RESEARCH ON PHYSIOLOGICAL RACES OF PIRICULARIA ORYZAE (PRELIMINARY REPORT) - Japan, 1961

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By Kazuo Goto and Tatsu Yamanaka

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A. Experimental Materials and Methods

The experimental rice plants were cultivated in tin boxes of 5x10 cm in size, which were painted with tar (Figure 1A), and kept in a glass house at a temperature 20° to 30°C and occasionally as high as 40°C. The soil was brought from a rice pad in Kanto district at Nerima-ku, Tokyo, and was fertilized with 1 gm Ammonium sulfate, 1.5 gm Calcium phosphate, and 0.2 gm Potassium chloride per each box. The grains were sterilized in a mercurial preparation then germinated at above temperatures. Twenty grains in total were seeded in each box. Two different kinds of grains were used in some boxes 10 grains of each kind, or in some boxes 20 grains of the same kind were seeded. When they beared 3 or 4 leaves (on 20th or 30th day after seeding), the suspension solution of Piricularia Oryzae spores was sprayed through micro-mist glass spray (Figure 1B and kept at 24° to 28°C, mainly at 25° to 26°C for 20 to 24 hours after inoculation and then kept in the glass room at 26°C (the moisture was 60 to 80% during the day and 90 to 95% during the night).

Observation of the disease spots was started on the 3rd day after inoculation and on the 7th and 8th day the numbers and patterns of the propagating spots were investigated on each species. The spot patterns were classified as areas of toxication, necrosis, and destruction, after the classification of Toya (1955). In addition to this, information obtained about the relationship between the extension of the spots and the veins of the leaves were classified according to the classification of Goto et. al. (1950) who originally made these observations in a study made on Gomahakare disease of the rice plants. (B. see Experiment 1 on method of investigation).

Samples of Piricularia oryzae were collected from affected rice plants in various parts of Japan. These were then cultured and a pure culture of Piricularia oryzae was obtained using the method of Goto (1935) and preserved in sucrose potato agar medium. The spores were then cultured either in rice straw media which consisted of a mixture of 300 gm dried rice straw, 15 gm of sucrose and distilled water, and was then boiled under pressure for 30 minutes and then filtered; or in a barley grain media which consisted of equal amounts of whole grain and distilled water which had been sterilized under pressure. The spores were cultured in the rice straw media for 2 to 3 weeks, but in the barley grain media for 7 to 10 days. Microscopic examination of the obtained spore suspension revealed usually 5 to 10 spores in a 15x10 magnification field (approximately 0.5 to 1 million spores per 1 cc of the suspension). Approximately 40 to 50 cc of the suspension was sprayed over 200 rice plants; and the experiment was repeated 2 to 4 times.

B. Evaluation of Determination Methods

In order to evaluate the physiologic form of rust disease affecting the wheat, the first leaf of the wheat is inoculated and the pattern of the disease spots obtained is the basis for classification. For example, in the case of Puccinia graminis, f. sp Tritici the physiologic forms observed in affected plants could be classified in five different groups, depending on either the presence or absence and the size of summer conidiophores and the adjoining chlorosis and necrosis (Stakman et. al., 1944).

Our classification of the race of Piricularia oryzae is based on the presence or absence of disease spots and the pattern of the spots. The condition of experimental specimens, the condition of inoculated spores, the environment during the incubation period and the method of inoculation were also specifically evaluated.

1. Principles Applied in the Classification of the Races; Especially the Number and Patterns of Spots

The suspension of Piricularia oryzae spores was sprayed on rice plants (plant age 3 to 4) and the development of the spots and the propagation of the disease pattern were evaluated on high level leaves every day for 10 days after the inoculation. The plants were kept in an incubator for 20 to 24 hours at 24° to 28°C and preferably below 26°C (usually 25° to 26°C), and then were placed in a glass room at 26°C. The following results were obtained:

a. The Brown Dots (R-type)

The brown dots are the first to be noted grossly after the inoculation and usually appear after 2 to 3 days, although there are some variations among the different species. Some of the brown dots remain as fine dots (indicated by b) such as those caused by the less virulent race on "Asahi" species. Others may propagate to 0.5 to 1.0 mm in size (indicated by B) such as those on "Ishikarihakumo" species as shown in Figure 2-A, or stretch the line of necrosis up and down (B stretch, Ujihara and Nakamishi /1953/ a). Occasionally in some species a small area of central necrosis is observed on the brown spots after the sixth day. The brown dots appear first and necrosis occurs later. The brown dots usually appear in large numbers, but occasionally very few may appear and sometimes they are mixed with some other disease patterns.

b. The Arrested Small Spots M (= RS type)

These appear as small white dots or white spots (indicated by w) on the fourth day after inoculation and are surrounded by bluish-gray or purple-gray areas (p). After the fifth or sixth day brownish discoloration appears peripherally and extends until the area of necrosis becomes as big as the space in between the first branches of the leaf-veins. The number of spots are usually small, however it varies slightly among the different species of rice plants and among the different races of Piricularia (Figure 2-B).

c. The Arrested Medium Spots S (= MS Type).

The susceptibility of the plants to this type is probably greater than to the last type. The initial process is similar to that of the previous type, then on the sixth day after inoculation, brownish discoloration appears around the disease spots. The area of necrosis becomes arrested after it occupies a slightly wider space than that in between the first branches of the leaf-veins. As a rule the number of spots is less than with the previous types and occasionally spots are found at lower levels of leaves (Figure 2-c).

d. The Large Spots S (= S type)

The formation of these spots is similar to those of the medium size, however they are much larger and more virulent. The area of necrosis is larger than the space between the two first branches of the vein. At first the spot appears as a small whitish point or spot, then enlarges and forms a medium-to-large white spot around the fifth or sixth day (w^0-W) (Figure 2F). A grayish-blue or grayish-purple area develops around these spots (P) (Pw; PW), and finally after the seventh day a large infiltrating spot (PW) involving more than the two initial branches of the vein or an arrested large spot (bG) becomes apparent.

This type of disease spot usually has two different shapes: oval and elongated spindle shape (Figure 2-F and 2-D). The oval shape is specific of Japanese rice plants and in this type the brownish discoloration around the lesion usually appears only after 8 to 10 days. The spindle shape is characteristic of foreign plants; i.e., Chinese "Chokato" and "Yakeiko." The brownish discoloration in these usually appears on the 7th day. Therefore we can say that the susceptibility of the different rice plants is not the same. The disease spots of this type usually appear in multiple foci (Figure 3, I-N).

TABLE 1. DIFFERENTIATION OF TYPES OF DISEASE SPOTS

Type of Spots	Velocity of Circumferential Brownish Discoloration	Development of Types of Spots 3rd day, 4th, 5th, 6th, 7th, Thereafter	Number of Spots	Width of Spots** (at the area of necrosis)
H R type (nonvirulent type)	--		--	--
R type (resistant type)	Earliest and the disease spots immediately turn into brown	b b → B b → B → B spread (b → B ——→ bg)	Generally many	Appear as points within the initial leaf veins & not the spots
R S type (mildly susceptible type)	Discoloration is rapid & the small spots turn immediately into brown	w → pw → bg	Generally rare	Do not spread beyond the initial leaf veins. Limited within. (small spots)
M S type (moderately susceptible type)	Discoloration is more rapid than S type. Changes occur after the medium size spots.	w → pw' → bg' w → w' → pw' → bg'	Generally rare	Spread beyond a initial leaf vein over to the next one. (medium spots)
S type (strongly susceptible type)	Slowest and discoloration takes over after the formation of a large spot	w → w' → pw' → bG w → w' → pw' → PW → bG w → w' → W' → PW → bG	Generally many	Spread beyond two initial leaf veins occupy three spaces in between (large spots)

* bB ......Indicate brownish points; b indicates a fine point and B indicates brownish points of 0.5 ~ 1.0 mm.
w, w', W.....Indicate whitish spots of each small, medium and large.
pw, pw', pW.....Indicate infiltrating spots of each small, medium and large.
bg, bg', bG.....Indicate arrested type spots of each small, medium and large.

** The area of intoxication is excluded from the actual disease spots.

TABLE 2. THE NUMBER OF DISEASE SPOTS ACCORDING TO THE TYPES ON RICE SPECIES

Species	Oryzae Strain / Type of Spots	53—11	53—33	54—14	54—15	54—05	P—2	54—09	53—01	54—08	54—10	54—17	54—20	54—13
Asahi No. 1	R	1.4	5.8	0.6	2.1	1.6	1.1	1.4	0.8	0.2	0.7	0.8	6.0	1.5
	M[1]	1.3	1.1	0.6	1.1	2.5	0.7	0.4	1.1	0.7	0.5	0.3	4.8	0.5
	S	13.[illegible]	28.4	17.7	15.[illegible]	11.[illegible]	6.4	10.[illegible]	4.[illegible]	5.[illegible]	[illegible]	6.7	15.5	4.4
	Hajita oryzae	0.7	0.3	0.3	1.4	0.5	0.1	0.6	0.5	0.2	0.4	0.8	0.7	0.7
Norin No.17	R	2.2	4.9	1.4	0.9	0.2	1.0	2.1	1.7	0.2	0.6	1.0	4.8	2.2
	M	0.4	1.1	1.4	1.3	2.1	0.4	0.7	1.5	0.3	0.3	0.5	3.0	0.7
	S	12.1	17.[illegible]	10.[illegible]	10.3	[illegible]	8.1	7.[illegible]	5.1	1.7	4.[illegible]	5.1	10.4	[illegible]
	Hajita oryzae	0.3	0.3	0.5	1.0	0.2	0.1	0.8	0.7	0.1	0.4	0.9	0.7	1.1
Higashi-yama No 38	R	1.9	6.0	0.4	1.3	0.7	0.5	2.2	0.8	0.2	0.5	1.1	5.0	1.3
	M	0.5	1.6	0.8	0.8	1.5	0.3	0.6	0.8	0.3	0.3	0.4	3.2	0.4
	S	14.4	20.[illegible]	17.7	7.[illegible]	[illegible]	6.5	[illegible].2	3.4	4.[illegible]	1.7	[illegible].7	17.[illegible]	[illegible]
	Hajita oryzae	0.1	0.3	0.5	1.0	0.2	0*	0.4	0.2	0*	0*	0.4	0.4	1.2
Ginea	R	4.9	7.4	3.5	2.7	1.0	0.8	1.1	0.4	0.3	1.0	0.6	3.6	1.4
	M	0.5	1.6	1.8	1.0	1.9	0.6	1.4	0.4	0.2	[illegible]	1.4	5.6	0.7
	S	7.2	[illegible].1	[illegible]	4.2	4.[illegible]	1.5	2.2	1.[illegible]	1.2	0	0.2	7.7	2.8
	Hajita oryzae	0*	0*	0*	0.1	0.1	0	0.1	0.1	0.1	0	0.1	0.3	0.1
Kanto No 54	R	1.2	4.7	0.4	0.1	0.2	0*	0.9	0.1	0*	0.1	0.3	[illegible].5	[illegible]
	M	0.1	0.8	5.5	[illegible].1	[illegible].2	[illegible]	1.1	[illegible]	[illegible].2	1.[illegible]	1.2	0	0*
	S	18.5	18.7	0.1	0	0	0	0*	0	0*	0	0*	0	0
	Hajita oryzae	1.3	0.6	0.1	0	0*	0	0.1	0.3	0*	0.2	0.1	0	0
Kanto No 51	R	3.7	8.5	0.5	0.3	0	0	0.6	0.1	0	0.2	0.4	[illegible].2	0*
	M	0.6	1.4	7.[illegible]	[illegible].4	[illegible].1	0*	1.2	1.1	[illegible].5	1.[illegible]	1.[illegible]	0	0*
	S	19.[illegible]	25.4	0.1	0	0	0	0	0	0	0	0	0	0
	Hajita oryzae	0.6	0.3	0.1	0	0	0	0.1	0	0	0*	0.1	0	0
Spore Concentration x10000/1cc		70	50~100	100~150	100	20~50	100~200	70	20~50	20~50	50	50	100	100

(Table 2 continued)

(Table 2 continued)

Species	Oryzae Strain / Type of Spots	54-19	54-22	54-25	53-40	54-07	54-12	54-01	54-01	53-39	54-18	54-02	54-24	53-12
Asahi No 1	R	5.4	2.1	2.2	1.8	4.6	5.6	0.6	3.2	3.5	18.0	0.3	4.8	0
	M1)	1.8	0.7	0.4	0.1	1.5	1.3	0.9	0.8	0	0.3	0.1	0.2	0
	S	22.4	5.8	8.1	8.4	14.0	12.1	3.8	14.2	0	0	0	0	0
	Hajita oryzae	1.2	0.7	0.8	0.5	1.3	0.5	0.4	0.8	0	0*	0	0	0
Norin No 17	R	2.1	2.3	2.2	3.4	8.8	11.5	0.5	7.1	5.7	15.6	0.2	4.0	0.5
	M	0.5	1.4	1.5	0.5	1.9	2.6	0.4	5.2	0	0.1	0	0.3	0
	S	10.0	7.2	7.5	10.7	10.6	7.0	5.0	0.4	0	0	0	0	0
	Hajita oryzae	0.8	0.6	0.6	0.3	1.1	0.7	0.2	0*	0	0	0	0	0
Higashi-yama No 38	R	2.9	3.1	2.4	2.8	7.8	0.4	0.4	1.6	2.3	3.1	0.6	1.6	0.1
	M	0.9	1.1	0.5	0.1	1.0	1.2	0.7	1.3	0.6	1.2	0	0.3	0
	S	12.1	11.0	8.3	10.0	14.3	2.8	2.1	0	10.1	10.0	2.1	4.4	0
	Hajita oryzae	1.1	0.9	0.4	0.4	1.0	0	0.1	0	0.4	0.7	0	1.2	0
Ginga	P	1.3	1.2	2.7	1.4	3.7	1.3	0	1.2	2.0	3.5	0.8	1.4	[illegible]
	M	1.4	1.3	2.2	0.6	4.4	1.5	0.3	0*	0.9	2.6	0.3	0.5	0
	S	7.5	5.2	5.0	2.8	0.3	0.2	0.2	0	4.5	3.0	1.8	3.7	0
	Hajita oryzae	0.2	0*	0*	0	0.1	0	0	0	0	0.2	0	0	0
Kanto No 54	R	0.4	0.4	0*	0.1	0.3	0.1	0	0.1	[illegible]	1.7	0	0.2	0
	M	0	0	0*	0	0*	0	0*	0	0	0.5	0	0*	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	0
	Hajita oryzae	0	0	0	0	0	0	0	0	0	0	0	0	0
Kanto No 51	R	0.2	0.1	0.1	0.1	0.4	0.1	0	0.4	0.1	[illegible]	0	0.1	0.1
	M	0.1	0.1	0	0	0*	0	0	0*	0.3	0*	0	0*	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	0
	Hajita oryzae	0	0	0	0	0	0	0	0	0	0	0	0	0
Spore Concentration x10000/1cc		100	50~100	50	20~30	100	70~100	20~50	100	70~100	150	50	50~70	150

Remarks: M1) indicates the co-existance of both R S type and M S type.

0* indicates less than 0.1

The numbers written in Gothic indicate the representative types of spots manifested on species of rice plants by each oryzae strains.

In summary we can say, that the brown spots appear within 2 to 3 days after inoculation; while the other types of spots appear as light purplish or whitish spots within 3 or 4 days. After the 10th day of inoculation almost all of the disease spots cease to propagate. The disease process is more rapid in the case of the more resistant lesions produced by the brownish spots and it ceases to spread after 4 or 5 days, while the lesions of less resistance last for 5 to 7 days and the lesions of least resistance (S) keep spreading for 7 to 10 days until the brownish discoloration appears around the lesion.

The above observations were carried out in a glass room at 26°C. Changes in growth conditions, the degree and stage of the growth of the plants, these will alter the process of the disease and even the reaction of the different types of lesion. The types of lesions are shown in Table 1.

When the susceptibility of the plant is high, as shown in Table 1, the lesion that appears as a white spot will spread in size for a longer period and the brownish discoloration around the lesion will appear later, thus the disease spot becomes larger.

In case of type MS, the lesion sometimes enlarges for a longer period of time and becomes larger as previously described.

Toya previously proposed that the circumferential area of intoxication (y) would be a good characteristic on which to base the classification of the lesions. However, according to experiments performed on immature plants, this area of intoxication varies greatly depending upon the amount of sunshine that these plants were exposed to before and after inoculation. For example: experiments performed without any sunshine for 3 to 4 days after inoculation showed a remarkable increase in the area of intoxication around the lesion, and therefore this manifestation was excluded upon evaluation of the experiments.

Twenty-six different strains of Piricularia oryzae were inoculated upon six different kinds of rice plants (Asahi No1, Norin No 17, Higashiyama No 38, Ginga, Kanto No 54, and Kanto No 51). After seven days the number of disease spots was studied according to their particular patterns. (Results in Table 2.)

The number of the disease spots has an intimate correlation with the concentration of the inoculating spores. However, many S type spots are seen, whenever there is a formation of S type spots (pathogen S), which are considered to be the most susceptible type (more than 5 to 10 spots per each individual specimen), and M and R types may also coexist. However in the case of the moderately susceptible M type spots (pathogen M) a smaller number of spots is seen (less than five spots per specimen), and S type spots rarely coexist, although some R type spots are encountered.

In the case of pathogen R, almost no formation of disease spots (HR) is seen, but occasionally few to many formations of R type spots occur.

The following explanation is given for the co-existence of various types of disease spots.

In general, the highest leaf of the plant is considered to be the most susceptible to the disease, and co-existence of different disease spots is rarely seen, while a specific response can be easily seen. The lower the leaf is, the greater is its resistance to the disease. Therefore, when the total number of disease spots are examined, co-existence of various types of disease spots are encountered.

2. Age of Plants, Levels of Leaves, and Number of Disease Spots

In order to classify the races of Piricularia oryzae, depending upon the number of disease spots and the pattern of infection, it is more convenient to use smaller rice plants for these experiments. An experiment was done to see how the number of disease spots and pattern of infection were altered depending upon the size of the rice plant. Rice plants with one to four true leaves were used and the number of disease spots and their patterns were investigated (Table 3).

TABLE 3. THE AGE OF RICE PLANTS AND THE NUMBER OF SPOTS

True Leaves / Experimental Number	Number of spots of susceptible type on leaves (per a specimen)				Number of spots of susceptible type on leaf sheaf (per a specimen)				Number of spots of resistant type on leaves (per a specimen)			
	4	3	2	1	4	3	2	1	4	3	2	1
I	2.7	3.7	3.9	0.7	0	0	0	0.4	0.8	0.4	0.1	0
II	7.5	4.3	0.7	0.5	0	0	0*	0*	2.0	0.5	0	0
III	7.6	6.0	3.3	2.2	0	0	0*	1.4	2.7	1.9	0.6	0.1
IIII	13.2	7.7	3.9	2.9	0	0	0.2	2.5	2.1	1.1	0.4	0.1
Mean Values	7.8	5.4	3.0	1.6	0	0	0.1	1.1	1.9	1.0	0.3	0.1

The experiments shown in Table 3, were performed in the early stages of this research. The susceptible disease spots are represented by the disease spots with central destruction (mainly a combination of S and M type spots); and the resistant type spots are represented by disease spots with brownish points or brownish spread (R type spots) and no central necrosis. As shown in Table 3, the older the rice plant, the greater is the number of susceptible and resistant type spots on the leaves. In cases of young rice plants the susceptible type spots predominate and the total number of spots is much smaller.

In case of the susceptible type spots on rice plants with one or two true leaves, the number of disease spots is smaller, the process of brownish discoloration of the spots is faster and the growth of the spots ceases while they are still rather small. On the other hand, rice plants with three or four true leaves bear more disease spots, since the leaves are bigger and form larger disease spots. The disease spots formed on the leaf sheath of rice plant with only one or two true leaves are rather obscure in their color and extension and therefore their evaluation for classification was difficult. Therefore it was thought to be best to use rice plants with 3 or 4 true leaves in evaluation of races.

Two different strains of Piricularia oryzae were inoculated to nine different species of rice plants, which bore one to four true leaves, and the number and pattern of disease spots were evaluated (Table 4). The result was exactly the same as that of Table 3. Each species of rice plants, excluding a few species, formed a large number of disease spots when they had four true leaves, but few spots were formed on plants with less than three leaves.

TABLE 4. AGE OF RICE PLANTS AND THE TYPES OF DISEASE SPOTS (1956)

Oryzae Strains	Experimental Specimen \ True leaves / Type of Spots	4			3			2			1		
		R	M	S	R	M	S	R	M	S	R	M	S
P-2	Tetep	-	-	-	±	-	-	-	-	-	-	-	-
	Kyoshiko	+	+	-	±	±	-	±	±	-	-	-	-
	Kanto No 51	+	±	-	-	±	-	-	±	-	-	-	-
	Ishikarihakumo	+++	-	-	+++	-	-	++	-	-	+	-	-
	Homarenishiki	++	+	+~++	+	-	++	-	-	+~++	±	±	-
	Ginga	++	+	++	++	+	++	±	+	+	±	±	-
	Norin No 22	++	+	+++	++	+	+++	±	-	++~+++	±	±	±
	Aichiasahi	++	-	+++	+	-	+++	-	-	+	-	±	±
	Norin No 20	++	-	+++	++	-	+++	-	-	++	±	-	+
54-25	Tetep	-	-	-	-	-	-	-	-	-	-	-	-
	Kyoshiko	±	-	-	-	-	-	-	-	-	-	-	-
	Kanto No 51	-	-	-	-	-	-	-	-	-	-	-	-
	Ishikarihakumo	++	-	-	++	-	-	+	-	-	±~+	-	-
	Homarenishiki	+	±	+	±	±	±	±	-	±	±	-	±
	Ginga	+	±	+	-	±	+	±	-	±	±	±	±
	Norin No 22	+	±	++	±	-	+~++	±	-	±	±	±	±
	Aichiasahi	+	-	++	±	-	+~++	±	-	±	±	-	±
	Norin No 20	-	-	+~++	-	-		-	-	±~+	-	-	±

Remarks: -, ±, +, ++, +++ indicate the total number of disease spots per each specimen. -·····0, ±···less than 1, +·····1~4, ++····5~9, +++·····more than 10

TABLE 5. DISTRIBUTION OF DISEASE SPOTS ON EACH LEVELS OF LEAVES

Strains of Oryzae	Levels of Leaves	Types of Disease Spots	Asahi No 1	Norin No 17	Higashi-yama No 38	Ginga	Kanto No 54	Kanto No 51
53-33	1st leaf	R	1.5	0.2	0.9	0.5	1.0	0.6
		M	0.1	0	0.1	0	0.1	0.1
		S	0.1	0	0.1	0	0.2	0
	2nd leaf	R	1.3	1.9	2.8	4.2	0.5	4.5
		M	0.4	0.5	0.7	0.7	0.3	0.6
		S	6.6	0.9	3.0	0.4	4.5	5.2
	3rd leaf	R	1.7	0.7	0.8	3.3	0.5	3.8
		M	0.5	0.2	0.2	0.4	0	0.6
		S	17.6	14.1	10.8	5.0	12.2	14.7
54-10	1st leaf	R	0.2	0	0.1	0.2	0	0
		M	0	0	0	0	0	0
		S	0	0	0	0	0	0
	2nd leaf	R	0.2	0.1	0.1	1.1	0	0
		M	0.4	0.2	0.3	0.7	0	0.1
		S	2.2	0.8	0	0	0	0
	3rd leaf	R	0.6	0.6	0.2	0	0.1	0.1
		M	0.3	0.3	1.2	0.3	2.1	1.7
		S	5.2	3.9	0.1	0	0	0
54-04	1st leaf	R	1.5	1.1	0	0.5	0.1	0.2
		M	0.1	0	0	0	0	0
		S	0.3	0	0	0	0	0
	2nd leaf	R	1.3	4.0	0.6	0.2	0.1	0.3
		M	0.4	1.7	0	0	0	0.1
		S	4.7	0	0	0	0	0
	3rd leaf	R	1.6	4.9	1.6	0.5	0	0
		M	0.6	5.1	1.5	0.1	0	0
		S	14.5	0.3	0	0	0	0
54-18	1st leaf	R	0.4	0.9	0.4	1.4	0.6	0.1
		M	0	0	0	0	0	0
		S	0	0	0	0	0	0
	2nd leaf	R	4.7	5.0	2.2	2.7	0.5	0.3
		M	0	0	0.2	0.3	0	0
		S	0	0	1.1	1.8	0	0
	3rd leaf	R	20.9	16.0	1.3	2.1	1.2	0.3
		M	0.2	0	0.3	2.2	0.7	0
		S	0	0	11.6	3.1	0	0

Then six different species of rice plants with three leaves were inoculated with four different strains of oryaze, and the distribution of disease spots, according to the levels of leaves was studied and the results are shown in Table 5.

According to Table 5, a greater number of disease spots is seen on higher level leaves. When a large number of S type spots are seen on the leaves of the highest level only a few M and R type spots are observed; however, on the lower level leaves the opposite is true. This indicates the susceptibility to Piricularia oryzae is maximum on leaves of the highest level and is less on leaves of the lower levels.

In case of strain No 54-18, a large number of R type spots are seen on the highest leaf of rice plant of "Asahi" series (Asahi No 1 and Norin No 17) but almost none are seen on the lower leaves. This indicates that Piricularia oryzae easily affect the highest level leaf, but the process terminates soon and only brown spots remain, while they are either unable to affect the lower level leaves or it is only subclinically manifested. This explains that the higher leaves are more susceptible to the disease than the lower leaves. In view of the above facts it seems reasonable to say that the manifestation of disease spots on the highest leaves represents the resistance of each specimen against the disease. Therefore, the classification of the physiological races of Piricularia oryzae can be most reasonably done by types of disease spots manifested on the highest leveled leaves of rice plants with three to four true leaves.

3. The Condition of the Applied Spores

a. Spore concentration and manifestation of the disease.

The influence of the spore concentration upon the disease manifestation was investigated by using different concentrations of spore solutions, such as 10, 20, and 40 spores in a microscopic field of 15 x 10 magnification (50, 100, and 200 or 400 millions of spores per each cubic centimeter). Aichiasaki and Kanto No 54 plants were inoculated with Race P-No 2 and the number of susceptible type disease spots (a combination of S and M types) and the number of resistant type disease spots (R type) were investigated. The results are shown in Table 6. The P-No 2 manifests S type pathogen on Aichiasahi and M or R type pathogen on Kanto No 54. It is obvious, according to the results shown in this table, that the number of the susceptible type disease spots correlates with the concentration of the spore suspension (mostly S type spots). When the spore concentration is 0.5 millions per cubic centimeter, 3.3 disease spots are seen per specimen. Thus the determination of races is easily conducted on Aichiasahi. However on Kanto No 54 which forms M type spots, only 0.3 spors are seen when the spore concentration is four million, and 0.1 or no spots when the concentration is less than two million. Therefore in order to determine the race of Piricularia oryzae which maintains M type pathogen, it seems that a higher concentration of spores is essential. On the other hand however, if the concentration is too high, the disease type becomes altered and is not practical for experiments.

TABLE 6. THE SPORE CONCENTRATION AND NUMBER OF DISEASE SPOTS (1957)

Experimental Specimen	Experiments \ Spore Concentration/1cc*	Susceptible Type Spots 4 mil	Susceptible Type Spots 2 mil	Susceptible Type Spots 1 mil	Susceptible Type Spots 0.5 mil	Resistant Type Spots 4 mil	Resistant Type Spots 2 mil	Resistant Type Spots 1 mil	Resistant Type Spots 0.5 mil
Aichi-asahi	I	27.3	26.0	6.8	2.9	11.5	13.4	3.0	1.8
	II	23.9	13.7	6.9	6.7	1.5	0.5	0.1	0.1
	III	2.4	2.6	0.4	0.2	1.5	1.4	0.1	0
	Mean Value	17.8	14.1	4.7	3.3	4.8	5.1	1.1	0.6
Kanto No 54	I	0.4	0.2	0.1	0	0.2	0	0.1	0.2
	II	0.4	0.1	0.3	0	0.1	0.1	0	0
	III	0	0	0	0.1	0	0	0	0
	Mean Value	0.3	0.1	0.1	0.0	0.1	0.0	0.0	0.1

* Million per cubic centimeter

Eighteen different strains of oryzae that produce S type reaction were inoculated to Asahi No 1, Norin No 17 and a part or all of the Ginga; and eight strains which produce M type reaction were inoculated to Kanto No 54 and Kanto No 51, and the number of disease spots were evaluated for each experiment, in which three different spore concentrations were applied; concentrations of 1 million, 0.9 to 0.5 million, and 0.4 to 0.2 million per cubic centimeter. The results are shown in Tables 7 and 8.

As shown in Table 7, where there is formation of S type spots on Asahi No 1 and Norin No 17, approximately 6.8 and 6.6 spots are seen per individual plant if a spore concentration of 0.4 to 0.2 million per cubic centimeter was used, however, as in the case of a medium resistant Ginga, 3.5 spots were seen per individual plant. The above results seem to be good enough to determine the races. On the other hand, the number of disease spots show a rapid decrease as concentration goes down as in the case of M type spots on Kanto No 54 and No 51. For instance, if the spore concentration is 0.9 to 0.5 million the number of disease spots is 1.3 per individual plant, and if the spore concentration is 0.4 to 0.2, the number of disease spots is less than 1. Thus the evaluation of races becomes rather difficult, and a spore concentration greater than 0.9 to 0.5 million per cubic centimeter is necessary. However, in the case of strain 54-15, which produces M type on Kanto No 54 and No 51, very few M type disease spots are seen even if the spore concentration is above

TABLE 7. THE SPORE CONCENTRATION AND THE NUMBER OF SPOTS
(S TYPE SPOTS)

Rice Species / Spore Concentration per cc* / Strain Numbers	Asahi No 1			Norin No 17			Ginga		
	more than 1mil	0.9~ 0.5 mil	0.4~ 0.2 mil	more than 1mil	0.9~ 0.5 mil	0.4~ 0.2 mil	more than 1mil	0.9~ 0.5 mil	0.4~ 0.2 mil
53-11		13.6			12.1			7.2	
53-33'		26.4			17.8			9.1	
54-14	17.7			10.8			9.8		
54-15	15.0			10.3			4.2		
54-05			11.3			9.8			4.0
54-09.		10.8			7.6			2.2	
53-01.			4.9			5.1			1.8
54-08			5.6			1.7			1.2
54-10		6.9			4.0				
54-17		6.7			5.1				
54-20	15.5			10.4			7.7		
54-13	4.4			6.3			2.8		
54-19	22.4			16.9			7.5		
54-22		5.8			7.2			5.2	
54-25		8.1			7.5			5.6	
53-40.			8.4			10.7			2.9
54-07	14.6			10.6					
54-12		12.1			7.6				
Mean Value	14.8	11.3	6.8	10.9	8.6	6.6	6.4	5.9	2.5

* Same as in Table 6.

one million. This is also the case with strain No P-2. The explanation for this is that the virulence of these strains is probably weaker than that of the other strains. The high concentration is essential to some extent for the inoculation. Spore formation is sometimes very poor depending upon the type of strain, however it is necessary to use a spore suspension of a concentration greater than 0.5 to 0.9 million per cubic centimeter.

b. The age of spores and the number and pattern of disease spots.

No P-2 strain was cultured in a wheat grain medium and the amount of spore formation was determined. The spore formation takes place on the third day, reaches its maximum on the 7th to 14th day, and gradually slows down after the 18th day. Spores therefore were collected on the 4th, 7th, 14th, and 18th day and a particular concentration was used for inoculation to ten different rice species (Table 9)

TABLE 8. THE SPORE CONCENTRATION AND THE NUMBER OF SPOTS (M TYPE SPOTS)

Rice Species / Spore Concentration per cc* / Strain Numbers	Kanto No 54			Kanto No 51		
	more than 1 million	0.9~0.5 million	0.4~0.2 million	more than 1 million	0.9~0.5 million	0.4~0.2 million
54-14	5.5			7.8		
54-15	0.1			0.4		
54-05			0.2			0.1
54-09		1.1			1.2	
53-01			0.6			1.1
54-08			0.2			0.5
54-10		1.6			1.6	
54-17		1.2			1.0	
Mean Value	2.8	1.3	0.3	4.1	1.3	0.6

*Same as in Table 6.

A certain number of disease spots are seen when spores collected after 4, 7 and 14 days of culture are used, and no significant difference is noted in the number and the pattern of the disease spots. However, if spores collected after 18 days are used, fewer disease spots are produced and in the case of P No 1 and Ginga the less virulent type spots predominate. For instance, in the cases of P No 1 and Ginga, S type spots are most prevalent in areas of inoculation with spores collected 4 to 14 days after the culture, and M type spots are seen mostly with spores collected after 18 days of culture. This probably indicates that the virulence of spores decreases as the number of spores increases after longer periods of culture.

Therefore it is important to use young spores, which are freshly formed, in order to investigate the races.

c. Virulence of spores cultured in different culture media.

Pathogen P-No 2 was cultured in rice straw media (14 days culture), in barley grain media (7 days culture), and yeast agar media (7 days culture), and the spores were then inoculated upon ten different species of rice plants, in order to investigate the number and patterns of the disease spots. The results are shown in Table 10. The experiment also included spores which formed on the diseased leaves of a rice plant (Aichiasahi), and the results are also included in Table 10. The spore concentration used in the experiment was approximately one million per cubic centimeter. As shown in Table 10, there are some differences in the number of disease spots between the spores cultured in barley grain media and those in yeast agar media, however, no difference is noted in the type of disease spots. In the case of the spores taken from the diseased leaves, the disease spots appeared about a half day earlier, and they seemed to be slightly more virulent on Ginga, Homarenishiki and Norin No 22.

TABLE 9. THE AGES OF SPORES AND THEIR PATHOGENESIS

Days After Culture / No & Type of Spots / Names of Species	4th Day		7th Day		14th Day		18th Day	
	No of Spots	Type of Spots	No of Spots	Type of Spots	No of Spots	Type of Spots	No of Spots	Type of Spots
Tetep	+	M	±	M	+	R	±	M
Pi no. 1	+++	S~M	++	S~M	++ ~ +++	S~M	+	M(S)*
Kyoshiko	-	R	-	R	-	R	-	R
Kanto no. 51	-	R	-	R	-	R	-	R
Ishikarihakumo	+++	R	+++	R	+	R	+	R
Ginga	++ ~ +++	S~M	++	S	+~++	S	±~+	M(S)
Homarenishiki	+	M	+	S~M	+	M	±~+	M
Norin no. 22	+++	S	++ ~ +++	S	+++	S	+	S
Aichiasahi	++	S	++	S	++	S	+	S
Norin no. 20	++	S	++ ~ +++	S	++	S	+	S

* ( ) indicates the number of disease spots of such a type less than three on each ten species of plants.

4. Difference in Environment after Inoculation and the Numbers and Patterns of Disease Spots.

The relationship between the difference in environment after inoculation and the number and the patterns of disease spots produced was evaluated under the following conditions: in a glass room at 26°C, in a glass room at 26°C with shading (80% shading), in an unstable glass room (at 24° to 35°C), and in a stable glass room at 20°C. When an unstable glass room is used, the brownish discoloration appears sooner and the reaction is usually of the resistant type. This is probably due to rise in temperature above 30°C frequently during the day. In shaded areas the circumferential area of intoxication is marked. In a glass room at the constant temperature of 20°C, the propagation of disease spots is slow, and the propagation of disease spots is delayed for about 3 to 4 days at temperatures below 26°C. Experiments were also done outdoors in a sunny place, but evaluation of results was difficult because of poor growth of plants, which are frequently injured by the winds and often die.

Therefore the ideal conditions for these experiments would be to keep the plants either in a glass room at 26°C or outdoors where there was some sunshine but no wind and at temperatures less than 30°C.

TABLE 10. THE PATHOGENESIS OF SPORES FORMED ON DIFFERENT CULTURE MEDIA

Culture Media / No & Types of Spots / Species	Rice Straw Media		Barley Media		Yeast Agar		Spores on Disease Spots	
	No of Spots	Type of Spots	No of Spots	Type of Spots	No of Spots	Type of Spots	No of Spots	Type of Spots
Tetep		R		R		R		R
Pi no. 1	+~++	S~M	+~++	S~M	+~++	S~M	+	S~M
Kyoshiko		R(M)*		R		R		R(M)*
Kanto no. 51		R		R		R		R
Ishikarihakumo		R		R		R		R
Ginga	+~++	S~M	++	S~M	++	S	+~++	S
Homarenishiki	+~++	S~M	+~++	S~M	+~++	S~M	+~++	S
Norin no. 22	+~++	S~M	++~+++	S~M	++	S~M	++	S
Aichiasahi	++	S	++	S	+++	S	+	S
Norin no. 20	++~+++	S	++	S	+++	S	++	S

* ( ) indicates the number of disease spots of such a type less than three on each ten species of plants.

5. Evaluation of Methods of Inoculation.

Inoculation was done by spray method and by direct injection into the sheaths of the leaves, and the results were compared as to the patterns of disease spots produced. Ten different species of rice plants with different resistances and six different strains of spores with different virulences were used in this experiment and the results are shown in Table 12.

As shown in Table 12, the virulence is usually much stronger with the direct injection method than with the spray method. The above tendency was most marked with the rice species such as Sensho Kanto No 51 and 54, and less evident but still present with Modan and Pi No 1. Within the limit of the available oryzae strains, the virulence is generally increased with the direct injection method, if not equal to that of the spray method. However, in no instance, the virulence was greater with the spray method than with the injection method. This is probably due to the fact that the immature leaves protected by the sheath are less resistant to the invasion and propagation of the disease than the mature leaves.

TABLE 11. THE CONDITION OF ENVIRONMENT AND THE DISEASE SPOTS AFTER INOCULATION

Condition After Inoculation / No & Type of Spots / Species	Stable Glass Chamber at 26°C		Glass Chamber of Uncontrolled Temperature		Shading (26°C)		Stable Glass Chamber at 20°C	
	No of Spots	Type of Spots	No of Spots	Type of Spots	No of Spots	Type of Spots	No of Spots	Type of Spots
Tetep	++	M	+	M	±	M	++	M
Pi no. 1	++	S~M	+~++	S~M	++	S~M	++	S~M
Kyoshiko	-	R	-	R	-	R	-	R
Kanto no. 51	-	R	-	R	-	R	-	R
Ishikarihakumo	+~++	R	+~++	R	+	R	++	R
Ginga	+~++	S~M	++	M	+	M	+~++	S
Homarenishiki	+	S~M	++	S~M	++	S~M	+	S~M
Norin no. 22	+++	S	+++	S~M	++	S	++	S
Aichiasahi	+++	S	++	S	+++	S	+++	S
Norin no. 20	+++	S	++	S	+++	S	++	S

When the highest leaves which were barely appearing on Sensho and Modan rice plants were inoculated by the spray method, the leaves showed tiny infiltrating disease spots. This corresponds with the fact that weak disease spots are produced by direct injection into these two species. These species have fair susceptibility to the disease while the immature leaves are still covered with a sheath. However, as soon as they become exposed to the air, they become resistant to the disease. When M pathogen is produced by inoculation with the spray method, S pathogen becomes the more predominate after inoculation by direct injection. For example, the strain No 54-10 forms M pathogen on Kanto No 51 and 54 if inoculation is done by the spray method, but S pathogen is formed after inoculation by direct injection method.

The strain No 54-25 was considered to be a pure isolated strain, but the following experiments indicate that some other strain coexists to which Kanto No 51 and 54 were very susceptible.

6. Summary

In view of the results obtained from the experiments performed, the best methods for the investigation and determination of the races of Piricularia oryzae are listed below:

TABLE 12. COMPARISON OF INOCULATION METHODS

Strains of Oryzae / Inoculation Method / Species	53-33		54-10		54-25		54-01		54-04		53-39	
	Spray 1)	Injection 2)	Spray	Injection	Spray	Injection	Spray	Injection	Spray	Injection	Spray	Injection
Tetep	R	M	R	R	R	R	R	R	R	R	R	R
Pi no. 1	R	S	R	R	R	M	M	M	R	M	R	R
Modan	M	S	R	M	R	M	R	M	R	M	R	R
Sensho	M	S	R	S	M 3)	S	R	S	R	S	R	S
Kanto no. 51	S	S	M	S	R (S)	S	R	M	R	R	R	M
Kanto no. 54	S	S	M	S	R (S)	S	R	M	R	R	R	M
Ginga	S	S	M	M	S	S	M	M	R	M	S	S
Higashiyama no. 38	S	S	S	S	S	S	S	S	M	M	S	S
Norin no. 17	S	S	S	S	S	S	S	S	M	M	R	R
Asahi no. 4	S	S	S	S	S	S	S	S	S	S	R	R

Remarks: 1) Spray inoculation method
2) Leaf sheath injection inoculation method
3) ( ) indicates the number of disease spots of such a type as within less than 1~3 per each ten species

a. The rice plants used for the experiments are grown in an environment similar to that on a farm, using plant speciments of 3 to 4 of age. When many different species are grown it is advisable to use separate boxes for planting to prevent confusion. (Figure 1-A).

b) Spore concentration of 0.05 to 0.1 million per cubic centimeter was used and 50 cc of the suspension was sprayed on 200 rice plants. It is preferable to use young formed spores. No significant difference was noted by using different culture media providing a satisfactory spore formation was obtained.

c. The inoculation was done by spray method using a spray machine with a very small tip to obtain a thin and even spread.

d. After the inoculation it is desirable to keep the plants in a glass room at 25° C if possible (at least below 30°C). Too much shade and strong winds are undesirable.

TABLE 13. INVESTIGATION FOR IDENTIFYING SPECIES OF RICE PLANTS (1955)

Strains of Oryzae / Species	53-33	53-11	54-14	54-15	54-10	54-20	54-25	54-04	54-18	Species With Identical Reactions
Zenith	R	R	R	R	R	R	R	R	R	Tetep, Tadukan, Pi no. 1 Pi no. 2, Usen, Rexoro, Boshito
Hyaknichito	R(M)	R(M)	R(M)	R(M)	R(M)	R	R	R	R	Modan, Rokujunichi-ko, Kannonsen
Yakeiko	M	M	M	M	R	R	R(M)	R	R	
Kyoshiko	S	S	M	M	M	R	R	R	R	Toto, Kanto no. 51, Kanto no. 52, Kanto No. 53, Kanto No. 54, Kanto No. 55
Amareliyo	M	M	M	M	M	M	R		R	
Sensho	M	M	R	M	M	M	M	R	R	
Norin no. 34	S	S	M	M	M	M	M	R	R	Fujisaka no. 5
Ishikarihakumo	S	M	M	M	M	M	M	M	R	
Ginga	S	S	S	S	M	S	S	R	S	Norin no. 22, Norin No. 23
Ayanishiki	S	S	M	M	M	S	S	M	R	
Norin no. 17	S	S	S	S	S	S	S	M	R	Ohanazawa no.1
Aichiasahi	S	S	S	S	S	S	S	S	R	Asahi no. 1, Senbonasahi, Shinasahi, Norin no. 18
Aikoku	S	S	S	S	S	S	S	M	S	Higashiyama no. 38, Rikuu no. 132, Norin no. 1 Norin no. 6, Norin no. 8
Caloro	S	S	S	S	S	S	S	S	S	Arkrose

e. The standard in classifying the races is based on the disease spots (R, M, and S type) appearing on the highest leaves of plants.

C. The Search for a Rice Species that Would Help to Identify the Physiological Races

To identify the physiological races of the red rust disease of wheat, Mains and Jackson (1926), proposed to use eleven different species of wheat, but Johnston and Mains (1932) excluded three species from the previous eleven as they revealed an identical pattern of infection to all physiologic races. Then Chester (1946) excluded three more species, which were very prone to variable responses due to changes in conditions. Thus five species were finally selected, i.e.: Malakoff, Webster, Loros, Mediterranean, and Democrat.

In order to select the species of rice plants which will differentiate the races of oryzae, after a period of preparations in 1954, a series of experiments were conducted from 1955 to 1957. In 1955, 44 different species of rice were inoculated by nine different strains of oryzae, 27 species of rice were inoculated by two different strains of Piricularia in 1956, and 72 species of rice were used for inoculation by 26 strains of Piricularia in 1957, and all the obtained results were evaluated (Table 13 to 15).

In our experiments done in 1955, 44 species of rice plants used were categorized into seven groups, 27 species into nine groups in 1956 and 72 species into 10 groups in 1957. From the results obtained in this period of three years, 82 species of rice were thus categorized according to the patterns of disease spots as shown in Table 16. Except for eight species which revealed non-specific reactions (among these Futaba and Hokushin No 1, which seemed to have a variable resistance to the disease), the species of rice were categorized into 10 major groups and 17 subgroups.

The groups, I, II, III, and IV consist mainly of foreign species of rice; group III and IV are either Chinese species or a mixture of Chinese and Japanese species. The groups V, VI, VII, VIII, IX, and X consist mainly of Japanese species, exclusive of Rikutosensho and Amareljyo of group VI, and Caloro and Arkrose of group X. In general it can be said that the smaller the group number, the stronger the resistance against the disease. Each species that belong to a certain group, have either an identical mating, combination of parents, or at least one identical parent. For example, Pi No. 1, 2, 3, and 4 in group II have Tadukan for their parent, Kanto No. 53, 54, and 55 in group IV have Kyoshiko as their parent, and all the species in group VI have Sensho as their parent. The trait of Norin No 22 is very prevalent in group VIII, for instance; Norin No 23 and 37, and Higasiyama No 38 have the same parental combination as Norin No 22, and also Honenwase, Chokei, Shinyamabuki, Shioji, Shimotsuki and etc., have Norin No 22 as their parent. Group IX consists of either Asahi species or species resulting

TABLE 14. INVESTIGATION FOR IDENTIFYING SPECIES OF RICE PLANTS (1956)

Strains of Oryzae / Species	55-70	53-33	55-75	55-69	55-52	54-10	55-61	55-62	55-63	55-64	Kita Po-1	Kita Po-58	Kita Po-59	55-66	55-48	54-04	55-37	55-36	Kita Po-19	P-2	55-65	54-25	55-14	54-20	Ai21	Species With Identical Reaction
Zenith	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Tetep	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	M	R	R	R	R	R	Usen
Tadukan	M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	M	R	R	R	R	R	
Pi no. 1	M	R	R	R	R	R	R	R	R	R	M	M	M	R	R	R	R	R	R	S	R	R	R	R	R	Pi no. 2
Kannonsen	R	M	M	M	R	R	R	R	R	R	R	R	R	M	R	R	R	R	R	R	M	M	R	R	R	
Chokato	R	S	S	S	R	M	R	R	R	R	R	R	R	R	R	R	R	R	R	(S)	M	R	R	R(M)	R(M)	Toto
Yakeiko	R	S	S	S	M	M	M	S	S	S	R	R	R	R	R	R	R	R	R	(S)	M	R	R	R(M)	R	
Kyoshiko	R	M	M	S	M	M	M	M	M	M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Kanto no. 51	R	S	S	S	M	M	M	S	S	S	R	R	R	R	R	R	R	R	R	M, R	(S)	(S)	(S)	(S)	(S)	Kanto no. 54 Kanto no. 55
Ishikarihakumo	R	S	M	M	M	S	R	R	R	R	S	M	M	R	R	R	R	R	R	M, R	M, R	M	R	R	R	
Sensho	R	M	M	M	M	R	R	R	R	R	M	M	M	M	R	R	R	R	R	M	M, R	M	R	M	M	Amarelyo
Homarenishiki	S	S	S	S	S	M	R	R	R	R	S	S	S	S	M	M	M	R	R	S~M	M	S	M	S	M	Ayanishiki
Ginga	S	S	S	S	S	M	M	M	M	S	S	S	S	S	M	R	R	S	R	S~M	M	S	M	S	M	
Norin no. 22	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	M	M	S	R	S	S	S	S	S	S	Norin No. 37
Norin no. 17	S	S	S	S	S	S	R	R	R	R	S	S	S	S	S	M	M	R	R	S	S	S	S	S	S	Eiko
Aichiasahi	S	S	S	S	S	S	R	R	R	R	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	Asahi no. 4
Norin no. 20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	M	S	R	S	S	S	S	S	S	

TABLE 15. INVESTIGATION FOR IDENTIFYING SPECIES OF RICE PLANTS (1957)

Species \ Strains of Oryzae	55-73	P-2b	53-33	55-69	55-75	54-10	55-44	55-63	55-64	Kita Po-1	Kita Po 1-59	54-20	55-48	54-04	55-29	55-35	53-12, 53-39, 54-24, Kita 35-71, Po 9	55-46	55-65	54-25	55-66	Ai P-21	Species with Identical Reactions
Zenith	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Modan
Usen	M	M	R	R	R	R	R	R	R	R	R (M)	R	R	M	R	R	R	R	R	R (M)	R	R	Hiyake-usen
Tadukan	M	M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	Tetep(55-73 for R)
Pi no 1	S	S	R	R	R	R	R	R	R	R (M)	R (M)		R	R (S)	R	R	R	R		R	R	R (M)	Pi no.3, Pi no.4
Chokato	R	R	S	S	S	M	M	R	R	R	R	R	R	R	R	R	R	(M)	(S)	R	R	R	Toto
Hokuyu no 2	R	R	M	M	M	R	R	R	R	R	R			R	R	R	R	R		R	R	R	
Yakeiko	R	R	S S~	S S~	S S~	R~ (M)	M	M	S~ M	R	R	R	R	R	R	R	R	(M)	(S)	(S)	(S)	(S)	
Choshiko	R	R	M	M	M	M	M	M	M	R	R	R	R	R	R	R	R	(M)	(M)	(S)	(S)	R	Kanto no 53 & 54
Kanto no 51	R	R (M)	S	S	S	M	S	S	S	R	R	R	R	R	R	R	R	(S)	(S)	(S)	(S)	(S)	Imochishirazu
Ishikarihakumo	R	R	S	S	S	S	S	R	R	S	R (M)	R (M)	R	R (M)	R (M)	R (M)	R	S	S	R (M)	M	S	Norin no 34
Sensho	M	R	M	M	M	R	R	M	M	R	M	S~ M	R	R (M)	M	M	R	(M)	M	M	M	M	Amareliyo
Homarenishiki	S	M	S	S	S	M	M	R	R	S	S	S	R (M)	R (M)	R	R	R	S~ M	S	S	S	S	Ayanishiki, Shuho, Fugisaka no 5, (P-2b for R), Hosenbou, Kotobukimochi, Senbonyu
Ginga	S	M	S	S	S	M	M	S	S	S	S	S	M~R	R (M)	S	S	R	M	S	S	S	S	
Norin no.22	S	S	S	S	S	S	S	S	S	S	S	S	S	M	S	S	R	S	S	S	S	S	Norin no 37-25, Higashiyama no 38 Koganami, Honenwase, Chokai Aikoku, Norin no 8, Shinyama-buki, Shioji, Shimotsuki, Ginbozu, Oitamitsui no 120

(Table 15 continued)

(Table 15 continued)

Norin no 20	S	S	S	S	S	S	S	R	R	S	S	S	S	M	R	R	R	S	S	S	S	S	Norin no 12, Hatsukoda, Mikonishiki, Kuseshirazu, Yubae, Akebono, Kinnanpu (54-10, 55-44, 55-46 for M) Senbonasahi, Takara, Zuiho, Zikkoku, Kogyoku, Norin no 18 21
Aichiasahi	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R	R	S	S	S	S	S	
Norin no 20	S	S	S	S	S	S	S	S	S	S	S	S	S	S~M	S	S	R	S	S	S	S	S	Norin no 6, 1 & 35, Rikuce no 132 Caloro
Java no 4	R (M)	(M)	R	R	R	R	R	R	R	R	M			M	R	R	R	R		M	M	R	
Rexoro	M	M	M	M	M	R	R	R	R	R	M			R	R	R	R	R		M	R	R	
Hiyakunichito	S	M	R	R	M	R	R	R	R (M)	R	R	R	R	R	R	R (M)	R	R	M	R (M)	R (M)	R	
Cody	M	M	M	M	M	M	M	M	M	M	S~M			M	S	S	R	M		M	M	M	
Russia no 32	S	M	M	M	M	S	S	M	R	M	M			M	S	S	R	S		M	S~M	S~M	

from mating combinations with Asahi species. However, Kanto no 51 and No 52 have Bokuto as a parent, which really belongs to group III. The reason for this is not clear. It may be due to the other parent (Ginbozu-chusei). Group I includes species strongly resistant to all races of oryzae. Group X includes the susceptible species that manifest S type reaction and occasionally M type reaction to all the races of oryzae, except for those that possess no pathogenicity.

Each group is divided into subgroups a, b, c, etc., (the resistance is arranged in order as a, b, c), because the reaction of each species to the pathogen is slightly different, though the resistance of the species in one particular group seems to be almost the same. Rikutosensho of group VI is more resistant than the other species in subgroup b, such as Homarenishiki etc. For instance, the race that gives S type reaction to the species in subgroup b, will give S type or M type reaction to Sensho, and the race that gives M type reaction to the species in subgroup b, will give either M type or R type reaction to Sensho.

Among these species there are some that are unstable as far as the resistance is concerned, and the identification of the patterns of the disease spots is difficult. The species suitable for identification of the races oryzae are the following: Zenith, Tadukan, Chokato, Kyoshiko, Kanto No 51, Ishikasihakumo, Homarenishiki, Ginga, Norin No 22, Aichi-asahi, and Norin No 20. Zenith, however, has variable heredity and further investigation should be done.

D. The Experiments for Identifying the Races

Together with the experiments done to identify the species of rice, experiments for identifying the races of Piricularia were conducted over a period of four years beginning in 1954. In 1954, 17 strains of Piricularia were inoculated upon six different species of rice (Kyoshiko, Kanto No 51, Higashiyama No 38, Chuseiginbozu, Norin No 17 and Aichi-asahi). In 1955, 27 strains of Piricularia were inoculated upon six different rice species (Kanto No 51 and 54, Ginga, Higashiyama No 38, Norin No 17 and Asahi No 2). In 1956, 61 strains of Piricularia were inoculated upon ten different species of rice (Usen, Pi No 2, Kanto No 51 and 55, Ishikarihakumo, Ayanishikiginga, Norin No 22 and No 17, and Aichiasahi). In 1957, 75 strains of Piricularia were inoculated upon 12 different rice species (Tetep, Usen, Pi No 2, Yokeiko, Kyoshiko, Kanto No 51, Ishikarihakumo, Homarenishiki, Ginga, Norin No 22, Sasashigure, and Aichiasahi).

Thus identification of races was done according to the patterns of disease spots as previously mentioned and the results are shown in Tables 17 to 20.

TABLE 16. CLASSIFICATION OF RICE SPECIES ACCORDING TO THE REACTION AGAINST PIRICULARIA ORYZAE

Group of Species	Names of Rice Species
I	Zenith, Modan
II	a. Usen, Hiyakeusen, Boshito b. Tadukan, Tetep c. Pi no 1, Pi no 2, Pi no 3, Pi no 4
III	a. Hokuyu no 2 b. Chokato, Toto, Kannonsen
IV	a. Yakeiko b. Kyoshiko c. Kanto no 51, 52, 53, 54 and 55, Imochishirazu
V	Ishikarihakumo, Norin no 34
VI	a. Rikutosensho, Amareliyo b. Homarenishiki, Ayanishiki, Shuho, Fugisaka no 5, Hosenbou, Kotobukimochi, Senbouyu
VII	Ginga
VIII	Norin no 22, 23 and 37, Higashiyama no 38, Koganenami, Honenwase, Chokei, Shinyamabuki, Shioji, Shimotsuki, Aikoku, Norin no 8, Oitamitsui no 120, Ginbozuchusei
IX	a. Norin no 17 and 12, Hatsukoda, Mihonishiki, Kuseshirazu, Yubae, Akebono, Eiko, Sasashigure, Ohanazawa no 1 b. Aichiasahi, Asahi no 1 and 4, Shinasahi, Senbouasahi Norin no 18 and 21, Kogyoku, Takara, Zuiho, Zikkok, Kinnanpu
X	Norin no 20, 1, 6 and 35, Rikuu no 132, Caloro, Arkrose
Unidentified Group	Java no 4, Rexoro, Cody, Russia no 32, Hyakunichito, Rokujunichiko, Hutaba, Hokushin no 1

TABLE 17. CLASSIFICATION OF RACE (1954)

Strains of Oryzae / Species	53 - 33	53 - 01	53 - 04	53 - 39	53 - 14
Kyoshiko	S	M	R	R	R
Kanto no 51	S	M	R	R	R
Higashiyama no 38	S	S	S	S	R
Chuseiginbozu	S	S	S	S	R
Norin no 17	S	S	S	R	R
Aichiasahi	S	S	S	R	R
Strains that Manifest the Identical Pathogenicity	53-11 53-12	53-03 53-13 53-16	53-08 53-09 53-17 53-18 53-20 53-22 53-40		

TABLE 18. CLASSIFICATION OF RACE (1955)

Strains of Oryzae / Species	53-33	54-14	54-10	54-20	54-01	54-04	53-39	53-12
Kanto no 51	S	M	M	R	R	R	R	R
Kanto no 54	S	M	M	R	R	R	R	R
Ginga	S	S	M	S	M	R	S	R
Higashiyama no 38	S	S	S	S	S	M	S	R
Norin no 17	S	S	S	S	S	M	R	R
Asahi no 1	S	S	S	S	S	S	R	R
Strains that Manifest the Identical Pathogenicity	53-11	P-2 54-05 54-15	53-01 54-08 54-09 54-17	53-40 54-13 54-19 54-22 54-25	54-07 54-12		54-02 54-18 54-24	53-14

TABLE 19. CLASSIFICATION OF RACE (1956)

Species \ Strains of Oryzae	55-73	53-33	55-07	54-10	55-12	55-47	55-61	55-68	Kita Po-1	55-46	54-01	54-20	55-48	54-04	55-36	53-12	55-65	54-26	Ai 21
Usen	M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pi no 2	M	R	M	R	R	M	R	R	M	R	R	R	R	R	R	R	R	R	R
Kanto no 51	R	S	M	M	M	M	S	R	R	R	R	R	R	R	R	R	(S)	(S)	R
Kanto no 55	R	S	M	M	M	M	S	R	R	R	R	R	R	R	R	R	(S)	(S)	(S)
Ishikarichakumo	R	S	S	S	S	S	R	S	S	S	S	R	R	R	R	R	S	R (M)	R (M)
Ayanishiki	S~M	S	S	M	M	M	R	S	S	M	M	S	M	M	R	R	S	S	M
Ginga	M	S	S	M	M	M	S	S	S	M	M	S	M	M, R	S	R	S	S	M
Norin no 22	S	S	S	S	M	M	S	S	S	S	S	S	S	M	S	R	S	S	S
Norin no 17	S	S	S	S	S	S	R	S	S	S	S	S	S	S~M	R	R	S	S	S
Aichiasahi	S	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	S	S	S
Strains that manifest identical pathogenicity	55-70	55-60, 55-67, 55-69, 55-74, 55-75		53-01, 55-16, 55-19, 55-22, 55-24, 55-39, 55-41, 55-42, 55-44, 55-46			55-62, 55-66, 55-64		Kita Po-58		[illegible], 55-14, 55-40, 55-54, 55-59, 55-71	53-40, 55-05, 55-25, 55-32, 55-52, 55-63, Kita Po-58		55-26, 55-27, 55-35, 55-37	53-39, 54-18, 55-15, 55-20	Kita Po-19			

TABLE 20. CLASSIFICATION

Strains of Oryzae / Species	55 - 73	P - 2b	53 - 33	57 - 04	54 - 10
Tetep	R	M	R	R	R
Aisen	M	M	R	R	R
Pi no 1	S	S	R	R	R
Yakeiko	R	R	S	M	M
Kyoshiko	R	R	S~M	M	M
Kanto no 51	R	R	S	M	M
Ishikarihakumo	R	R	S	S	S
Homarenishiki	S	M	S	S	M
Ginga	S	M	S	S	M
Norin no 22	S	S	S	S	S
Sasashigure	S	S	S	S	S
Aichiasahi	S	S	S	S	S
Strains that manifest the identical pathogenicity			55-69 55-75	57-14	55-44 57-36

OF RACE (1957)

55 - 63	57 - 06	54 - 20	55 - 48	54 - 04	55 - 29	53 - 12	57 - 17
R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R
S	R	R	R	R	R	R	R
S~M	R	R	R	R	R	R	R(M)
S	R	R	R	R	R	R	R(M)
R	S	R	R	R	R	R	S
R	S	S	M	M	R	R	M
S	S	S	M	M, R	S	R	M
S	S	S	S	M	S	R	S
R	S	S	S	S	R	R	S
R	S	S	S	S	R	R	S
55-64 57-77 57-78 57-79 57-80 57-81 57-85	57-05 57-07 57-10 57-11 57-15 57-16 57-18 57-76 57-82 57-87 57-94 57-102 Kita Po-1 Cho 310	57-03 57-37 57-53 57-56 57-59 57-61 57-63 57-83 57-96 57-98 57-114 57-115 57-120 Kita Po-59	57-132 57-140 57-141 57-142	57-55 57-84 57-92 57-95 Ai 49	55-35 57-54 57-57 57-60	53-39 54-24 55-71 57-13 Kita Po-19	57-131 57-134 57-135 57-136

In experiments done in 1954, six species of rice plants from group IV (Kyoshiko and Kanto No 51), group VIII (Higashiyama No 38 and Ginbozuchusei) and group IX (Norin No 17 and Aichiasahi) were applied to identify the races of oryzae, and 17 different strains of oryzae were categorized into five races. In 1955, 27 different strains of oryzae were categorized into eight races by the same method using six species of rice plants from the following groups: group IV (Kanto No 51 and No 54) group VII (Ginga), group VIII (Higashiyama No 38), and group IX (Norin No 17 and Asahi No 1). In 1956, 61 different strains of oryzae were again categorized into 14 races using ten species of rice plants: from group II (Usen and Pi No 2), from group IV (Kanto No 51 and 55), from group V (Ishikarihakumo), from group VI (Ayanishiki), from group VII (Ginga), from group VIII (Norin No 22), and from group IX (Norin No 17 and Aichiasahi). Besides these 14 races of oryzae, there seemed to be a type (X type), which consisted of more than two different races of oryzae.

In 1957 12 more races of oryzae were identified by an experiment similar to that of 1956, except for a few changes in the rice plants used i.e., Tetep added to group II, Yakeiko and Kyoshiko instead of Kanto No 55 to group IV and Sasashigure instead of Norin No 17 of group IX. In this experiment a few races were also found to be present which were called X type.

The classification of races of oryzae is based upon the results obtained from the above experiments using 11 different species of rice plants, as shown in Table 21. It required four years to perform these experiments and this knowledge about the experimental species of rice plants is shown in Tables 13 to 15.

Actually the races of oryzae were classified into three major groups: I, C, and N, according to the decision made at a conference in 1959, although a detailed classification reveals 14 different races. Group I is pathogenic to Tadukan and shows S type reaction on Pi no 1-4, which are mixed species of Tadukan and Japanese species.

Group C is not pathogenic to Tadukan, but is pathogenic to Chokato, Kyoshiko etc., which are Chinese species. Group C seems to consist of five different races of oryzae: one that is strongly pathogenic to Chokato etc., one that is not pathogenic to Chokato and Japanese species Ishikarihakumo, Homarenishiki and Aichiasahi, but is strongly pathogenic to Kyoshiko and Kanto No 51, and three other species which are moderately pathogenic (M) to Chinese species and give three different reactions upon Japanese species.

Group N consists of two different species that are either pathogenic or non-pathogenic to Ishikarihakumo. The pathogenic group is further classified into three different races depending upon characteristic manifestations they produce upon other Japanese rice species. The nonpathogenic group consists of four different races. Thus group N includes seven different races. There is one that shows no pathogenicity against any of these species and is called 0 race.

Thus, group I consists of one race, group C consists of five races, and group N consists of seven races, and thus a total of 14 different races was recognized, including the 0 race.

A few strains of oryzae were also recognized which manifested a reaction similar to that of type X in case of the rust disease. However, there might be a co-existance of races, which were strongly pathogenic to the Chinese species, although the experiments were performed using isolated strains cultured from a single spore. The author et. al. actually encountered a virulent race, which strongly affected Chinese species sorting out of a strain (55-65) in another experiment.

Some strains such as 53-12, 55-71, 53-39, 54-18, and 54-24 lose their virulence after the mono-spore isolation culture in potato-agar media and the toxicity of spores becomes altered from that of strains immediately after the isolation. Some oryzae strains maintain satisfactory spore formation immediately after the isolation, but it markedly decreases after six months to one year in agar culture media. Therefore, in order to identify the races of Piricularia oryzae, the experiments should take place immediately after the isolation of strains.

E. Summary

1. When rice plants with three to four true leaves were used, the differentiation of the types of disease spots was much easier and the number of spots was larger than in cases of rice plants with only one to two true leaves. Moreover, the distribution of disease spots on different levels of leaves was examined and the largest number of disease spots was found to be present on the highest leveled leaf, regardless of the types of spots.

2. Three characteristic types of disease spots (S, M, and R) were found to be present on the highest leveled leaves, and these were applied as standard types in the classification of races of oryzae.

3. The ideal spore concentration was thought to be 0.5 to 1 million spores per cubic centimeter and 50 cc of the suspension was used to spray two hundred rice plants. The sooner the spores were applied from the culture, the larger the number of disease spots produced. There was no significant difference between the spores cultured in different culture media.

4. The injection inoculation into the sheath of leaves revealed a stronger virulence as compared with the spray inoculation method, and this tendency is more marked with some rice species.

TABLE 21. CLASSIFICATION

Group of Race / Number of Race / Species	Group T	Group C					
Zenith	R	R	R	R	R	R	R
Tadukan	M	R	R	R	R	R	R
Chokato	R	S	M	M	R	M	R
Kyoshiko	R	S~M	M	M	S~M	M	R
Kanto no 51	R	S	M	M	S	M	R
Ishikarihakumo	R	S	S	S	R	S	S
Homarenishiki	S~M	S	S	M	R	M	S
Ginga	S~M	S	S	M	S	M	S
Norin no 22	S	S	S	S	S	M	S
Aichiasahi	S	S	S	S	R	S	S
Norin no 20	S	S	S	S	S	S	S
	p-2b 55-70 55-73	53-11 (53-12) 53-33 55-60 55-67 55-69 55-74 55-75	55-07 57-04 57-14 54-05 54-15	53-01 54-08 54-09 54-10 54-17 55-16 55-19 55-22 55-24 55-39 55-41 55-42 55-44 55-46 57-36	55-61 55-62 55-63 55-64 57-77 57-78 57-79 57-80 57-81 57-85	55-12 55-47	55-68 57-05 57-06 57-07 57-10 57-11 57-15 57-16 57-18 57-76 57-82 57-87 57-94 57-102 Cho 310 Kita Po-1 Kita Po-58

Remarks: () indicates, with the strain number inside, the complete loss of

OF RACE (1954~1957)

Group N						Group O	X Type		
R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	(S)	(S)	
R	R	R	R	R	R	R	(S)	(S)	(M)
R	R	R	R	R	R	R	(S)	(S)	(M)
S	M	R	R	R	R	R	S	R(M)	S
M	M	S	M	M	R	R	S	S	M
M	M	S	M	M, R	S	R	S	S	M
S	S	S	S	M	S	R	S	S	S
S	S	S	S	S	R	R	S	S	S
S	S	S	S	S	S	R	S	S	S
55-46	54-01 55-03 55-14 55-49 55-54 55-56 (55-71)	54-20 53-40 55-06 55-25 55-32 55-52 55-66 57-03 57-37 57-53 57-56 57-59 57-61 57-63 57-83 57-96 57-98 57-114 57-115 57-120 Kita Po-59	55-4r 57-132 57-140 57-141 57-142	54-04 55-26 55-27 55-36 55-37 57-55 57-84 57-92 57-95 Ai 49	(53-39) 54-02 (54-18) (54-24) 55-15 55-29 55-35 55-54 57-57 57-60	53-14 57-13 Kita Po-19	55-65	54-25	57-17 57-131 57-134 57-135 57-136

pathogenicity of a strain during culture.

5. The optimal temperature was thought to be $26^{\circ}C$ in a stable glass chamber or lower than $30^{\circ}C$ in a glass room.

6. Eighty-six species of rice plants were categorized into ten major groups according to the reaction against oryzae; and 11 species of rice were used for identification of races of oryzae: Zenith, Tadukan, Chokato, Kyoshiko, Kanto No 51, Ishikarihakumo, Homarenishiki, Ginga, Norin No 22, Aichiasahi, and Norin No 20.

7. The experimental strains of oryzae were categorized into three major groups of races according to their disease manifestation upon each species of rice plants and then further categorized into 14 races. A few strains of oryzae that showed X type reaction were also observed.

- END -