshino, R. and Lee, E. J. 1987. Epidemiological studies of rice blast disease caused by *Pyricularia oryzae* Cavara. II. Sporulation and conidia release from naturally infected lesions. *Korean J. Plant Prot.* 26:37-41

Kuribayashi, K. and Ichikawa, H. 1953. Outbreak of rice blast disease and conidia release. *Plant Prot. Japan* 7:245-249.

Suzuki, H. 1969. Studies on the behaviour of rice blast fungus spore and application to outbreak forecast of rice blast disease. *Bull. Hokuriku Agr. Exp. Sta.* 10:1-118.

Tokunaga, Y., Kato, H. and Koshimizu, Y. 1965. Studies on the relationships between metabolism of the rice plant and its resistance to blast disease. II. Metabolism of phosphorus and resistance of leaf blast. *Bull. Tohoku. Agr. Exp. Sta.* 32:61-88.

Toyota, S. and Suzuki, N. 1952. Histochemical studies on the lesions of rice blast caused by *Pyricularia oryzae* Cav. I. Some observations on the sporulation on lesions of different types occurring on leaves of the same variety. *Ann. Phytopathol. Soc. Jpn.* 17:1-5.