Potential for Conidium Formation of Pyricularia oryzae in Lesions on Leaves and Panicles of Rice

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ABSTRACT

The potential for conidium formation by *Pyricularia oryzae* in lesions on rice leaves increased rapidly and decreased slowly with time. Conidium formation reached a peak from 3 to 8 days after appearance of lesions, when they developed a central grey zone with a dark purple-brown margin. Sporulation was heavier in lesions when expanding leaves were inoculated than when inoculations were made 3 or 4 days after they became fully expanded. Lesions on the upper five leaves of each culm could produce conidia at the initial heading stage of a population of rice plants. The peak of sporulation in lesions on the penultimate leaf inoculated during its expansion coincided with the initial stage of heading. In spikelets, the presporulation period was shorter than in the rachis and in the neck node. The peak of potential for sporulation of spikelets and of neck nodes inoculated at the time of their emergence occurred from the 4th to the 8th day, and from the 8th day after appearance of lesions, respectively. Spikelets and neck nodes inoculated 10 days after their emergence had much lower yield potential than ones inoculated just after emergence. Maximum conidium production on the infected rachises occurred from 10 to 20 days after lesions appeared. These diseased organs on the panicle could supply conidia to neighboring panicles. Phytopathology 60:608-612.

Infection of panicles of the rice plant *Oryza sativa* L. by the blast fungus, *Pyricularia oryzae* Cav., causes loss in yield of grain. Grain requires 35 to 60 days in different varieties to ripen after panicles emerge. Loss of grain depends upon when infection occurs. Therefore, it is important to know when and where inoculum is produced and how the quantity of inoculum changes with time.

The outbreak of panicle blast is closely correlated with the amount of leaf blast (6, 12, 14, 18). Lesions on leaves (6, 19) and on the collar auricle and ligule (15) supply conidia for infection of panicles. Although conidia are produced on some diseased weed grasses whether the plants live or die (2, 16), it is not clear whether they function as inoculum sources for panicle infection or not. On the other hand, some spore-trapping studies demonstrated a rapid increase in conidia after heading occurred, even though levels of leaf blast were low. For example, an outbreak of panicle branch blast before harvest brought about an unexpected loss in yield in 1960 in the southern part of Akita-ken in northern Japan (8). Although conidia are probably rapidly produced on the panicle itself, the extent to which this happens is unknown.

To obtain such information, the time and level of conidium production on different organs were studied.

MATERIALS AND METHODS.—Two cultivars of rice were used. Cultivar Fujiminori is moderately susceptible, and Otori is highly susceptible to isolate TH 61-33 of race JN-1; that is, IG-1, of *Pyricularia oryzae*. Seedlings were grown in a water-logged seedbed for 44 days, and transplanted in a flooded lowland of the Experiment Farm in Omagari on 8 June 1962 and 5 June 1963. The field was fertilized with 12,000 kg compost, 300 kg $(NH_4)_2SO_4$, 300 kg $Ca(H_2PO_4)_2$, and 120 kg KCl/hectare. Planting was on a 23 × 23 cm spacing, or 20 plants/m^2. Each inoculation test involved three subplots, each containing nine plants.

Fresh conidia were produced by inoculating leaves of pot-grown rice plants of the cultivar Sasashigure. Detached leaves were incubated overnight in a moist chamber, and conidia were dislodged for use in field inoculation. These were suspended in water, and the suspension was sprayed on test plants by means of an atomizer. Inoculated plants were enclosed in a vinyl-covered frame wrapped with a wet straw mat for about 40 hr. For inoculation of the rachis, the spore paste method (20), which is a modification of Misawa's pulp method (13), was used. To a mixture of conidia and powdered cellulose, a small amount of a suspension of sodium carboxymethyl cellulose was added. The resulting sticky paste was applied as inoculum to plant tissues. Plants were then sprayed with water and incubated in a moist chamber.

The system used for designating specific leaves during experiments was that of Katayama (9). Leaves along a main culm were numbered in order from the primary leaf, except for the coleoptile and the imperfect leaf. The main culm itself was indicated by 0. Thus, a designation of 10/0 indicated the 10th leaf along the main culm. The leaves between the 10/0 leaf and the flag leaf were marked to identify each of them. The terminal flag leaves were identified as 14/0 in Fujiminori and 16/0 in Otori, respectively. The apical leaf is indicated here by "n", and successive lower leaves by "n-1", "n-2", etc. Leaves on the main culm were inoculated either at the middle stage of expansion (ME) or on the 3rd or 4th day after expansion (AE) in 1962. Neck nodes were inoculated 3 days after emergence of panicles, just when the neck node was emerging from the flag leaf sheath, or 13 days after panicle emergence. The term "panicle emergence" means the time when the apical spikelet appeared from the sheath of the flag leaf. In 1962, panicles used for inoculation of spikelets or neck nodes emerged from stems of Fujiminori on 10 August, and in Otori on 18 August. Inoculation was done 3 or 13 days after emergence. In 1963, the rachises of the panicles which emerged on 3 August on Fujiminori and on 20 August on Otori were inoculated 3, 16, or 26 days after emer-

gence. Inoculation was confined to a zone one-third the length of the main axis of the panicle. Throughout these experiments, diseased organs were placed in the field, so that both production of conidia and their release occurred under field conditions.

Samples from inoculated plants were collected around 4 PM. Lesions on leaves, neck nodes, or panicle axes were wiped with wet cotton, and each was placed against the inner wall of a small glass tube 1.2 cm in diam and 2.7 cm high, containing 0.2 ml of distilled water. Six tubes containing samples were placed into larger tubes 2.8 cm in diam and 5.5 cm high, containing 5 ml water, and seven larger tubes were held in a dish 9 cm in diam and 7 cm high, with 50 ml water. The cover was lined inside with moistened filter paper. Samples of spikelets detached from pedicels were laid on healthy culms in a petri dish with moistened filter paper. All samples in their containers were incubated in darkness at 27 C for 15 hr. The capacity of infected tissues to produce conidia under such conditions per unit time was called the potential for sporulation.

Before conidia were counted, 0.2 ml of 0.2% Neoesterin was added to the sample in the tube. This preparation had a hydrophile-lipophile balance value of about 15.5. Spikelets were transferred to the small tube, and 0.4 ml of 0.1% solution were added before counting. The tissues were washed with a syringe, and density of the resulting conidial suspension was determined with a hemocytometer. Preliminary determination of sample size for each counting was made on the basis of the quantity necessary to yield more than 50 conidia/mm^3. Samples in the early or late stages of development consisted of several lesions, whereas samples in the middle stage of development consisted of a single lesion. The number of conidia was assessed on the basis of the average of 6 aliquots of 0.4 mm^3 each.

RESULTS.—*Potential for sporulation on infected leaves.*—The relationship between potential for sporulation and time of initiation of heading is shown in Fig. 1-A for the cultivar Fujiminori. The temperature ranged from 14.8 to 34.5 C during these experiments. Small, purplish-black spots appeared 4 days after inoculation in every test. Conidium production increased rapidly and decreased gradually with time. When each leaf on a main culm was inoculated during expansion, the peak period was detected 3 to 8 days after appearance of lesions. The maximum intensity of sporulation was associated with lesions having a grey zone in the center with a margin changing in color from dark purple to brown. Fewer conidia were borne in the lesion when inoculated at the AE stage than when inoculated at the ME stage. Potential was maintained for more than 20 days after lesion appearance. The initial stage of heading in the rice population coincided with the maximum conidium production in lesions on the n-1 leaf inoculated at the ME stage. Little potential for sporulation in lesions on the n-3 leaf inoculated at the ME stage was still observed at the initial stage of heading. The number of conidia produced in lesions was greater on leaves in the tillering stage than in the stage after the panicle primordium was formed.

On the cultivar Otori, potential for sporulation was similar (Fig. 1-B). The lesions on the n-4 leaf inoculated at either the ME or the AE stage were bearing conidia at the initial stage of heading. The n-1 leaf was inoculated at the time it expanded. The initiation of sporulation on this leaf corresponded to the initial stage of heading, and the peak period coincided with the first half of the heading period.

Potential for sporulation on infected neck nodes.—When the neck nodes were inoculated immediately after their emergence, conidia were produced on the 2nd day after their appearance and then rose to a peak 14 to 25 days after they appeared in Fujiminori, and 8 to 15 days afterward in Otori (Fig. 2). Then conidium production declined, but had not ceased by harvest time, namely, at least for 34 days in Fujiminori and 37 days in Otori. The maximum number of conidia formed was

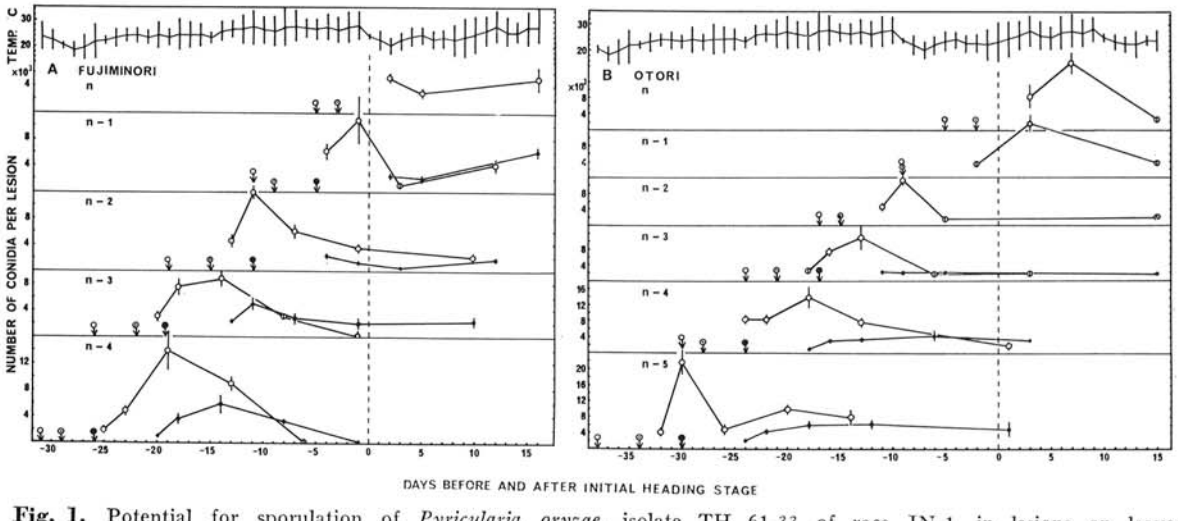

Fig. 1. Potential for sporulation of *Pyricularia oryzae,* isolate TH 61-33 of race JN-1, in lesions on leaves of moderately susceptible Fujiminori and highly susceptible Otori rice. Each leaf on a main culm was inoculated either at the middle stage of expansion (♀) or after expansion (♀). The flag leaf is indicated by n and successive lower leaves by n-1, n-2, etc. The time of full expansion of each leaf is designated by (♀). The initial stage of heading in the rice population is indicated by the vertical dotted line. All points are an average of 8 samples with the standard errors.

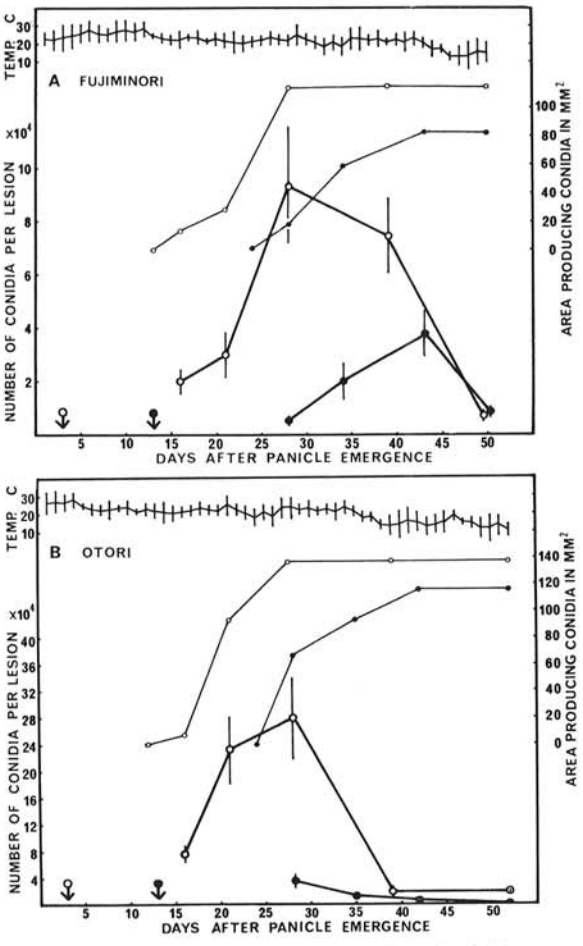

Fig. 2. Potential for sporulation of *Pyricularia oryzae* in lesions on neck nodes inoculated 3 days (○) or 13 days (●) after panicle emergence in Fujiminori and Otori rice. When the apical spikelet appeared from the sheath of the flag leaf, the culm was at the panicle emergence stage.

9.3×10^4/lesion in Fujiminori and 28×10^4 in Otori cultivars. The development of lesions in Otori was greater than in Fujiminori under nearly equal climatic conditions. The temperature ranged from 3.5 to 34.2 C. The surface area of a lesion was calculated from measurements of stem diam and the length of area changing in color. The number of conidia per square mm was 1.6×10^3 in Fujiminori and 2.4×10^3 in Otori at the peak period. When neck nodes were inoculated on the 10th day after their emergence, potential for sporulation was less than when inoculated at time of emergence. The incubation period in the case of newly emerged neck nodes was 11 days; for nodes inoculated 10 days later in Fujiminori it was 12 days. Comparable incubation periods for Otori were 10 days and 12 days. During these periods, the temperature ranged from 13.2 to 33.8 C. The tissues around infected neck nodes changed from green to brown in color, and brownish-purple lines developed at this time. The disease sometimes ceased developing at this stage. Therefore, the incubation period was defined as the first appearance of a lesion in the shape of a pseudomorph or a triangle upward or downward to the neck node. The end of the incubation period was arbitrarily defined as the stage when the total number of stems having such lesions exceeded one-third of the number of inoculated stems.

Potential for sporulation of infected spikelets.—The spikelets in which both lemma and palea were infected were sampled. Spikelets having only infected empty glumes were discarded. Spikelets inoculated 3 days after panicle emergence produced a few conidia on the night when the lesion appeared and a maximum number of conidia on the 8th day after lesion appearance in Fujiminori and on the 4th day in Otori (Fig. 3). The temperature ranged from 13.2 to 34.2 C. A spikelet yielded 8×10^4 of conidia at the maximum in both cultivars. When inoculated at the milky stage, on the 13th day after panicle emergence from the stem, spikelets had lower potential. The maximum production of conidia was less than 10^4, and there was no distinguishable peak. Potential was maintained for more than 20 days. The incubation periods were from 5 to 7 days during these trials, and the temperature ranged from 14.5 to 33.8 C.

Potential for sporulation on infected rachises.—The symptom appeared on the rachis from 10 to 12 days after inoculation. The temperature ranged from 6.3 to 31.0 C during these experiments. The number of conidia formed was from 2×10^3 to 10^4 on the 2nd to the 5th day after lesion appearance (Table 1). The peak was detected from 10 to 20 days after lesion appearance. The highest number of conidia counted was 6×10^4. Potential to produce conidia was maintained for more than 20 days. When the lesion involved the nodes of the primary branches, the number of conidia was abruptly increased in some cases.

DISCUSSION.—The strength of inoculum potential varies both in respect to time and space. Under temperatures ranging from 14.8 to 34.5 C in the field, the

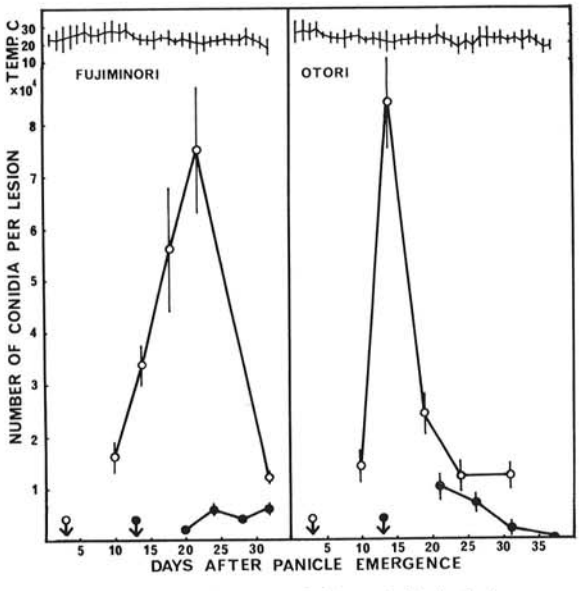

Fig. 3. Potential for sporulation of *Pyricularia oryzae* in lesions on spikelets inoculated 3 days (○) or 13 days (●) after panicle emergence in Fujiminori and Otori rice.

TABLE 1. Potential for sporulation of *Pyricularia oryzae* on the infected rachis of rice

Cultivars	1st Inoculation[a]		2nd Inoculation		3rd Inoculation	
	Days after lesion appearance	No. conidia/ lesion[b]	Days after lesion appearance	No. conidia/ lesion	Days after lesion appearance	No. conidia/ lesion
		$\times 10^3$		$\times 10^3$		$\times 10^3$
Fujiminori	2	9.9 ± 2.0	5	2.4 ± 0.4	7	0.3 ± 0.07
	10	38.1 ± 4.3	14	8.8 ± 1.6	17	6.0 ± 2.4
	20	30.1 ± 4.6	24	14.7 ± 1.8		
Otori	2	9.9 ± 1.3	2	2.0 ± 0.5	3	2.7 ± 0.7
	12	19.7 ± 6.4	11	1.4 ± 0.5	13	23.7 ± 1.9
	22	22.5 ± 5.8	21	9.5 ± 2.9		

[a] The inoculations were conducted 3, 16, or 26 days after panicle emergence, respectively. A zone one-third the length of the main axis of the panicle was inoculated by spore paste method.
[b] Numbers are an average of 8 samples with the standard error.

incubation period of leaf blast was 4 days, and conidia were produced on the 2nd day after appearance of lesions. Therefore, the presporulation period of leaf blast was 6 days under these conditions. The same result was reported under constant optimum temperature by Andersen et al. (1). The potential for sporulation on lesions increases rapidly with time, attains maximum, then decreases gradually. The maximum production of conidia occurs from 7 to 12 days after infection, i.e., from 3 to 8 days after appearance of spots. Potential was maintained for more than 25 days after inoculation. The maximum production is associated with lesions having gray centers with margins changing in color from dark purple to brown. This result was also noted when the number of conidia was determined in the different developing types of lesions that were collected at the same time (6, 7, 11, 12, 21). The relation of the amount of conidium production to age of lesions as found in this study is also similar to the results in previous studies (3, 4). The maximum number of conidia varied with the combinations of host cultivars, fungus isolates, and the growth stage of plants. This is in agreement with the data by Barksdale & Asai (3) and Ou & Ayad (17).

We can estimate the relation between the source strength of inoculum and the vertical distribution of inoculum sources. According to the nature of the disease gradient, infection occurred on leaves during and soon after leaf expansion in the field, giving rise to a progressive series of lesions. If a leaf emerges in 5 days, as is the case during tillering, lesions on the second (n-1) or third leaf (n-2) down the culm in a descending order will have the maximum potential. When leaves require 9 days for expansion, as occurs during the booting stage, the inoculum they produce will be the maximum in potential on the first n or the second leaf n-1. During the incubation period, growth of leaves elevates them so that conidia are liberated from lesions at a higher position than when infection occurred. This is termed "a lifting of the inoculum source by a host". Some conidia that are liberated from an elevated position settle on young, developing tissues.

When a source of inoculum is able to supply propagules directly to specified organs or tissues of a host, the source is said to have "a direct effect" on the given parts. When the propagules from a source must pass through a cycle of infection and produce a second generation of spores before infecting the specified tissues, the source is said to have "an indirect effect". In the developing plant, conidia produced on the fourth leaf, n-3, below the apex in Fujiminori and the fifth leaf, n-4, in Otori had a direct effect upon the emerged panicle at the initial stage of heading, when leaves were infected during their expansion. When infected in the 3rd or 4th day after their expansion, leaves on a main culm immediately below also directly affected panicles. Thus, the boundary time of infection of these leaves was 25 to 30 days before the initial heading stage in northern Japan.

The presporulation period is of shorter duration in a spikelet than in a panicle axis or in a neck node. When the temperature ranged from 13.2 to 33.8 C, sporulation did not occur until 5, 10, and 13 days in lesions on spikelets, panicle axes, and neck nodes, respectively. According to Katsube (10), when infection of a neck node or a rachis occurred by the 25th day after emergence from the flag leaf n, the infection caused some losses of grain yield. A certain period of about 10 days is required for heading. Hence, for loss of yield the final time of infection is 35 days after the initial heading stage in a population of rice plants. A knowledge of the presporulation period of various organs and the term of their sporulation permits a statement as to whether a given organ can be the inoculum source which brings about yield loss in the same rice population (Fig. 4).

Emergence of each panicle requires from 3 to 5 days from protrusion of an apical spikelet to appearance of a neck node from the sheath of a flag leaf. If both the apical spikelet and the neck node on the same culm are infected immediately after they emerge from the sheath of the terminal leaf, there is a difference of 11 to 13 days in the times when conidia would be formed on each tissue. Sporulation on lesions of the panicle branch may begin earlier than on the neck node, but may begin later from a lesion on the apical spikelet when each organ receives a shower of conidia during the emerging period. Surveys that have measured increase in lesions on different parts of the panicle have been conducted under different field conditions. These studies showed that the number of lesions per unit field area was greater in spikelets than in panicle branches and neck nodes during the first 2 weeks after the middle stage of heading (H. Kato,

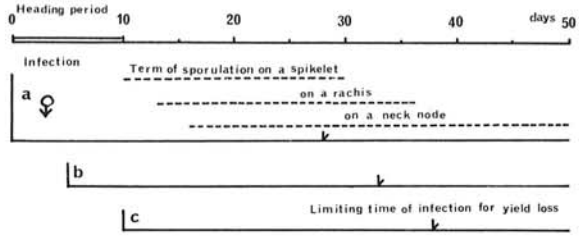

Fig. 4. The relation between potential for sporulation of *Pyricularia oryzae* on infected panicles of rice, and the final time of infection that would result in loss in yield. Yield loss would be expected when the panicles, which emerged on the first (a), middle (b), and last (c) day during the heading period, respectively, were infected by the respective time indicated by each arrow. Thus, if panicles of the (a) group were infected on the day of neck node emergence (♀), each organ of the panicle would supply conidia during the term shown by the dotted lines.

T. Sasaki, & Y. Koshimzu, *unpublished data*). On occasions when spikelets on a panicle emerge early and are infected early, they may produce conidia that infect newly emerged panicles in the same population. Therefore, at first the spikelet is an important source of inoculum during the ripening of grains. The panicle branches and the neck nodes follow it. Though the ontogenetic disease proneness of rice leaf against the given fungus prominently decreases during the period following the formation of a panicle primordium, all parts of a young panicle, especially spikelets, have higher proneness. The number of available sites of infection suddenly increases when panicles begin to appear in a rice population. As long as a small amount of mycelia survives on leaves, the produced conidia would be distributed to these sites, and thereafter inoculum would increase rapidly. When both early and late maturing cultivars are grown in nearby fields, the source of inoculum would be on the panicle of the early-maturing cultivar, as suggested by Willis et al. (22). The same principle may be presumed to apply when different plantings are made in one area during the growing season.

Symptoms of the infected panicle branch were classified into three categories by Hirano & Goto (5): primary panicle branch blast, secondary panicle branch blast, and rachis blast. In the present study, we have treated rachis blast only, because homogeneous materials are easy to obtain. Nodes of branches become infected often, and they die back from the spikelets frequently in the field (5). The potential for sporulation of these other parts of the organ needs further detailed study.

LITERATURE CITED

1. Andersen, A. L., B. W. Henry, & E. C. Tullis. 1947. Factors affecting infectivity, spread, and persistence of *Piricularia oryzae* Cav. Phytopathology 37:94-110.
2. Asuyama, H. 1965. Morphology, taxonomy, host range, and life cycle of *Piricularia oryzae,* p. 9-22. *In* The rice blast disease, Int. Rice Res. Inst. Symp., Johns Hopkins Press, Baltimore, Md.
3. Barksdale, T. H., & G. N. Asai. 1961. Diurnal spore release of *Piricularia oryzae* from rice leaves. Phytopathology 51:313-317.
4. Hagiwara, Y., & K. Nakamura. 1960. Application time of mercuric compound for protection of rotten neck and panicle branches of rice. [In Japanese] Chugoku Agr. Res. 17:19-30.
5. Hirano, K., & K. Goto. 1963. Pathogenesis and ecology of panicle branch blast of rice plant. [In Japanese, English summary] Bull. Nat. Inst. Agr. Sci. Ser. C. 16:1-58.
6. Hori, M. 1963. Studies on the forecasting of rice blast. With special reference to the experimental forecasting. [In Japanese, English summary] Special Res. Rep. on Disease and Insect Forecasting, Min. Agr. Forest., Japan. 14:1-76.
7. Ichikawa, H., T. Harada, & M. Kondo. 1959. Studies on the relationship between sporulation and leaf infection types of rice blast. [In Japanese] Kanto Plant Prot. Soc. Proc. 6:3.
8. Ishigooka, T. 1961. Relation of climatic factors to the epidemic of rice blast in Akita in 1960. [In Japanese] Ann. Rep. Plant Prot. North Japan 12: 32-34.
9. Katayama, T. 1951. Studies on tillering of rice, wheat and barley. [In Japanese] Yokendo Publ. Co., Tokyo.
10. Katsube, T. 1961. Some observations on susceptibility of panicles of rice to the blast fungus. [In Japanese] Ann. Rep. Plant Prot. North Japan 12:34-36.
11. Kobayashi, Y. 1963. Studies on the forecasting method of the rice blast by means of chemical analysis of leaves. [In Japanese] Special Res. Rep. on Disease and Insect Forecasting, Min. Agr. Forest., Japan. 15:1-100.
12. Kuribayashi, K., & H. Ichikawa. 1952. Studies on the forecasting of the rice blast disease. [In Japanese] Nagano Agr. Exp. Sta. 13:1-229.
13. Misawa, T. 1959. A new technique for inoculation of the rice blast fungus. [In Japanese] Shokubutsu Boeki, Tokyo. 13:15-16.
14. Ono, K. 1963. Principles, methods, and organization of blast disease forecasting. p. 173-194. *In* The rice blast disease, Proc. Int. Rice Res. Inst. Symp., Johns Hopkins Press, Baltimore, Md.
15. Ono, K., & H. Suzuki. 1960. Studies on mechanism of infection and ecology of blast and stem rot of rice plant. [In Japanese, English summary] Special Res. Rep. on Disease and Insect Forecasting, Min. Agr. Forest., Japan. 4:1-160.
16. Ono, K., & H. Suzuki. 1963. Saprophytic multiplication of rice blast fungus in the paddy field. [In Japanese, English summary] Bull. Hokuriku Agr. Exp. Sta. 4:1-16.
17. Ou, S. H., & M. R. Ayad. 1968. Pathogenic races of *Pyricularia oryzae* originating from single lesions and monoconidial cultures. Phytopathology 58:179-182.
18. Rangaswami, G., & T. V. Subramanian. 1958. The correlation between the leaf and neck infections of the blast disease of rice. Indian Phytopathol. 11: 179-185.
19. Thomas, K. M. 1930. Some aspects of the control of blast disease of paddy. Madras Agr. J. 18:596-606.
20. Tokunaga, Y., H. Kato, & Y. Koshimizu. 1965. Studies on the relationship between metabolism of rice plant and its resistance to blast disease. 2. Effect of the phosphorus content in leaf blades during maturity on disease proneness to leaf blast. [In Japanese, English summary] Bull. Tohoku Agr. Exp. Sta. 32:61-88.
21. Toyoda, S., & N. Suzuki. 1952. Histochemical studies on the lesions of rice blast caused by *Piricularia oryzae* Cav. I. Some observations on the sporulation on lesions of different types occurring on leaves of the same variety. [In Japanese, English summary] Ann. Phytopathol. Soc., Japan. 17:1-4.
22. Willis, G. M., R. D. Allowitz, & E. S. Menvielle. panicle to *Piricularia oryzae*. Phytopathology 58: 1968. Differential susceptibility of rice leaves and 1072 (Abstr.).