Reports on the Physiological Specialization of Fusarium. I.

On the Differenciation of the Pathogenecity among the Strains of Rice-" Bakanae "-Fungus.[1)]

By

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Contents.

I. Introduction.

Investigations on the physiological specialization of fungi, which are pathogenic to the cultivated plants has become one of the common subject in the field of plant pathological studies. Numerous papers have been published on the specialization of species of the genus Fusarium. However there are comparatively few reports as to the Fusarium species infecting the fruits and fruit-trees as well as tropical Gramineae. Consequently the senior writer (Nisikado 1931a) worked on this problem of the fruit attacking species of Fusarium, during his stay in Germany under the direction of Dr. H. W. Wollenweber.

Studies on the physiological specialization of *Lisea Fujikuroi* Saw., the rice-" Bakanae "-fungus are also few, although it causes one of the most serious diseases of rice plant in Japan, so that the writers undertook the investigation on the physiological specialization of this fungus.

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1) Reproduced from the writers' article in Japanese published in the " Nôgaku Kenkyû ". Vol. 20, pp. 320—345, April 25, 1933.

As the first step of the studies the writers compared the pathogenecity of the various strains of the fungus isolated from the "Bakanae"-diseased rice plants. For this purpose it seems reasonable to experiment with rice plant. However according to the previous experiments (NISIKADO 1932, NISIKADO and MATSUMOTO 1932), it was found that the pathogenecity of this fungus is manifested very distinctly on the corn seedlings and the strains showing strong overgrowth of corn seedlings are also strong to produce the overgrowth of rice seedlings. Consequently the present writers compared experimentally the pathogenecity of the various strains of the "Bakanae"-fungus to the corn seedlings.

By the attack of this fungus, the growth of rice and corn seedlings is not only abnormally promoted but also sometimes much retarded, as shown by KUROSAWA (1930), SETO (1928, 1932) and ITO and SHIMADA (1931). Moreover the pathogenecity of a fungus varies during its culture, even within the same strains. Therefore the comparison of pathogenecity tested by the abnormal overgrowth of the seedlings, offers a room for discussions. When a difference in the abnormal overgrowth among seedlings is observed, it may be assumed that it is caused by the difference in the virulency of the strains tested. For these reasons, the writers have adopted the degree of the abnormal overgrowth as the category for the comparative test of pathogenecity.

II. Source of the Cultures Studied.

A majority of the strains of the rice-"Bakanae"-fungus studied were secured through the courtesy of Prof. R. TAKAHASHI, Miye Imperial College of Agriculture and Forestry. Some of the cultures were sent by Mr. K. KUWAZUKA, the plant pathologist of the Aiti Prefectural Experiment Station. For the sake of comparison, some closely related species of Fusarium which were kindly given by Dr. H. W. WOLLENWEBER, Oberregierungsrat, Biologische Reichsanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, were also studied. The writers wish to express their hearty thanks to the above named gentlemen for their kind supply of the fungus-cultures.

The description of the strains studied are given below:

Strain No. 414, *Lisea Fujikuroi* SAW., isolated from a "Bakanae"-diseased rice plant in Formosa; No. 415 the same isolated in Kyôto; No. 483—487, isolated from a "Bakanae"-diseased rice plant collected in Aiti Prefecture; No. 488, isolated from an ascospore produced on a diseased rice culm, which was in the previous season heavily affected by the "Bakanae"-disease in the same prefecture; No. 602, isolated from a conidium collected in the same locality. (In the following only the name of the prefecture, whence the strain was secured, is briefly given.) No. 603, secured from Tiba; No. 604 from Sizuoka; No. 605 from Kanagawa; No. 606 from Totigi; Nos. 607—608 from Isikawa; Nos. 609—610 from Hukusima; Nos. 611—616 from Miye; Nos. 617—619 from ascospores collected in Miye ; No. 620 from Nagano ; Nos. 621—622 from Nagasaki ; No. 623 from Kumamoto ; No. 624 from Ôita ; No. 625 from Ehime ; Nos. 626—627 from Kôti ; No. 628 from Kagawa ; No. 629 from Yamaguti ; Nos. 630—631 from Hyôgo ; Nos. 623—633 from Tottori ; No. 634 from Simane ; Nos. 635—636 from Wakayama ; No. 637 from Nara ; No. 638 from Siga ; Nos. 639—646 from Miye ; Nos. 647—650 from ascospores collected in Miye ; Nos. 651—653 from Formosa ; Nos. 654—658 from Miye and No. 667 from Okayama.

Strain No. 410 is *Fusarium moniliforme* SH. var. *majus* WR. et RG., the causal fungus of the "Pokkah boeng"-disease of sugar cane, isolated in Java. No. 411, another strain of the same fungus isolated in Mexico. No. 412 is *Fusarium moniliforme* SH. isolated from diseased corn in Illinois and No. 413, another strain of the same fungus isolated in Minnesota, U. S. A. No. 589 is *Fusarium moniliforme* SH. isolated from diseased cotton plant in Turkey.

III. Methods and Results of Experiments.

a) Experiment I.

The above mentioned strains of the "Bakanae"-fungus as well as its related species were grown on boiled stems of Hubum-clover (*Melilotus alba* DESR.). After the mycelial mats and conidia well developed, the cultures were used for inoculation experiments on the corn seeds. Each set of 33 grains of the corn seeds in a Petri dish was treated with a mixture of equal volume of 50 per cent alcohol and 0.1 per cent solution of corrosive sublimate for two or three minutes and washed in sterilized water. After the surface of seeds was disinfected, the seeds were put in a sterilized Petri dish, to which a piece of the above said cultures on boiled stems of Hubum-clover was then added. Then the Petri dish was shaken for two or three minutes to attach the conidia to the surface of the corn seeds to be tested.

Galvanized-iron pots, 20 cm. high and 16 cm. in diameter, and containing 5 kg. of fine sand in each, were sterilized under a pressure of 20 pounds for two hours. Then 0.1 per cent KNOP's solution was added. Care was taken to keep the moisture content of sand in the pots almost similar. Two of these pots thus prepared were used for each of the strains studied.

On September 9, 1932, the corn seeds were inoculated and on the next day, 16 inoculated seeds were sown in a pot and 17 in another pot of the set. After sowing, the pots were kept in the nethouse in the daytime and in the glasshouse at night. When the corn seedlings in the control grew and attained to the length of about 15 cm., the seedlings were all pulled out from the pots for length-measurement. The length from the lowest node at the base of a culm to the top of the longest leaf was measured. The unit used was one cm., the fractions over one half (inclusive) being counted as one and the rests disregarded. The average length of the seedlings thus measured are given in the fourth column of Table I.

Table I.

Results of the Inoculation-Experiments of Corn-Seedlings with Various Strains of *Lisea Fujikuroi* Sawada, the Rice-" Bakanae "-Fungus. (I.)

The corn seeds were inoculated on September 9, 1932 and sown on the next day. The results were examined on September 26, 1932.

Nos. of strains studied	Classes in length of the seedlings tested (cm.)												Mean length of the seedlings tested	Mean length of 10 overgrown seedlings	Percentage of germination	Overgrowth index
	1 – 5	6 – 10	11 – 5	16 – 20	21 – 5	26 – 30	31 – 5	36 – 40	41 – 5	41 – 50	51 – 5	Total	cm.	cm.	%	
No. 410	—	2	4	10	11	1	—	—	—	—	—	28	18.67	23.2	84.9	105.0
411	—	3	6	8	5	—	—	—	—	—	—	22	16.86	21.4	66.7	96.8
412	—	3	6	7	8	1	—	—	—	—	—	25	17.25	23.0	75.8	104.1
413	—	3	7	11	7	—	—	—	—	—	—	28	17.11	21.4	84.9	95.8
414	—	—	3	6	11	7	2	—	—	—	—	29	21.90	26.9	87.9	121.8
415	—	—	4	6	10	6	1	—	—	—	—	27	21.67	27.4	81.8	124.0
483	1	1	6	10	9	—	—	—	—	—	—	27	17.56	21.2	81.8	95.9
484	—	1	1	3	6	5	10	5	—	—	—	31	28.06	34.2	94.0	154.8
485	1	2	2	6	9	6	—	—	—	—	—	26	20.50	26.2	78.8	118.6
486	—	—	3	2	9	8	1	—	—	—	—	23	23.96	28.6	69.7	129.4
487	—	—	2	2	5	9	2	5	—	—	—	25	27.44	34.3	75.8	155.3
488	1	4	2	1	8	5	4	—	—	—	—	25	21.20	29.4	75.8	133.1
602	—	—	4	5	14	3	—	—	—	—	—	26	21.12	25.1	78.8	113.6
603	—	1	7	6	9	6	—	—	—	—	—	29	19.82	25.0	87.9	113.2
604	—	—	1	5	8	10	4	—	—	—	—	28	24.44	29.6	81.8	134.0
605	—	—	—	1	17	8	2	—	—	—	—	28	24.96	27.8	84.9	125.8
606	—	—	2	3	12	8	2	1	—	—	—	28	27.35	28.7	84.9	125.4
607	—	—	—	5	9	11	3	1	—	—	—	29	26.13	31.5	87.9	142.6
608	—	—	—	1	6	9	8	3	1	—	—	28	29.50	35.7	84.9	161.6
609	—	—	2	10	10	4	—	—	—	—	—	26	20.50	25.2	78.8	118.6
610	—	3	1	3	7	2	—	—	—	—	—	16	19.37	23.4	48.5	105.9
611	—	—	1	2	9	8	4	1	—	—	—	25	25.84	30.7	75.8	139.4
612	—	1	4	4	11	2	—	—	—	—	—	22	20.09	24.3	66.6	110.0
613	—	—	1	1	19	8	1	—	—	—	—	30	23.73	27.2	90.9	123.2
614	—	1	4	6	4	3	—	—	—	—	—	18	18.72	23.7	54.6	107.3
615	—	—	1	0	9	14	4	0	1	—	—	29	27.38	32.0	87.9	144.8
616	—	—	1	4	8	7	3	—	—	—	—	23	24.70	29.7	69.7	134.4
617	—	1	2	6	15	2	—	—	—	—	—	26	20.81	24.7	78.8	111.8
618	—	—	3	11	9	1	—	—	—	—	—	24	19.75	23.2	72.7	105.0
619	—	1	6	7	8	3	—	—	—	—	—	25	19.28	24.0	75.8	108.7
620	—	1	0	2	9	11	1	—	—	—	—	24	24.83	28.2	72.7	127.6
621	—	—	—	1	9	12	6	1	—	—	—	29	27.59	31.1	87.9	140.8
622	1	2	2	6	10	—	—	—	—	—	—	21	18.29	23.1	63.6	104.6
623	—	—	2	3	10	14	3	—	—	—	—	32	24.13	29.9	97.0	135.4

Table I (Continued).

Nos. of strains studied	Classes in length of the seedlings tested (cm.)												Mean length of the seedlings tested	Mean length of 10 overgrown seedlings	Percentage of germination	Overgrowth index
	1–5	6–10	11–5	16–20	21–5	26–30	31–5	36–40	41–5	46–50	51–5	Total	cm.	cm.	%	
No. 624	—	—	—	3	0	6	6	5	4	4	—	28	33.79	41.7	84.9	188.9
625	—	1	0	1	4	11	7	6	2	—	—	32	30.13	37.4	97.0	169.2
626	—	—	—	3	15	7	3	—	—	—	—	28	24.41	29.6	84.9	134.1
627	—	—	—	1	4	10	7	3	1	—	—	26	30.23	35.6	78.8	161.2
628	—	—	—	—	6	11	8	2	3	—	—	30	30.77	37.0	90.9	167.5
629	—	—	5	11	5	2	—	—	—	—	—	23	18.39	21.8	69.7	98.7
630	—	—	—	1	8	10	7	1	1	—	—	28	27.46	35.0	84.9	158.6
631	—	—	1	2	9	7	6	3	2	2	—	32	29.81	39.3	97.0	177.9
632	—	—	—	4	7	13	2	—	—	—	—	26	25.00	29.1	78.8	131.7
633	—	—	—	6	8	15	1	1	—	—	—	31	25.23	30.5	94.0	138.2
634	—	—	—	1	5	6	6	4	—	—	—	22	29.73	34.5	66.7	156.1
635	—	1	1.	9	15	3	—	—	—	—	—	29	21.28	25.2	87.9	114.1
636	—	—	1	5	7	10	1	—	—	—	—	24	24.00	28.0	72.7	126.7
637	—	—	9	8	8	—	—	—	—	—	—	25	18.56	22.4	75.8	101.4
638	—	1	0	0	6	11	6	7	—	—	—	31	29.94	36.7	94.0	166.2
639	—	—	4	12	10	2	—	—	—	—	—	28	20.11	23.2	84.9	104.9
640	—	—	—	8	16	2	—	—	—	—	—	26	21.73	24.2	78.8	109.6
641	—	1	1	8	16	5	—	—	—	—	—	31	21.61	26.3	94.0	119.1
642	—	—	3	1	10	9	5	0	2	—	—	30	26.80	33.8	90 9	153.0
643	—	—	—	11	11	4	2	—	—	—	—	28	22.46	27.1	84.9	122.7
644	—	1	1	4	11	8	1	—	—	—	—	26	23.23	27.5	78.8	124.4
645	—	—	—	1	7	11	5	1	—	—	—	25	27.20	31.2	75.8	141.2
646	—	—	2	4	9	6	7	—	—	—	—	28	25.00	31.8	84.9	143.9
647	—	—	2	5	9	9	3	—	—	—	—	28	23.82	28.9	84.9	130.8
648	—	—	1	6	11	6	1	—	—	—	—	25	22.84	27.9	75.8	126.3
649	—	1	4	14	6	2	—	—	—	—	—	27	18.56	22.8	81.8	103.2
650	—	1	1	6	4	1	—	—	—	—	—	13	18.62	22.0	39.4	99.5
651	—	—	3	11	9	1	—	—	—	—	—	24	19.66	23.1	72.7	104.6
652	—	2	1	4	15	3	—	—	—	—	—	25	21.60	25.4	75.8	114.9
653	—	1	1	13	9	4	—	—	—	—	—	28	20.21	25.0	84.9	113.2
654	—	—	—	—	3	17	6	2	3	—	—	31	30.03	34.9	94.0	158.0
655	—	—	1	4	10	10	2	1	—	—	—	28	25.43	30.7	84.9	139.0
656	—	—	3	5	5	6	2	2	—	—	—	23	24.61	31.3	69.7	141.7
657	—	—	—	—	6	5	8	5	2	—	—	26	31.42	37.9	78.8	171.6
658	—	1	4	11	5	5	—	—	—	—	—	26	20.00	25.7	78.8	116.3
667	—	1	0	4	7	11	6	1	—	—	—	30	26.00	32.1	90.9	135.3
Control	1	5	8	19	8	1	—	—	—	—	—	42	16.64	22.1	84.0	100.0

According to the above table, there are pretty big differences in the length among the seedlings infected with various strains of the rice-"Bakanae"-fungus. It is easily perceived that the various strains are different in the power to produce the abnormal overgrowth of the corn seedlings. But it is very rare that all the seedlings in a pot show overgrowth even in the case of a strain provided with strong pathogenecity, so that the mean length of all the seedlings in a pot seems hardly to represent the real degree of the seedlings-overgrowth for the strain studied. To understand this relation more clearly, the measurements were grouped into classes of 1—5, 6—10, 11—15 cm. etc. and the frequency of these groups was also given. The result is shown in the second column of Table I. All the seedlings of the control belong to the class of 21—25 cm. or less, with one exception, which lies in the class of 26—30 cm.; while a majority of the seedlings of the following twenty-five strains belongs to the classes of 26—30 cm. or more : Strain Nos. 484 ; 487 ; 604 ; 607 ; 608 ; 611 ; 615 ; 620 ; 621 ; 623 ; 624 ; 625 ; 627 ; 628 ; 630 ; 631 ; 632 ; 633 ; 634 ; 638 ; 642 ; 645 ; 654 and No. 657. It is clear that these strains are able to cause the overgrowth of the corn seedlings. Both the strains No. 410 and No. 411 are *Fusarium moniliforme* SH. var. *majus* WR. et RG., the causal fungus of the "Pokkah boeng"-disease of sugar cane. These strains produced no overgrowth in this experiment, although the latter strain had caused a slight overgrowth on corn seedlings in the senior writer's experiment in Berlin-Dahlem. The strain Nos. 412 and 413, belonging to *Fusarium moniliforme* SH., caused no overgrowth of corn seedlings.

To compare the degree of the abnormal overgrowth of the corn seedlings more clearly, mean length of ten overgrown long seedlings (30 per cent of the total sown seeds) out of thirty-three seedlings was determined. The result is given in the fifth column of Table I. Then the ratios of the mean length of each of the inoculated seedlings to that of the control were also calculated and multiplied by 100. It is provisionally called "Overgrowth index", and is given in the last column of the same table. The examination of the table shows that the overgrowth index of fourty-two strains lies above 120, moreover that of twenty-three strains among them above 150. These strains may be concluded to have the power to produce the overgrowth of the corn seedlings.

b) Experiment II.

The cultures of above said seventy-one strains of the "Bakanae"-fungus and its related species on boiled rind of water melon[1] were used for inoculation of the corn seeds, as described previously. The inoculated corn seeds were sown in pots of galvanized iron, and the seedlings were grown under the same condition as in Experiment I. After 16 days cultivation, the seedlings were pulled out and the length was measured. As shown in Table II, the results were almost similar to those secured in the first experiment.

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1) The medium was prepared in the following manner: A piece of rind of water melon, about 30 mm. long, 7 mm. thick and 7 mm. wide, was inserted into a test tube with addition of small quantity of water, and sterilized in an autoclave at 15 pounds pressure for 30 minutes.

Table II.

Results of the Inoculation-Experiments of Corn-Seedlings with Various Strains of *Lisea Fujikuroi* Sawada, the Rice-"Bakanae"-Fungus. (II.)

The corn seeds were inoculated and sown on September 24, 1932.
Tne results were examined on October 10, 1932.

Nos. of strains studied	Classes in length of the seedlings tested (cm.)												Mean length of the seedlings tested	Mean length of 10 overgrown seedlings	Percentage of germination	Overgrowth index
	1–5	6–10	11–5	16–20	21–5	26–30	31–5	36–40	41–5	46–50	51–5	Total				
													cm.	cm.	%	
No. 410	—	1	28	1	—	—	—	—	—	—	—	30	12.72	14.1	93.8	90.4
411	—	6	19	1	—	—	—	—	—	—	—	26	11.96	13.4	81.3	85.9
412	1	3	18	3	—	—	—	—	—	—	—	25	12.84	14.7	78.2	94.2
413	—	3	24	2	—	—	—	—	—	—	—	29	12.69	14.5	90.6	92.9
414	—	1	2	1	10	7	3	—	—	—	—	24	24.17	31.3	75.0	200.6
415	—	4	23	1	—	—	—	—	—	—	—	28	12.29	14.1	78 5	90.4
483	1	4	20	1	—	—	—	—	—	—	—	26	12.19	14.4	81.3	92.3
484	—	3	11	10	2	2	—	—	—	—	—	28	16.04	21.4	87.5	137.2
485	—	3	17	8	—	—	—	—	—	—	—	28	14.36	17.6	87.5	112.8
486	—	1	12	12	2	—	—	—	—	—	—	27	15.62	18.0	84.4	115.5
487	—	1	3	11	6	7	1	—	—	—	—	29	21.83	30.4	90.6	194.9
488	—	7	24	—	—	—	—	—	—	—	—	31	11.65	13.3	96.9	80.2
589	—	1	29	—	—	—	—	—	—	—	—	30	12.83	14.2	93.8	91.0
602	—	5	24	—	—	—	—	—	—	—	—	29	21.17	13.9	90.6	89.1
603	—	2	27	2	—	—	—	—	—	—	—	31	12.74	14.7	96.9	94.2
604	—	—	1	0	5	14	5	—	—	—	—	25	27.00	31.1	78.2	199.4
605	—	—	7	15	4	—	—	—	—	—	—	26	17.46	20.5	81.3	131.4
606	—	—	2	16	11	1	—	—	—	—	—	30	20.03	23.0	93.8	147.4
607	—	—	8	15	2	1	—	—	—	—	—	26	17.46	20.5	81.3	131.4
608	—	—	2	8	15	6	—	—	—	—	—	31	22.06	23.9	96.9	153.2
609	—	—	—	1	25	4	—	—	—	—	—	30	23.10	25.5	93.8	99.4
610	—	1	0	1	12	11	5	—	—	—	—	30	26.33	30.9	93.8	198.1
611	—	1	7	16	1	—	—	—	—	—	—	25	16.16	18.2	78.2	116.7
612	1	2	24	1	—	—	—	—	—	—	—	28	12.14	14.1	87.5	90.4
613	3	4	11	11	—	—	—	—	—	—	—	29	13.24	16.9	90.6	108.3
614	—	3	25	—	—	—	—	—	—	—	—	28	12.14	13.8	87.5	88.4
615	—	1	9	12	5	1	—	—	—	—	—	28	17.00	20.7	87.5	132.7
616	—	—	4	14	4	0	1	—	—	—	—	23	18.43	21.7	71.9	139.1
617	—	2	20	2	—	—	—	—	—	—	—	24	13.33	14.6	75.0	93.5
618	1	2	17	7	—	—	—	—	—	—	—	27	12.85	16.0	84.4	102.6
619	2	5	19	2	—	—	—	—	—	—	—	28	12.25	14.9	87.5	95.5
620	—	1	3	8	9	2	1	—	—	—	—	24	20.17	26.1	75.0	167.4
621	—	1	1	15	9	4	—	—	—	—	—	30	20.33	25.6	93.8	164.1
622	—	3	17	8	—	—	—	—	—	—	—	28	14.04	16.3	87.5	104.5
623	1	0	2	6	11	11	—	—	—	—	—	31	22.74	28.3	96.9	181.4
624	—	—	2	8	7	10	5	—	—	—	—	32	24.13	30.3	100.0	194.3

Table II (Continued).

Nos. of strains studied	Classes in length of the seedlings tested (cm.)												Mean length of the seedlings tested	Mean length of 10 overgrown seedlings	Percentage of germination	Overgrowth index
	1–5	6–10	11–5	16–20	21–5	26–30	31–5	36–40	41–5	46–50	51–5	Total	cm.	cm.	%	
No. 625	1	1	19	9	—	—	—	—	—	—	—	30	14.40	16.6	93.8	106.4
626	—	2	18	8	—	—	—	—	—	—	—	28	15.14	17.1	87.5	109.6
627	—	—	6	13	5	1	—	—	—	—	—	25	17.76	21.8	78.2	139.8
628	—	3	22	4	—	—	—	—	—	—	—	29	13.52	15.5	90.6	99.4
629	—	1	21	9	—	—	—	—	—	—	—	31	14.23	16.1	96.9	103.2
630	—	2	5	18	4	1	—	—	—	—	—	30	17.93	21.3	93.8	136.6
631	—	1	24	4	—	—	—	—	—	—	—	29	14.10	15.8	90.6	101.3
632	—	—	6	18	2	1	—	—	—	—	—	27	17.33	20.4	84.4	130.8
633	—	—	1	16	6	8	—	—	—	—	—	31	21.71	26.5	96.9	169.8
634	—	—	—	9	10	8	5	—	—	—	—	32	24.28	30.0	100.0	192.2
635	—	—	20	9	—	—	—	—	—	—	—	29	15.00	16.9	90.6	108.3
636	—	—	2	13	13	1	1	—	—	—	—	30	21.17	24.8	93.8	158.4
637	—	2	12	9	—	—	—	—	—	—	—	23	14.30	16.3	71.9	104.5
638	—	—	1	2	0	2	9	6	—	—	—	20	31.75	35.3	62.5	226.8
639	—	—	6	17	0	1	—	—	—	—	—	24	17.04	19.3	75.0	123.7
640	1	0	12	17	1	—	—	—	—	—	—	31	15.77	17.9	96.9	114.3
641	—	—	—	2	7	15	3	—	—	—	—	27	26.48	29.5	84.4	188.6
642	—	—	1	7	9	10	1	—	—	—	—	28	23.21	28.1	87.5	179.6
643	—	1	11	8	—	—	—	—	—	—	—	20	14.45	16.3	62.5	104.2
644	—	—	5	20	5	—	—	—	—	—	—	30	17.80	20.5	93.8	131.1
645	—	—	—	2	6	12	2	—	—	—	—	22	26.50	29.7	68.8	189.9
646	1	1	6	20	3	1	—	—	—	—	—	32	17.31	21.3	100.0	136.1
647	—	1	0	0	9	10	7	1	—	—	—	28	27.04	32.0	87.5	204.5
648	—	2	2	7	9	4	—	—	—	—	—	24	21.00	25.7	75.0	164.3
649	—	1	10	20	—	—	—	—	—	—	—	31	16.10	18.2	96.9	116.4
650	—	—	18	11	—	—	—	—	—	—	—	29	15.10	16.8	90.6	112.4
651	—	1	24	—	—	—	—	—	—	—	—	25	13.08	14.3	78.2	91.4
652	—	2	26	—	—	—	—	—	—	—	—	28	12.50	14.0	87.5	89.4
653	—	1	5	16	5	2	—	—	—	—	—	29	18.34	23.0	90.6	147.1
654	—	3	17	8	—	—	—	—	—	—	—	28	13.86	16.1	87.5	102.8
655	—	2	3	10	13	—	—	—	—	—	—	28	19.43	23.2	87.5	148.2
656	—	—	2	16	10	0	1	—	—	—	—	29	19.90	23.6	90.6	150.8
657	—	—	1	5	6	12	3	—	—	—	—	27	24.78	30.1	84.4	192.4
658	—	3	25	4	—	—	—	—	—	—	—	32	13.78	16.7	100.0	106.8
667	—	1	0	3	7	8	1	—	—	—	—	20	23.90	25.1	62.5	160.4
Control	—	2	54	5	—	—	—	—	—	—	—	61	13.46	15.6	95.3	100.0

c) Experiment III.

The pots of galvanized iron containing sand, used in the first experiment, were once more employed in this experiment. After the corn seedlings and pieces of the roots grown in the first experiment were sieved off, the pots were autoclaved under 15 pounds pressure for 30 minutes. The fungus cultures tested, were grown on boiled rice in test tubes, and were added to the disinfected corn seeds in Petri dishes and shaken thoroughly. The inoculated corn seeds were sown on September 29, 1932, and the results were observed on October 13, 1932. They are given in Table III.

Table III.

Results of the Inoculation-Experiments of Corn-Seedlings with Various Strains of *Lisea Fujikuroi* Sawada, the Rice-"Bakanae"-Fungus. (III.)

The corn seeds were inoculated on September 28, 1932 and sown on the next day. The results were examined on October 13, 1932.

Nos. of strains studied	Classes in length of the seedlings tested (cm.)												Mean length of the seedlings tested	Mean length of 10 overgrown seedlings	Percentage of germination	Overgrowth index
	1–5	6–10	11–5	16–20	21–5	26–30	31–5	36–40	41–5	46–50	51–5	Total	cm.	cm.	%	
No. 410	—	15	10	—	—	—	—	—	—	—	—	25	10.00	11.8	78.2	85.5
411	—	6	15	—	—	—	—	—	—	—	—	21	11.14	12.4	65.6	89.8
412	2	17	5	—	—	—	—	—	—	—	—	24	8.42	10.2	75.0	73.9
413	1	3	27	—	—	—	—	—	—	—	—	31	11.65	13.4	96.9	97.1
414	—	—	2	5	4	6	1	—	—	—	—	18	22.72	27.0	56.3	195.7
415	—	—	3	6	17	2	—	—	—	—	—	28	21.29	24.9	87.5	180.5
483	—	9	16	—	—	—	—	—	—	—	—	25	10.68	12.1	78.2	87.7
484	—	—	—	3	5	15	1	—	—	—	—	24	25.50	28.5	75.0	206.7
485	—	1	1	0	11	7	1	—	—	—	—	21	23.81	27.3	65.6	197.8
486	—	1	0	2	5	7	2	—	—	—	—	17	24.82	28.7	53.2	208.0
487	—	1	1	1	2	14	5	—	—	—	—	24	26.92	30.9	75.0	224.0
488	1	7	20	—	—	—	—	—	—	—	—	28	11.11	12.9	87.5	93.4
589	—	8	7	—	—	—	—	—	—	—	—	15	10.40	11.2	46.9	81.8
602	—	4	26	—	—	—	—	—	—	—	—	30	11.87	13.3	93.8	96.3
603	—	5	17	1	—	—	—	—	—	—	—	23	12.09	13.8	71.9	100.0
604	—	—	—	1	9	9	11	—	—	—	—	30	27.87	32.6	93.8	236.2
605	—	—	5	7	9	11	—	—	—	—	—	32	22.66	28.6	100.0	207.3
606	—	1	1	4	5	7	3	—	—	—	—	21	24.14	28.4	65.6	206.0
607	—	—	1	4	5	13	3	—	—	—	—	26	25.39	30.1	81.3	218.2
608	—	—	2	4	3	7	6	—	—	—	—	22	25.20	30.6	68.8	221.8
609	—	1	6	6	7	—	—	—	—	—	—	20	17.80	21.6	62.5	156.6
610	—	2	3	5	2	6	5	1	—	—	—	24	22.50	32.0	75.0	232.0
611	—	2	1	5	6	15	2	—	—	—	—	31	23.50	28.6	96.9	207.2
612	—	7	24	—	—	—	—	—	—	—	—	31	11.26	12.9	96.9	93.3
613	—	—	15	10	—	—	—	—	—	—	—	25	15.20	16.8	78.2	121.8
614	—	5	25	—	—	—	—	—	—	—	—	30	12.13	13.7	93.8	99.2
615	—	—	1	2	8	13	4	—	—	—	—	28	26.70	30.2	87.5	219.0
616	—	—	—	—	2	8	16	4	—	—	—	30	31.50	35.2	93.8	255.2

Table III (Continued).

Nos. of strains studied	Classes in length of the seedlings tested (cm.)												Mean length of the seedlings tested	Mean length of 10 overgrown seedlings	Percentage of germination	Overgrowth index
	1–5	6–10	11–5	16–20	21–5	26–30	31–5	36–40	41–5	46–50	51–5	Total	cm.	cm.	%	
No. 620	—	—	4	12	1	1	—	—	—	—	—	18	17.60	19.8	56.3	143.5
621	—	—	2	8	2	11	1	—	—	—	—	24	22.83	28.3	75.0	205.1
622	—	1	27	3	—	—	—	—	—	—	—	31	13.36	15.1	96.9	109.4
623	—	2	8	6	2	4	—	—	—	—	—	22	17.54	23.0	68.8	166.7
624	—	—	2	1	6	9	5	—	—	—	—	23	26.19	30.2	71.9	218.9
625	—	—	—	—	3	10	13	1	—	—	—	27	30.20	33.6	84.4	243.5
626	—	—	1	2	6	8	5	—	—	—	—	22	26.45	31.3	68.8	226.9
627	—	—	2	3	5	8	—	—	—	—	—	18	22.83	26.7	56.3	193.6
628	—	3	29	—	—	—	—	—	—	—	—	32	11.78	12.5	100.0	90.6
629	—	6	22	—	—	—	—	—	—	—	—	28	11.18	12.6	87.5	91.3
630	—	1	1	3	8	4	5	—	—	—	—	22	23.59	28.7	68.8	208.0
631	—	—	2	4	3	4	1	—	—	—	—	14	22.69	25.6	43.8	185.5
632	—	—	4	6	4	7	3	—	—	—	—	24	22.92	29.7	75.0	215.3
633	—	—	—	6	9	8	—	—	—	—	—	23	23.65	27.6	71.9	200.0
634	—	—	2	6	11	5	3	—	—	—	—	27	23.90	29.0	84.4	210.3
635	—	—	1	5	9	8	—	—	—	—	—	23	23.43	26.9	71.9	194.9
636	—	1	0	2	6	8	3	—	—	—	—	20	25.30	29.5	62.5	213.8
637	—	3	26	1	—	—	—	—	—	—	—	30	12.36	14.3	93.8	103.7
638	—	1	3	3	5	5	3	—	—	—	—	20	22.40	28.3	62.5	205.1
639	—	2	25	2	—	—	—	—	—	—	—	29	13.20	15.3	90.6	110.8
640	—	2	7	4	1	—	—	—	—	—	—	14	15.21	17.0	43.8	123.2
641	—	1	5	4	4	5	1	—	—	—	—	20	20.60	24.9	62.5	180.4
642	—	1	1	3	7	11	1	1	—	—	—	25	24.71	30.1	78.2	218.2
643	—	—	5	10	5	3	1	—	—	—	—	24	19.90	25.0	75.0	181.2
644	—	—	2	2	15	7	—	—	—	—	—	26	23.20	26.2	81.3	189.9
645	—	—	2	3	4	10	4	—	—	—	—	23	24.43	29.4	71.9	213.1
646	—	—	—	5	10	11	2	—	—	—	—	28	24.53	29.4	87.5	213.1
647	2	1	2	10	10	—	—	—	—	—	—	25	18.32	22.8	78.2	165.3
648	—	—	6	11	8	1	—	—	—	—	—	26	19.38	23.4	81.3	169.7
649	—	7	19	—	—	—	—	—	—	—	—	26	11.50	13.1	81.3	94.9
650	—	3	19	—	—	—	—	—	—	—	—	22	11.91	13.0	68.8	94.2
651	—	4	8	10	1	—	—	—	—	—	—	23	14.65	18.0	71.9	130.5
652	—	1	21	10	—	—	—	—	—	—	—	32	14.02	16.7	100.0	121.0
653	—	1	3	5	4	7	2	—	—	—	—	22	22.22	28.3	68.8	205.0
654	—	2	7	8	2	—	—	—	—	—	—	19	14.94	17.4	59.4	126.1
655	—	—	—	3	10	14	3	—	—	—	—	30	25.10	29.2	93.8	211.6
656	—	—	1	3	4	14	1	—	—	—	—	23	26.03	29.7	71.9	215.3
657	—	1	0	2	4	16	4	—	—	—	—	27	26.53	30.5	84.4	221.1
658	1	6	4	3	—	—	—	—	—	—	—	14	11.57	13.4	43.8	97.1
667	—	—	2	0	6	14	2	1	—	—	—	25	26.64	31.0	78.2	224.7
Control	—	—	47	—	—	—	—	—	—	—	—	47	12.38	13.8	73.4	100.0

The results given in Table III, are almost similar to those of the two previous experiments. But the abnormal overgrowth is much more prominent. In this experiment the overgrown seedlings were more slender, lanky and yellowish than those in the previous experiments. They were easily distinguished from the normal grown seedlings not only by the length but also by the color and shape of seedlings. Moreover the degree of the overgrowth was much greater than that in the previous experiments. While the overgrowth indexes in two previous experiments were all under 200, those in this experiment were above 200 in case of the following thirty strains : Strain Nos. 484 ; 486 ; 487 ; 604 ; 605 ; 606—608 ; 610—611 ; 615 ; 616 ; 621 ; 624—626 ; 630 ; 632—634 ; 636 ; 638 ; 642 ; 645 ; 646 ; 653 ; 655—657 and 667.

As stated above, the same sand in the pots used in the first experiment was repeatedly used in the third experiment, although they were autoclaved. For this reason, the pathogenic substances produced in the first experiment might have remained in the pots and doubled the pathogenic action.

The above given results show that there are great differences in the pathogenecity among various strains tested, and also that the pathogenic substances are not destroyed by heat, at last, by the temperature under 120°C., as reported by some authors.

IV. Discussions.

The results of the above given experiments may be now considered conclusively. For the sake of comparison, the overgrowth index of the three experiments is tabulated and given in Table IV, in which the average of three index is given. The sames are also given graphically in Figure I, in which the overgrowth index is given on the ordinate, and the number of the strains tested are placed on the abssissa in order of the mean value of the overgrowth index of the three experiments.

(See Table IV on 124—125 and Figure I on 126—127.)

Table IV as well as Figure I show that the overgrowth index of thirty-one strains are above 120 throughout three experiments and those of twenty-four strains above 130. On the other hand, some of the strains show none or a little overgrowth. Among the strains which show no overgrowth, strain, Nos. 412, 413 and 589, belonging to *Fusarium moniliforme* Sh. and Nos. 410 and 411 of *Fusarium moniliforme* Sh. var. *majus* Wr. et Rg., are found. It is also noteworthy that the growth of the seedlings inoculated with the last stated five strains is rather hindered.

Table IV.

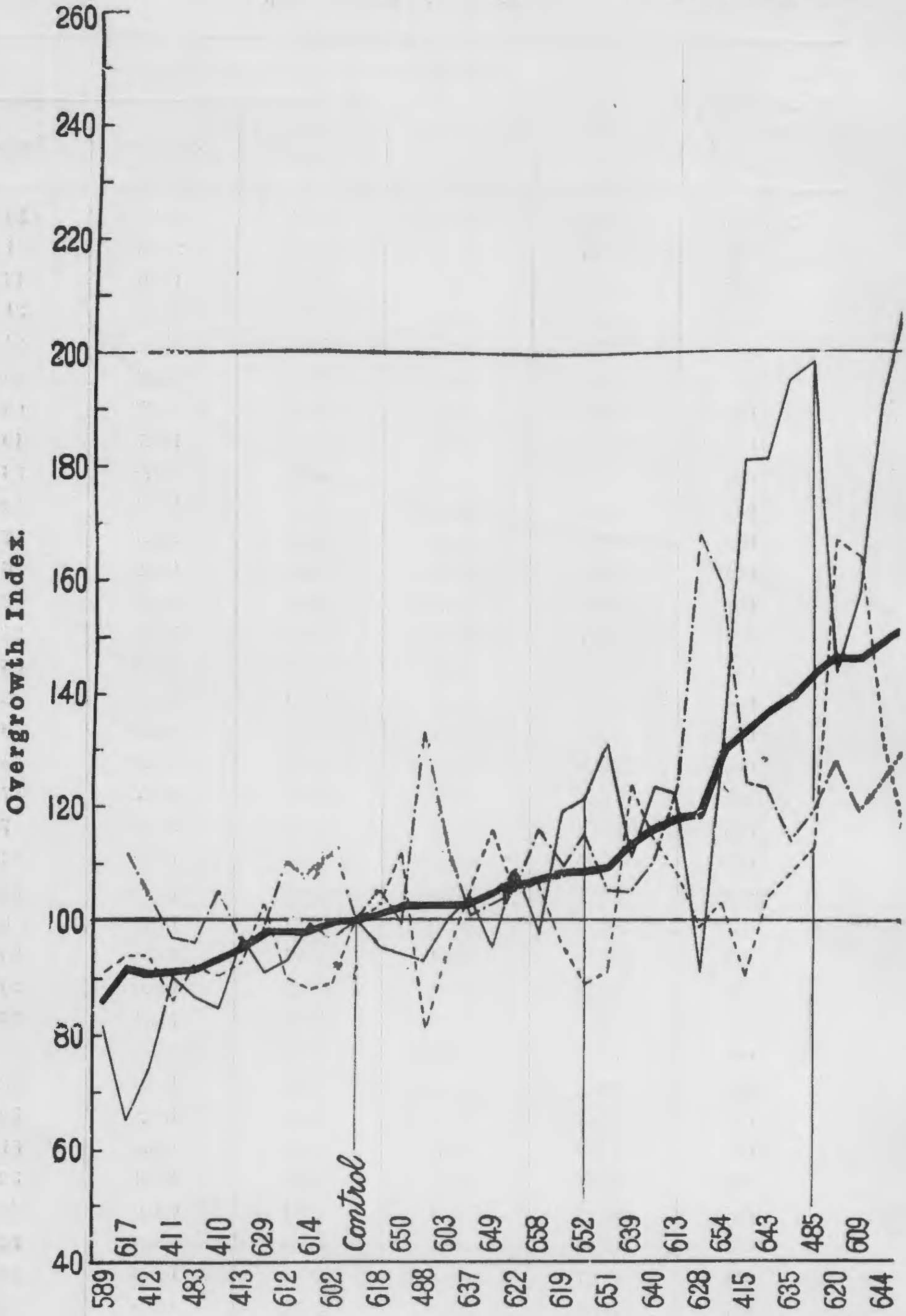
Summary of the Comparisons of the Overgrowth Index of the Corn-Seedlings, resulted by the Inoculations of Various Strains of *Lisea Fujikuroi* Sawada, the Rice-" Bakanae "-Fungus.

Nos. of strains tested	Overgrowth Index of corn seedlings				
	Experiment I	Experiment II	Experiment III	Average	Order
No. 410	105.0	90.4	85.5	93.6	67
411	96.8	85.9	89.8	90.8	69
412	104.1	94.2	73.9	90.7	70
413	95.8	92.9	97.1	95.3	66
414	121.8	200.6	195.7	172.7	14
415	124.0	90.4	180.5	133.1	44
483	95.9	92.3	87.7	91.7	68
484	154.8	137.2	206.7	169.6	16
485	118.6	112.8	197.8	143.1	41
486	129.4	115.5	208.0	150.9	37
487	155.3	194.9	224.0	191.4	4
488	133.1	80.2	93.4	102.2	58
589	—	91.0	81.8	86.4	72
602	113.6	89.1	96.3	99.7	62
603	113.2	94.2	100.0	102.5	57
604	134.0	199.4	236.2	189.9	5
605	125.8	131.4	207.3	154.8	34
606	125.4	147.4	206.0	159.6	29
607	142.6	131.4	218.2	164.1	26
608	161.6	153.2	221.8	178.9	9
609	118.6	163.5	156.6	146.2	39
610	105.9	198.1	232.0	178.7	10
611	139.4	116.7	207.2	154.4	35
612	110.0	90.4	93.3	97.9	64
613	123.2	108.3	121.8	117.8	47
614	107.3	88.4	99.2	98.3	63
615	144.8	132.7	219.0	165.5	23
616	134.4	139.1	255.2	176.2	11
617	111.8	93.5	65.2	90.2	71
618	105.0	102.6	94.9	100.8	60
619	108.7	95.5	118.9	107.7	52
620	127.6	167.4	143.5	146.2	40
621	140.8	164.1	205.1	170.0	15
622	104.6	104.5	109.4	106.2	54

Table IV (Continued).

Nos. of strains tested	Overgrowth Index of corn seedlings				
	Experiment I	Experiment II	Experiment III	Average	Order
No. 623	135.4	181.4	166.7	161.2	28
624	188.7	194.3	218.9	200.6	1
625	169.2	106.4	243.5	173.0	13
626	134.1	109.6	226.9	156.9	31
627	161.2	139.8	193.6	164.9	24
628	167.5	99.4	90.6	119.2	46
629	98.7	103.2	91.3	97.7	65
630	158.6	136.6	208.0	167.7	19
631	177.9	101.3	185.5	154.9	33
632	131.7	130.8	215.3	159.3	30
633	138.2	169.8	200.0	169.3	17
634	156.1	192.2	210.3	186.2	6
635	114.1	108.3	194.9	139.1	42
636	126.7	158.4	213.8	166.3	21
637	101.4	104.5	103.7	103.2	56
638	166.2	226.8	205.1	199.4	2
639	104.9	123.7	110.8	113.1	49
640	109.6	114.3	123.2	115.7	48
641	119.1	188.6	180.4	162.7	27
642	153.0	179.6	218.2	183.6	7
643	122.7	104.2	181.2	136.0	43
644	124.4	131.1	189.9	148.5	38
645	141.2	189.9	213.1	181.4	8
646	143.9	136.1	213.1	164.4	25
647	130.8	204.5	165.3	166.9	20
648	126.3	164.3	169.7	153.4	36
649	103.2	116.4	94.9	104.8	55
650	99.5	112.4	94.2	102.0	59
651	104.6	91.4	130.5	108.8	50
652	114.9	89.4	121.0	108.4	51
653	113.2	147.1	205.0	155.1	32
654	158.0	102.8	126.1	129.0	45
655	139.0	148.2	211.6	166.3	22
656	141.7	150.8	215.3	169.3	18
657	171.6	192.4	221.1	195.0	3
658	116.3	106.8	97.1	106.7	53
667	135.3	160.4	224.7	173.5	12
Control	100.0	100.0	100.0	100.0	61

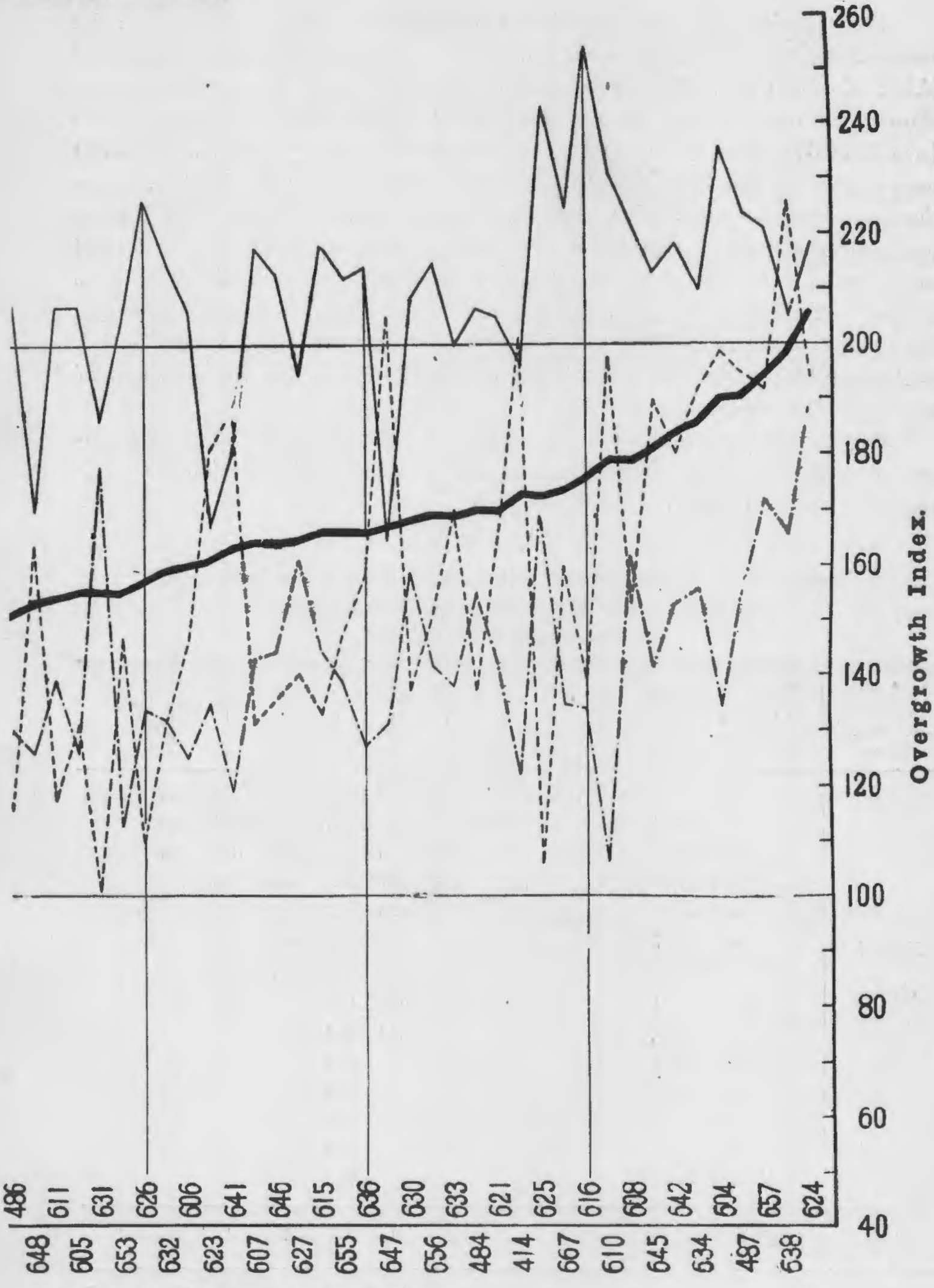
Fig.
Comparisons of the Overgrowth Index of Strains of the Rice-



Remarks: Overgrowth index is given on the ordinate and the strain number of the fungus-cultures tested are placed on the abssissa in the order of the mean value of the overgrowth index. The mean value of the

I.

the Corn-Seedlings, inoculated with Various "Bakanae"-Fungus.



of the "Bakanae"-Fungus Studied.

three experiments is expressed by the heavy line (▬▬▬), the result of Experiment I by the chain line (-·-·-), Experiment II by broken line (--------) and Experiment III by thin line (———).

Many strains which showed strong pathogenecity, were secured from the conidia. On the other hand, some strains isolated from the conidia, [e. g. No. 483 (Aiti), No. 602 (Aiti), No. 614 (Miye), No. 612 (Miye) and No. 629 (Yamaguti)] showed no overgrowth. Among the strains isolated from ascospores, some (e. g. No. 647) showed strong pathogenecity, while the some others (e. g. No. 650) were contrary. The differentiation seems to be indifferent by the locality, where the strains were secured. For example, the strain No. 657, No. 656 and No. 655 produced prominent overgrowth, the overgrowth index being 195, 169.3 and 166.3 respectively, while the strain No. 658 no overgrowth, although all these were isolated in Tu, Miye Prefecture. Among the strains secured from Aiti Prefecture, the pathogenecity of No. 487 and No. 484 was strong and No. 483 and No. 602, very weak, the overgrowth index being 191.4 and 169.6 in the former and 91.7 and 99.7 in the latter respectively.

Further the strains studied were grouped after the index number of abnormal overgrowth into classes, such as 100—110, 110—120, 120—130 etc. The result is given in Table V.

Table V.

Distribution of the Overgrowth Index of the Corn-Seedlings, resulted by the Inoculation of Various Strains of the "Bakanae"-Fungus.

Classes of overgrowth index	* 80 – 90	90 – 100	100 – 110	110 – 120	120 – 130	130 – 140	140 – 150	150 – 160	160 – 170	170 – 180	180 – 190	190 – 200	200 – 210
		483	488	613	654	415	485	486	484	414	604	487	624
		602	603	628		635	609	605	607	608	634	638	
		612	618	639		643	620	606	615	610	642	657	
		614	619	640			644	611	623	616	645		
		617	622					626	627	621			
Nos. of		629	637					631	630	625			
strains			649					632	633	667			
tested			650					648	636				
			651					653	641				
			652						646				
		410	658						647				
		411							655				
		412							656				
	589	413	Control						658				
Total	0+1	6+4	11+1	4	1	3	4	9	13	7	4	3	1

Remarks: In this table the strain-numbers underlined are not the rice-"Bakanae"-fungus. In the line of total, the figures following plus sign (+) show the sum of the above said strains, not belonging to the "Bakanae"-fungus.

* In strict sence the classes 80—90, 90—100, etc., mean 81—90, 91—100, etc., respectively.

Table V shows two modes in frequency number of classes. One of the modes lies in the class of 100—110 in which eleven strains are found. In the group of this mode, six strains belong to the class 90—100 and four to the class 110—120. Above all twenty-one strains, which belong to this group, may be assumed as non-pathological to corn seedlings. Those strains of *Fusarium moniliforme* SH. and its variety *F. moniliforme* SH. v. *majus* WR. et RG. are found in the class 90—100.

The other modes lies in the class 160—170, in which thirteen strains are found. In this group, nine strains belong to the class 150—160, seven to the class 170—180, four to the both class 140—150 and 180—190, three to each of the class 130—140 and 190—200 and one to the classes of 120—130 and 200—210. These fourty-five strains are able to produce the abnormal overgrowth of the corn seedlings. Above all Table V shows that the strains studied in the present experiment may be clearly devided into two groups, one of which is non-pathogenic to the corn seedlings and the other strongly pathogenic. Physiological differentiation in the pathogenecity among various strains of the rice-" Bakanae "-fungus, *Lisea Fujikuroi* SAW., is clearly demonstrated in the writers' experiment.

V. Summary.

1) The present paper is the first series of the reports on the physiological specialization of species of Fusarium, and deals with the differentiation in the pathogenecity of strains of the rice-" Bakanae "-fungus, *Lisea Fujikuroi* SAW.

2) In this experiment, sixty-six strains of the rice-" Bakanae "-fungus, collected from various localities in Japan, were used for inoculation to corn seeds. For the sake of comparison, five other strains of the form species *Fusarium moniliforme* SH. and *F. moniliforme* SH. v. *majus* WR. et RG. were also tested.

3) As already reported by the senior writer, the rice-" Bakanae "-fungus is able to show a more distinct symptom of the disease, or an abnormal overgrowth, on the corn seedlings rather than on the rice seedlings. Therefore the corn seedlings, inoculated with the pure cultures of the above said strains, were sown in sand in the pots, which had been sterilized in an autoclave. The corn seedlings from the inoculated seeds were compared as to their abnormal overgrowth.

4) The result of the experiment shows great differentiation in the pathogenecity to the corn seedlings, among the strains of the rice-" Bakanae "-fungus. The differentiation seems to have little relation to the source of cultures, especially to the locality, whence the strains were obtained.

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