Spatial Distribution and Dissemination of Bacterial Grain Rot of Rice Caused by *Pseudomonas glumae*

Seiya Tsushima* and Hideki Naito**

Abstract

The distribution and spatial pattern of bacterial grain rot of rice, caused by *Pseudomonas glumae*, were determined by using Morisita's index *I* of distribution. An area of 5,000 hills (50 rows × 100 hills) 1 and 2 weeks after heading time and an area of 2,000 hills (20 rows × 100 hills) 3 weeks after heading time was assessed for disease incidence in 3 fields, A, B and C. Each assessment was done by using 5 quadrat sizes (rows × hills). The spatial pattern of the hills, whose disease incidences were 2 to 4, was clumped in field A a week after heading time as determined by Morisita's index, but not in fields, B, C. At 2 weeks after heading time, the shape of the *I* curve for the 3 fields was typical for a pattern with small clumps. The higher the severity of fields, the earlier was the formation of the aggregations. Hills, with a disease incidence of 4, were frequently surrounded by hills of lower disease incidence. The dissemination of this disease was studied in a paddy field by setting diseased hill planted in 1/5,000 a pots as a inoculum source at the center of 2.7 m × 2.88 m plots (9 rows × 19 hills) at heading time or a week after heading time. For all plots, the further the distance from the inoculum source, the lower was the disease incidence. The disease incidences of 8 plants surrounding the inoculum source were clearly largest for the plot in which a severely diseased hill was set as a inoculum source at heading time. Populations of *P. glumae* from severely diseased panicles, which were sampled from the paddy field and were incubated in a moist chamber, were clearly higher than those from slightly diseased panicles and symptomless panicles, moreover, the populations of *P. glumae* from diseased panicles depended on the incubation time in a moist chamber. The results of this experiment show that severely diseased panicles which were occurred earlier, were important in focal formation of bacterial grain rot of rice.

(Received May 16, 1990)

Key words: *Pseudomonas glumae*, spatial distribution, epidemiology.

INTRODUCTION

Bacterial grain rot of rice, caused by *Pseudomonas glumae* Kurita et Tabei, is an important disease of rice in Japan. This pathogen causes seedling rot in nursery boxes and grain rot in panicles after heading in paddy fields. In the case of grain rot of panicles, the symptoms generally appears only on the grains.

Yoshimura et al. reported that diseased hills caused by this pathogen were frequently aggregated in paddy fields and severely diseased panicles, in which most grains were infected, were frequently observed at the center of the aggregations. This report suggests that severely diseased panicles were important in forming infection foci in paddy fields. However, they did not analyze the spatial pattern of this disease.

We reported that the infectious period of the grains was mainly from the day of flowering.
to about 5 days after flowering. The heading period in paddy fields is about 2 weeks. Thus, the infectious period of rice plants in paddy fields was estimated to be mainly from heading time, which is the day that 40-50% of the panicles in a field appear, to about 10 days after heading time\(^5\). This pathogen was recovered with approximately \(<10^7\) cfu/g fresh weight of leaf sheaths and symptomless grains but about \(10^9\) cfu/g fresh weight of visibly diseased grains\(^4\). From these results, it was suggested that the severely diseased panicles which were heading at an earlier period might be important as primary inoculum.

The objective of this study was to analyze spatial data obtained from rice plants with bacterial grain rot in naturally infested fields and to clarify the role of severely diseased panicles in the development of this disease.

**MATERIALS AND METHODS**

**Spatial distribution.** Three fields, located at Mitsuhashi-cho in Fukuoka Prefecture, were surveyed in 1987. Cultivar Koganebare was mechanically planted with 0.16 m spacing in 0.3 m rows in fields A and B, and cultivar Tsukushibare was mechanically planted with the same spacing in field C. An area of 5,000 hills (50 rows × 100 hills) were randomly chosen in these fields. The disease incidence was recorded 1 and 2 weeks after heading time of rice plants in each of 5,000 hills (50 rows × 100 hills) and 3 weeks after heading time in each of 2,000 hills (20 rows × 100 hills).

Disease incidence was determined as follows:
1. only one slightly diseased panicle (less than 30% diseased grains) per hill.
2. 2 to 5 slightly diseased panicles per hill.
3. more than 6 slightly diseased panicles per hill.
4. more than one severely diseased panicle (more than 30% diseased grains) per hill.

**Morisita’s index\(^6\) of distribution.** \(I_B\) was computed according to the formula

\[
I_B = I_d(N-1/q)/(N-1)
\]

\[
I_d = q\left[\frac{q}{T(T-1)}\right]
\]

in which \(x_i\) is the number of diseased hills in a sampling unit, \(q\) is the number of sampling units, \(T\) is the total number of diseased hills and \(N\) is the number of hills in a sampling unit. Values of \(I_B\) correspond to three different interpretations of the distribution: \(I_B<1\) indicates a uniform distribution, \(I_B=1\) a random distribution, and \(I_B>1\) a clumped distribution.

**Dissemination.** Cultivar Asominori was hand planted in June of 1988 with 0.16 m spacing in 0.3 m rows. A diseased potted hill of rice (cv. Koganebare) was placed at the center of 2.7 m × 2.88 m plots (9 rows × 19 hills), at heading time or a week after heading time of cv. Asominori (Fig. 1) as an inoculum source. In plot A, a severely diseased hill was set as an inoculum source at heading time; in plot B, a severely diseased hill was set as an inoculum source a week after heading time; in plot C, a slightly diseased hill was set as an inoculum source at heading time; in plot D, a healthy hill was set in the center of the plot at heading time. The diseased hills of rice were inoculated with a bacterial suspension 2-3 weeks prior to setting them in the plots. “Severely diseased hills” had 2 panicles with more than 60% diseased grains and other panicles with less than 10% diseased grains. In “slightly diseased hills”, the percentages of diseased grains of all panicles were less than 10%.

The count was made for 10 panicles per hill 3 weeks after heading time of cv. Asominori. Disease incidence was determined as follows:

\(n_0=\) the number of panicles with 0% diseased grains
\(n_1=1-10\%\), \(n_2=11-30\%\), \(n_3=31-60\%\), \(n_4>61\%\)

\(N=n_0+n_1+n_2+n_3+n_4\)

Disease incidence = \((5n_1+20n_2+45n_3+80n_4)/N\)

The experiment was made in three replications.
Fig. 1. The plot used to study dissemination of bacterial grain rot. Rice (cv. Asominori) was hand planted with 0.16 m spacing in 0.3 m rows. An inoculum source (□) was established by setting diseased hills (cv. Koganebare) planted in 1/5,000 a pots at the center of 2.7×2.88 m plot. Disease incidence of 8 plants (□) surrounding an inoculum source in each plot was compared.

**Populations of P. glumae from diseased and symptomless panicles.** Four diseased panicles (A, B, C, D) and one symptomless panicle (E) were sampled from a paddy field, in which cv. Reiho was planted, approximately 10 days after heading time.

Sampled panicles were placed in a moist chamber at 28°C. At 0, 1 and 6 hr after inoculation, sampled panicles were placed in 20 ml of sterile distilled water in test tube and shaken for 2 min at 28°C. Aliquots of 0.1 ml from 10-fold dilutions of this suspension were pipetted onto S-PG medium. Bacterial colonies were counted 7 days after incubation at 28°C.

**RESULTS**

**Spatial distribution**

The percentages of the hills, whose disease incidence was 2 to 4, in field A was usually more than those in fields B and C and that of those hills 3 weeks after heading time were 42.3% (moderate) in field A, 5.8% (light) in field B and 7.8% (light) in field C, respectively.

For the bacterial grain rot of rice a week after heading time in field A, the $I_B$ was greatest (14.00) with the 2×4 quadrat size (rows×hills). The shape of the $I_B$ curve with increasing the quadrat size decreased until it reached a minimum of 1.65 with the 10×10 quadrat size. All $I_B$ values were significantly different from randomness at the 5% level, but the $I_B$ for fields B and C were both 0.0.

At 2 weeks after heading time, the shape of $I_B$ curves for fields B and C were similar to that of the $I_B$ for field A. This is typical for a pattern with small clumps. For field A, the hills with a disease incidence of 4 were frequently surrounded by hills of lower diseased incidence. The $I_B$ value with each quadrat size in field C was generally higher than that in fields A and B, and the $I_B$ value at each quadrat size in field A was generally the lowest.

At 3 weeks after heading time, the $I_B$ for field A was a minimum of 1.32 with the 2×4 quadrat size and was the greatest (1.63) with the 5×5 quadrat size and decreased with larger quadrat size. All $I_B$ values for field A are significantly different from randomness at the 5% level. The shape of $I_B$ curve for field B was similar to that for field C. All $I_B$ values for fields B and C were significantly different from randomness at the 5% level. The $I_B$ values in each quadrat size of fields B and C were generally higher than that in field A. For fields B and C, it was typical for a pattern with small clumps. For field A, it was typical for a pattern with large clumps. For fields B and C, the hills with a disease incidence of 4 were frequently surrounded by hills of lower disease incidence (Figs. 2 and 3).

A total of the severely diseased panicles with more than 30% diseased grains in field A 1 and 2 weeks after heading time was clearly more than that of fields B and C.
Fig. 2. The pattern of 3 classes of disease incidence for bacterial grain rot of rice in an area of 5,000 hills (50 rows × 100 hills) at 2 weeks and 2,000 hills (20 rows × 100 hills) at 3 weeks after heading time in 3 fields (A, B and C). •: the number of diseased panicles with more than 30% diseased grains was more than one per hill. ◻: the number of diseased panicles with less than 30% diseased grains was more than 6 per hill. ••: the number of diseased panicles with less than 30% diseased grains was 2 to 5 per hill.
Fig. 3. Morisita's index of distribution plotted against a series of quadrat sizes (rows × hills) used to analyze the incidence of bacterial grain rot, 1, 2 and 3 weeks after heading time in 3 fields (A, B and C). ●: field A, ○: field B, ■: field C.

**Dissemination**

For plot A in which the severely diseased hill was set as an inoculum source at heading time of cv. Asominori, the disease incidence of cv. Asominori was largest (16.5) at a distance of 0.16 m to the east of the inoculum source and decreased with further distance from the inoculum source. The shape of the disease incidence curve was similar to that to the west, south and north of the inoculum source. For plot B in which a severely diseased hill was set as an inoculum source a week after heading time and plot C in which a slightly diseased hill was set as an inoculum source at heading time, in general, the further the distance to the inoculum source, the lower was the disease incidence (Fig. 4).

For plots A, B, C and D, the disease incidences of 8 plants (Fig. 1) surrounding the inoculum source were clearly largest (7.4) in the plot A and were 2.1, 1.9 and 0.4 in the plot B, C and D, respectively (Fig. 5).

**Populations of P. glumae isolated from diseased and symptomless panicles**

Percentages of diseased grains of sampled panicles (A–E) at about 10 days after heading time were A: 71.4%, B: 64.8%, C: 10.3%, D: 1.2%, and E: 0.0%, respectively. *Pseudomonas glumae* was recovered more than 10⁴ cfu/ml from A and B samples, respectively, and about 10³ cfu/ml from C sample, but was not recovered from D or E when the pathogen was recovered from the panicles just after sampling in a paddy field. Populations of *P. glumae* from diseased panicles clearly increased with increasing disease severity. After 6 hr incubation in a moist chamber, *P. glumae* was recovered about 10⁷ cfu/ml from A and B, about 10⁶ cfu/ml from C and about 10⁴ cfu/ml from D, but was not recovered from E. Populations of *P. glumae* from diseased panicles clearly increased with longer incubation time in a moist chamber (Fig. 6).
Fig. 4. Dissemination of bacterial grain rot of rice observed in a paddy field in 1988. A: a severely diseased hill, planted in 1/5,000 a pot and inoculated 2-3 weeks before setting, was set as an inoculum source at heading time in the plot. B: a severely diseased hill was set as an inoculum source a week after heading time in the plot. C: a slightly diseased hill was set as an inoculum source at heading time in the plot. D: a healthy hill was set in the center of the plot at heading time. ○: disease incidence in the east of the inoculum source, □: disease incidence in the west of the inoculum source, ■: disease incidence in the north of the inoculum source, △: disease incidence in the south of the inoculum source. Vertical bars show the standard error of the mean of disease incidence.

Fig. 5. Disease incidence of bacterial grain rot of the 8 rice plants surrounding an inoculum source. A: a severely diseased hill, which was planted in a 1/5,000 a pot and inoculated 2-3 weeks before setting, was set as an inoculum source at heading time in the plot. B: a severely diseased hill was set as an inoculum source a week after heading time in the plot. C: a slightly diseased hill was set as an inoculum source at heading time in the plot. D: a healthy hill was set in the center of the plot at heading time. Vertical bars show the standard error of the mean of disease incidence.

Fig. 6. Populations of *Pseudomonas glumae* from different diseased panicles sampled in a paddy field with different incubation periods in a moist chamber. Percentages of diseased grains per total grains in a panicle were ○: 71.4%, ●: 64.8%, □: 10.3%, △: 1.2% and ■: 0%, respectively.
DISCUSSION

The distribution pattern of bacterial grain rot of rice was assessed by Morisita's index $I_B$ 1, 2 and 3 weeks after heading time in 3 paddy fields, the severity of fields being moderate on field A, light on fields B and C.

In this experiment, it was clear that the distribution of hills, whose disease incidences were 2 to 4, a week after heading time was typical for a pattern with small clumps for field A, but not for fields B, C and that it was typical for a pattern with small clumps for fields A, B and C 2 weeks after heading time. This result suggested that the higher the severity of fields, the earlier was the formation of the aggregations in fields.

Severely diseased panicles were typical of this disease. Since all of the infected grains cannot ripen, the severely diseased panicles remain standing during the period of ripening in paddy fields. From this result, it was suggested that relationship between the focal formation and the occurrence of severely diseased panicles should be examined.

Moreover we reported that the infectious period of plants to this pathogen in paddy fields was estimated mainly from heading time to about 10 days after heading time. Thus, if the severely diseased panicles are important as a primary inoculum, we should study on two considerations. First, the earlier the occurrence of severely diseased panicles, the larger is the focal formation. Second, the higher the disease severity of panicles, the larger is the focal formation.

The dissemination of bacterial grain rot was studied in a paddy field for clarifying the role of severely diseased hills as inoculum source. This experiment shows clearly that the shorter the distance to the inoculum source, the higher will be the disease incidence of the plant surrounding the inoculum source. This result suggests that diseased plant will be important as inoculum source in paddy fields. Moreover, this experiment shows that the earlier the setting time of the inoculum source or the higher the disease incidence of inoculum source, the larger will be the focal formation.

Populations of P. glumae from severely diseased panicles with more than 60% diseased grains were clearly larger than that from slightly diseased panicles with less than 10% diseased grains kept for 0 to 1 hr in a moist chamber. Populations of P. glumae from severely diseased panicles were clearly beyond the infection threshold (about 10^3 cfu/ml) of this disease. This result shows that the higher the severity of panicles, those panicles are important as the inoculum source. Moreover, the longer the incubation time of the diseased panicles in moist chamber, the higher was the population of P. glumae from diseased panicles. These results suggest that the dissemination of P. glumae also depends on a wetting period of diseased panicle as an inoculum source.

In this experiment, it is clear that the severely diseased panicles are important in forming infection foci in paddy fields and the earlier the occurrence of severely diseased panicles, the larger is the focal formation.

We wish to thank Dr. H. Kato, University of Kobe, for his critical reading of the manuscript and Dr. J.J. Gaspard, University of California (present address: Kyushu National Agricultural Experiment Station), for advice.

Literature cited


**和 文 摘 要**

對馬誠也・內藤秀樹：イネもみ枯細菌病の圃場における分布様式と発病株からの伝染

自然発病条件下の3圃場（A圃場：中発生；B, C圃場：少発生）において、イネもみ枯細菌病の発生経過を検証するため、*I*-指数を用い調査した。発病度2以上の発病株の分布について検討した結果、出穂期1週間後ではA圃場のみが集中分布を示したが、出穂期2週間後ではA圃場とも同様の*I*-曲線を描き、小集団をもつ集中分布を示した。出穂期3週間後では、B, C圃場で小集団をもつ集中分布を示したが、A圃場では病勢が著しく拡大し、大集団をもつ集中分布を示した。発病株が集中した箇所の中心部には重症株（発病程度30%以上）をもつ株が観察され、重症株が周辺株の発病に関与していることが示唆された。そこで、圃場試験により出穂期および出穂期1週間後に発病度の異なるイネ株を設置し、出穂期3週間後に設置株周辺イネ株の発病を調べた。その結果、設置した発病株に近い株ほど発病度が高くなり、設置した発病株が周辺株の発病を引き起こしていることが明らかとなった。また設置株が同一発病度の区では早期に設置した区ほど、また設置時期が同一の区では発病度の高い株を設置した区ほど周辺株の発病度が顕著に高かった。自然発生圃場から、発病程度の異なる穂を取り出し、発病穂の洗浄液からS-PG培地を用いた試験的に病原細菌を検出した結果、発病頻度が高い穂ほど病原細菌量が顕著に多かった。この結果は、発病頻度が高い穂ほど伝染源として果たす役割が大きいことを裏づけるものと考えられた。