STUDIES ON THE NUTRITIONAL PHYSIOLOGY OF *PIRICULARIA ORYZAE* CAVARA

By

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Contents

I. Introduction

Studies on the physiology of *Piricularia Oryzae* CAV., the causal fungus of "Imochi" disease of rice plants, have been published rather superfluously in Japan up to the present time. On the nutritional physiology of the fungus, however, only a few detailed studies have been carried out. This is true, possibly, because of some difficulties in the artificial culture of the fungus in synthetic nutrient solutions.

To throw light upon the problem of the fundamental nutritional

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physiology of the fungus, the authors carried out cultural studies in several nutrient solutions differing in kinds and amounts of the component compounds.

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II. Review of the previous works

A great many cultural studies of parasitic fungi in synthetic nutrient solutins have been carried out, and the nutritional physiology of numerous kinds of fungi has become fully known. On *Piricularia Oryzae,* however, although extensive studies have been made from several viewpoints of plant pathology, details of the nutritional physiology of the fungus yet remain unsolved in several respects. A majority of the many important cultural studies of the fungus have been carried out on agar media and mostly on vegetable ones.

N. SUEMATSU (1916) published a report on cultural studies of *Dactylaria parasitans* (= *Piricularia Oryzae*). He cultured the fungus on various artificial culture media and studied the growth in relation to the temperature, and to the reaction and concentration of the media. In his studies no synthetic culture solution was used. In 1917 and also in 1926, Y. NISHIKADO performed more detailed studies on the nutrition of this fungus, and reported that the fungus grew saprophytically on various agar media of plant decoctions and produced conidia positively. On the other hand, he cultured the fungus on synthetic agar media containing various carbohydrates, and reported that the medium containing 3% glucose was the best for the mycelial growth of the fungus, but in the corresponding liquid media it was less vigorous. In various synthetic solutions the fungus grew more or less, and he reported that in COON's solution and USCHINSKY's solution which contain organic salts of ammonium, the mycelial development was fairly good, but in RICHARDS' solution, PFEFFER's solution, CURRIE's solution and CZAPEK's solution it was only slight, while the peptone water was not suitable for the development of the fungus. In 1928, SUEDA reported a study on the relation of glucose concentrations in culture media to the mycelial growth of *Piricularia Oryzae.* He used a synthetic agar medium containing monopotassium phosphate 0.5 g., magnesium sulphate 0.25 g., am-

monium nitrate 1.0 g., ferric chloride trace, water 1000 cc., and various amounts of glucose. SUEDA found that the medium containing 2% glucose was the most suitable for the growth of the fungus. In 1931, S. SATOH studied the decomposition of cellulose by *Piricularia Oryzae*. He used a synthetic solution of the following composition: potassium nitrate 0.1 g., monopotassium phosphate 0.05 g., magnesium sulphate 0.025 g., ferric chloride trace, 10 cc. water, and a piece of filter paper or "umgefällter Zellulose" as the carbon source. In his experiments, *Piricularia Oryzae* grew slightly in the culture solutions and the decomposition of cellulose was recognized when "umgefällter Zellulose" was used. In 1936, F. SETO demonstrated the decomposition of cellulose by *Piricularia Oryzae* in comparative studies of cellulose decomposition in several plant pathogenic fungi. He used a synthetic mineral salt solution (monopotassium phosphate 1 g., magnesium sulphate 1 g., sodium chloride 1 g., calcium carbonate 2 g., ammonium nitrate 2 g., water 1000 cc.), containing a piece of filter paper as the carbon source for the culture of the fungi. It was found that *Piricularia Oryzae* grew in this culture solution and slightly decomposed the cellulose of the filter paper.

III. Materials and methods

The stock culture of *Piricularia Oryzae* CAV. used in the present studies was kindly provided by the courtesy of Mr. M. SAKAMOTO.

The glasswares used in the experiments were manufactures of the special "Kiku" hard glass which were expected to be satisfactory in minimizing the experimental errors owing to undesirable dissolving of alkali components of the glass. The ERLENMEYER's flasks of 200 cc. capacity were used for the fungus cultures. These glasswares were cleaned enough with potassium bichromate sulphuric acid cleaning fluid and soap, and after 3 days' boiling in a KOCH's steam sterilizer they were washed thoroughly with running water.

The redistilled water used for preparing the culture solutions was produced with SHIBATA's redistilling apparatus. The reaction of this redistilled water was about pH 6.0 under the condition of exposure to the air; it was proven to be entirely ammonia free by the test of NESSLER's reagent.

The chemicals used for the preparation of nutrient solutions were the products of the KOJIMA Chemical Company and were guaranteed in their qualities.

The culture solutions were sterilized in a KOCH's steam sterilizer for 30 minutes twice with one day's interval.

The fungus for the inoculation sources was cultured at 28°C. in a thermostat on agar plates of potato decoction medium containing 1% sucrose. After 10 days' incubation, a bit of growing mycelium in the border part of the colony was cut off together with agar medium as a small block of about 3 cubic mm., and it was used as the inoculum.

The cultures were continued for 30 days in a thermostat at 28°C., with the maximum fluctuation of the temperature ± 1°C.

The color designations follow RIDGWAY's "Color standards and nomenclature".

Hydrogen ion concentrations of the culture solutions were determined colorimetrically by KOLTHOFF's indicator method. The H-ion concentrations of the culture solutions were measured after sterilization first, and the final H-ion concentrations were measured after 30 days' culture.

The growth of the fungus was compared by dry weights of the developed mycelium. For this purpose the fungus colonies grown in the culture solutions were taken on a sheet of filter paper and washed with dilute HCl solution and warm distilled water. After a short dehydration at room temperature, the fungus mats were carefully separated from the filter paper, placed on clean glass plates and dried at 60°C.–70°C. in an electric oven for about 24 hours. After a complete desiccation in a $CaCl_2$ -desiccator, they were weighed on a balance.

IV. The standard nutrient solution

It has been generally known that *Piricularia Oryzae* grows better on agar media of plant decoctions than on synthetic nutrient ones, and also that agar media are more suitable than liquid ones. In a liquid medium the inoculum sinks, and the development of the mycelium is retarded more or less, possibly due to the interception of air supply. In studies on the detailed nutritional relation of the fungus, however, the use of synthetic nutrient solutions containing

chemically constant components is absolutely necessary, and agar media or plant decoctions should be avoided owing to their chemically unknown and inconstant characters.

Several cultural works of *Piricularia Oryzae* in synthetic solutions have been carried out by SUEMATSU (1916), MIYAKE and ADACHI (1922), NISHIKADO (1926) and SUEDA (1928). They reported that the fungus grows fairly well in synthetic nutrient solutions containing organic nitrogen compounds.

NISHIKADO (1926), however, found that the fungus growth was slight in RICHARDS' solution, PFEFFER's solution, MEYER's solution and CZAPEK's solution.

In the present studies preliminary cultural experiments were first carried out in the following 6 kinds of synthetic nutrient solutions in order to make choice of proper standard nutrient solution.

I. Culture solution I
NH_4NO_3 2.5 g., KH_2PO_4 5.0 g., $MgSO_4 \cdot 7H_2O$ 0.25 g., Sucrose 30.0 g., Redistilled water 1000 cc.

II. Culture solution II
NH_4NO_3 10.0 g., KH_2PO_4 5.0 g. $MgSO_4 : 7H_2O$ 2.5 g., $FeCl_3$ trace, Sucrose 30.0 g., Redistilled water 1000 cc.

III. Culture solution III
NH_4NO_3 5.0 g., KH_2PO_4 2.5 g., $MgSO_4 \cdot 7H_2O$ 1.25 g., Sucrose 30.0 g., Redistilled water 1000 cc.

IV. Culture solution IV
KNO_3 10.0 g., KH_2PO_4 5.0 g., $MgSO_4 \cdot 7H_2O$ 2.5 g., Sucrose 30.0 g., Redistilled water 1000 cc.

V. Culture solution V
KNO_3 2.0 g., KH_2PO_4 1.0 g., $MgSO_4$ $7H_2O$ 0.5 g., Sucrose 30.0 g., Redistilled water 1000 cc.

VI. Culture solution VI
KNO_3 2.0 g., K_2HPO_4 1.0 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., $CaCl_2$ 0.1 g., Sucrose 30.0 g., Redistilled water 1000 cc.

Thirty cubic centimeters of each of these culture solutions were taken in flasks, and after sterilization, the fungus was inoculated. The cultures were continued in a thermostat at 28°C. for 30 days. The results of the cultural experiments are given in Table I.

TABLE 1. Results of the cultural experiments in various nutrient solutions

Cultural solution	Initial pH	Growth of the fungus
I	4.5	slight
II	4.6	slight
III	4.5	a little
IV	4.6	slight
V	4.8	slight
VI	7.3	moderate

The culture solution VI seemed to be the most suitable for this fungus among these synthetic solutions tested. In this nutrient solution, the fungus grew moderately and the mycelial colonies were grayish olive in color and about 20 mm. in diameter.

For trial, the H-ion concentration in culture solution III was changed variously by the addition of sodium hydroxide solution. Somewhat concentrated culture solution III was prepared by adding 600 cc. of redistilled water instead of the normal 1000 cc. as a stock solution, and 30 cc. of this stock solution were taken in each flask.

After sterilization, the various amounts of sterilized sodium hydroxide solution and redistilled water were added to the solution in each flask as shown in the following Table 2.

TABLE 2. H-ion concentrations of culture solutions as altered by additions of NaOH-solution

Number of culture solution	Volume of stock solution (cc.)	NaOH-solution		Volume of H_2O added to make up the whole vol. 50 cc.	Hydrogen ion concentration (pH)
		Conc. (N)	Vol. (cc.)		
1	30	—	0	20.0	4.5
2	30	1/20	0.5	19.5	5.0
3	30	1/20	1.5	18.5	5.5
4	30	1/20	3.5	16.5	6.0
5	30	1/20	5.0	15.0	6.3
6	30	1/20	7.5	12.5	6.5
7	30	1/20	12.5	7.5	6.7
8	30	1/20	20.0	0	7.2
9	30	1/20	15.5	4.5	8.4

Each solution was prepared in triplicate. The culture was carried out in a thermostat at 28°C. for 30 days. The results are shown in the following Table 3.

TABLE 3. Fungus growth in culture solution III varying in the H-ion concentration

Number of culture solution	Initial pH of the culture solution	Degree of the Fungus development
1	4.5	+
2	5.0	++
3	5.5	++++
4	6.0	+++++
5	6.3	+++++
6	6.5	+++
7	6.7	+
8	7.2	±
9	8.4	−

The sign − means no glowth of the fungus, ± means a slight growth, + means a little growth, and ++ means about 6 mm. in diameter of the fungus colony.

The fungus grew fairly well at pH 5.5 to pH 6.5, and the best growth was attained at pH 6.0, in which the diameter of the mycelial colony was about 15 mm. The growth of the fungus in culture solution III seemed to be inferior to that in culture solution VI even at the most favourable hydrogen ion concentration.

In an attempt to set up a formula of suitable mineral salts solution for the culture of *Piricularia Oryzae*, the following seven combinations of several kinds of nutrient salts as shown in Table 4, following, were tried.

Thirty cc. of each of these seven kinds of nutrient solutions were taken in ERLENMEYER's flasks and sterilized in a KOCH's sterilizer. By this sterilization treatment precipitation took place in No. 4, No. 6 and No. 7 solutions. Cultures were continued in a thermostat at 28°C. for 30 days. The results are shown in the following Table 5.

From these results, it was clear that certain nutrient salts added to the cultural solutions directly influenced the development of the fungus or indirectly through the change of hydrogen ion

TABLE 4. Various combinations of nutrient salts in culture solutions

Solution / om-ponents*	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
KNO_3	2.0	2.0	2.0	2.0	2.0	2.0	2.0
$MgSO_4 \cdot 7H_2O$	0.5	0.5	0.5	0.5	0.5	0.5	0.5
KH_2PO_4	1.0	1.0			0 5	0.5	0.5
K_2HPO_4			1.0	1.0	0.5	0.5	0.5
$CaCl_2$		0.1		0.1		0.1	0.1
$FeCl_3$							trace**
Sucrose	30	30	30	30	30	30	30
Water	1000	1000	1000	1000	1000	1000	1000
pH value***	4.7	4.8	7.4	7.3	6.3	6.3	6.3

* Units of the volumes of the components are gram and cc.
** Five per cent $FeCl_3$ solution was added one drop per 30cc. of the culture solution.
*** Hydrogen ion concentrations of solutions were determined after sterilization.

TABLE 5. Results of cultural experiments with various cultural solutions varied in the combinations of nutrient mineral salts

Number of cultural solution	pH		Dry weight of mycelial colony (mg.)	Color of colony
	Initial	Final		
1	4.7	4.4	—	White
2	4.8	4.5	—	White
3	7.4	7.2	1.8	Olivaceous black or grayish olive
4	7.3	7.1	2.3	Grayish olive
5	6.3	6.0	3.9	Grayish olive
6	6.3	5.9	3.7	Grayish olive
7	6.3	5.9	4.1	Grayish olive or dark grayish olive

concentration of the solution, while some kinds of the salts seemed to exert almost no influence upon the fungus nutrition. For instance, K_2HPO_4 seemed to be more suitable than KH_2PO_4 for the mycelial development of the fungus, while $CaCl_2$ was by no means effective to the growth of the fungus either positively or negatively. The

initial H-ion concentration of No. 7 solution was pH 6.3, and it seemed probably the optimum for the mycelial growth of the fungus; the most vigorous development took place in this solution. Based upon the present experiments the writers recommend the synthetic nutrient solution of the following formula as a suitable liquid medium for the culture of *Piricularia Oryzae*:

Potassium nitrate	(KNO_3)	2.0 g.
Monopotassium phosphate	(KH_2PO_4)	0.5 g.
Dipotassium phophate	(K_2HPO_4)	0.5 g.
Magnesium sulphate	($MgSO_4 \cdot 7H_2O$)	0.5 g.
Calcium chloride	($CaCl_2$)	0.1 g.
Ferric chloride	($FeCl_3$)	trace *
Sucrose	($C_{12}H_{22}O_{11}$)	30.0 g.
Redistilled water	(H_2O)	1000.0 cc.

* One drop of 5% $FeCl_3$ solution for 30cc. of the culture solution.

This nutrient solution was adopted as the standard solution through the whole course of the present studies, though a slight precipitation takes place in the solution after sterilization in KOCH's steam sterilizer.

V. Cultural studies concerning carbon sources

In the present studies, the carbonydrates, organic acids and higher alcohols were used as carbon sources.

Culture solution No. 7 described in the previous chapter was used as the standard nutrient solution with a constitution of nutrient mineral salts as follows: potassium nitrate 2.0 g., magnesium sulphate 0.5 g., monopotassium phosphate 0.5 g., dipotassium phosphate 0.5 g., calcium chloride 0.1 g., ferric chloride trace, redistilled water 1000 cc.

By adding various amounts of each carbon compound to the standard mineral salts solution mentioned above the cultural solutions containing various carbon sources were prepared. Thirty cc. portions of each of the cultural solutions were taken in each ERLENMEYER's flask of 200 cc. capacity. They were all sterilized in a KOCH's sterilizer for 30 minutes twice with one day's interval. A bit of mycelium was inoculated and incubated in a thermostat at

28°C. After 30 days' incubation, all cultures were taken out from the thermostat, and the observations were made. These cultures were repeated two times, from July to August and from January to February, and each culture was carried out in triplicate.

1. Cultural experiments on carbohydrates

Carbohydrates are the most important source of carbon in the nutrition of fungi. It is supposed that the kinds and amounts of carbohydrates contained in the cell tissues of host plants sometimes have important connections with the parasitism of fungi and accordingly also with the severity of diseases.

In the present experiments, the following carbohydrates were used as carbon sources.

Monosaccharides: glucose, fructose, galactose
Disaccharides: sucrose, maltose, lactose
Polysaccharides: soluble starch, inulin, cellulose

Monosaccharides were given in concentrations of 0.7 mol., 0.5 mol., 0.3 mol., 0.1 mol. and 0.05 mol., and disaccharides 0.35 mol., 0.25 mol., 0.15 mol., 0.05 mol. and 0.005 mol. In the cases of polysaccharides, soluble starch and inulin were given in concentrations of 5%, 2%, 0.5%, 0.25% and 0.125%, and for cellulose pieces of filter paper were used.

The results of these cultures, except cellulose cultures which will be specially described, are shown in the following Table 6. The dry weights in the table represent averages of three cultures in each of two experiments.

In these cultures, the fungus generally grew well, and produced deep colored round colonies in the culture solutions. The development of aerial mycelium was hardly recognized with an exceptional case of the soluble starch cultures. The production of spores did not take place in any culture at all. The hydrogen ion concentrations of the solutions after 30 days' culture increased more or less in most cases except in the lactose cultures.

Among the monosaccharides, glucose was the most nutritive, and galactose and fructose were markedly less judging from dry weights of the developed mycelium and also from the macroscopic appearance of the developed colonies. In the culture solutions containing glucose, the growth of the fungus became gradually better

TABLE 6. Results of cultural experiments on various carbohydrates

Carbon source	Conc. (mol.)	pH of the culture-solution		Dry wt. of fungus mat (mg.)		Color of fungus mat
		Initial	Final		average	
Glucose	0.7	5.8	5.7 5.5	2.6 1.9	2.25	Olivaceous black or dark grayish olive
	0.5	5.9	5.7 5.5	4.2 3.6	3.90	Olivaceous black or dark greenish olive
	0.3	6.0	5.7 5.7	8.8 9.2	9.00	do.
	0.1	6.0	5.8 5.6	11.2 14.2	12.70	Dark grayish olive
	0.05	6.2	5.9 5.4	16.2 15.3	15.70	Light brownish or deep grayish olive
Fructose	0.7	5.9	5.1 5.4	1.6 1.1	1.35	Dark greenish olive or grayish olive
	0.5	6.0	5.2 4.6	1.8 1.3	1.55	Grayish olive
	0.3	6.1	5.3 5.4	2.0 1.4	1.70	Dark grayish olive or pale olive-buff
	0.1	6.1	5.8 5.9	2.4 1.5	1.95	Pale olive-buff or dark greenish olive
	0.05	6.1	5.8 5.9	2.6 1.3	1.95	do.
Galactose	0.7	5.9	5.7 5.8	2.2 1.8	2.00	Olivaceous black
	0.5	6.0	5.7 5.9	5.4 5.6	5.50	Olivaceous black or deep grayish olive
	0.3	6.0	5 7 5.8	3.6 2.9	3.25	do.
	0.1	6.1	5.8 5 8	1.8 2.3	2.05	do.
	0.05	6.2	5.8 5.9	2.0 1.4	1.70	Olivaceous black or smoke gray
	0.35	6.2	5.9 5.9	4.9 5.5	5.20	Olivaceous black or dark grayish olive

TABLE 6. (*Continued*)

Carbon source	Conc. (mol.)	pH of the culture-solution		Dry wt. of fungus mat (mg.)		Color of fungus mat
		Initial	Final		average	
Sucrose	0.25	6.2	5.9 5.7	7.3 6.2	6.75	do.
	0.15	6.2	5.8 5.9	6.5 4.9	5.70	do.
	0.05	6.3	5.9 5.9	5.2 3.3	4.25	Olivaceous black or light grayish olive
	0.005	6.3	6.1 5.9	2.7 1.4	2.05	do.
Lactose	0.35	6.2	6.3 6.2	5.3 5.2	5.25	Olivaceous black or light olive gray
	0.25	6.2	6.2 6.2	6.6 5.1	5.85	Olivaceous black or light mouse gray
	0.15	6.2	6.0 6.2	5.1 4.2	4.65	Olivaceous black
	0.05	6.2	6.2 6.0	3.8 2.8	3.30	Olivaceous black or deep olive
	0.005	6.3	6.2 6.3	3.5 2.2	2.80	Light olive gray or pale olive buff
Maltose	0.35	5.9	4.0 4.6	19.2 18.6	18.90	Deep olive buff or smoke gray
	0.25	6.0	4.2 4.3	31.1 35.0	33.05	Olive buff or smoke gray
	0.15	6.0	4.2 4.3	15.9 16.8	16.35	Deep olive buff
	0.05	6.1	4.4 4.6	5.6 6.9	6.25	Dark olive buff or white
	0.005	6.3	6.1 6.1	1.3 1.1	1.20	Pale olive buff or white
	5%	5.8	4.8 4.6	29.8 32.6	31.20	Light grayish olive or olive buff
	2%	6.0	4.9 4.7	21.3 18.4	19.85	do.

TABLE 6. (*Continued*)

Carbon source	Conc. (mol.)	pH of the culture-solution		Dry weight of fungus mat		Color of fungus mat
		Initial	Final	(mg.)	average	
Soluble starch	0.5%	6.2	5.4 5.7	15.4 14.8	15.10	Light grayish olive
	0.25%	6.2	5.9 5.9	8.3 9.7	9.00	Grayish olive
	0.125%	6.3	6.2 6.0	5.1 6.4	5.75	Grayish olive or white
Inulin	5%	5.8	5 5 5.9		+	White *
	2%	6.0	5.6 5.7	2.5 2.2	2.35	do.
	0.5%	6.3	5.8 6.2	2.0 1.8	1.90	do.
	0.25%	6.3	5.6 5.7	1.4 1.4	1.40	do.
	0.125%	6.3	6.2 6.2	1.0 —	0.50	do.

* The fungus grew only a little, and irregularly shaped small colonies developed adhering tighly to the bottom of flasks.

according to the decrease of concentrations of the sugar in the scope of the present experiment, and the solution containing 0.05 mol. glucose was the most favourable for the mycelial development of the fungus. In the solutions containing galactose, the fungus grew well at rather higher concentrations of the sugar, and at the concentration of 0.5 mol. the best development of the mycelium was attained. Fructose seemed to be less suitable for the growth of the fugus, and in the solutions containing this sugar as the carbon source the mycelium developed poorly and the colonies were pale in color and small in both size and dry weight.

Among the disaccharides, maltose was the most nutritive for this fungus, while sucrose and lactose were markedly inferior in this respect. In the culture solutions containing maltose the fungus developed remarkably well into large, thick and pale-colored

colonies, but neither aerial mycelium nor spore was produced. In the solution containing 0.25 mol. maltose the most vigorous mycelial development was attained, but it was far less either in higher or in lower concentrations of maltose than this point. In the media containing sucrose, the fungus developed well into apparently compact and deep colored colonies and the best growth was attained also at 0.25 mol. concentration, but the mycelial development in sucrose cultures was generally far inferior to that in the maltose cultures. Accordingly it may be said that maltose is a more suitable sugar than sucrose for the mycelial growth of the present fungus. In the culture solutions containing lactose, the fungus growth were almost similar to those in sucrose cultures.

For trial, the production of reducing sugars in the culture solution containing 0.1 mol. sucrose by the fungus development was examined by Micro-Bertrand's method after BIERRI and GOIRAN using FEHLING'S solution. For the control 0.1 mol. sucrose culture solution was incubated at 28°C. for 30 days without inoculating the fungus. The results of this experiment are shown in the following Table 7.

TABLE 7. Showing the production of reducing sugars in the culture solution by an increase of the amount of cuprous oxide due to the reduction of FEHLING'S solution

Sample	Amount of cuprous oxide (mg. / 3 cc.)
Stale culture solution (the fungus developed)	32.37
Control culture solution (no fungus)	10.72

The data represented the average of 3 tests.

From these results, it was clear that reducing sugars were markedly increased in the culture solution by the development of the fungus. Sucrose was decomposed into glucose and fructose, and considering the results obtained in monosaccharide cultures it is highly probable that glucose would be utilized more readily than fructose by this fungus.

In the case of maltose, one molecule of it produced two molecules

of glucose by hydrolysis, which should be the reason for the particularly luxuriant growth of the fungus in the cultural solutions containing maltose.

It is interesting that the fungus grew well at a low concentration of glucose, while in the case of maltose cultures the best growth was attained at a comparatively high content of the sugar in the culture solution. This was probably due to gradual and durable supply of glucose by progressive hydrolysis of maltose corresponding to the development of the fungus.

Among the polysaccharides, soluble starch was the most nutritive while inulin and cellulose were far less suitable for this fungus as carbon sources. In the solutions containing soluble starch, the fungus grew as vigorously as in the maltose cultures and moreover the aerial mycelium developed well in general, but the production of spores could not be recognized.

Inulin is known as a condensation product of fructose, and it was clear in the results of cultural experiments with monosaccharides that fructose is not highly nutritive for this fungus, and this should be the reason for less vigorous growth of the fungus in the culture solution containing inulin as the carbon source.

Cellulose, the most important component of cell membrane of plants, is one of the polysaccharides. It is a highly stable compound but can be decomposed and utilized by many fungi as a carbon source. SATOH (1930) and SETO (1936) already reported that some plant-pathogenic fungi could decompose cellulose, and that *Piricularia Oryzae* also did slightly.

The writers examined the nutritive value of cellulose as a carbon source in the present cultural studies of *Piricularia Oryzae*. The cellulose used in this experiment was the "Toyo" filter paper manufactured for general use in quantitative chemical analysis. Fifty cc. of sterilized standard solution were poured into a PETRI-dish which contained a sheet of the filter paper under a strictly germ free condition. The filter paper was rested on a VAN TIEGHEM cell to prevent it from sinking and to keep it situated just at the surface of the culture solution. The fungus was inoculated at the centre of the filter paper. Cultures in triplicate were incubated in a thermostat at 28°C. for 30 days.

After the culture, the solution was strained through filter paper, and the filtrate was examined by adding FEHLING's solution to

ascertain the existence of reducing sugars. If the decomposition of cellulose had taken place in the culture solution by the growth of the fungus, the occurrence of the reducing sugars should be proved by an addition of FEHLING's solution. For control, filter paper was immersed in the nitrogen free cultural solution which was prepared by eliminating potassium nitrate from the standard mineral salts solution. The results of the experiments are shown in the following Table 8.

TABLE 8. The reduction of FEHLING'S solution due to reducing sugars produced by the decomposition of cellulose

Culture solution	pH		Reduction of FEHLING'S solution	Growth of the fungus
	Initial	Final		
Standard mineral salts solution	6.3	6.3	+	+
Containing no N-compound (control)	6.3	6.0	−	−

In the culture solution containing potassium nitrate, the fungus grew in drab-gray colored colonies on the immersed filter paper. By an addition of FEHLING's solution to the filtrate of the stale culture solution reddish precipitations of cuprous oxide was caused. This should be the proof of the production of reducing sugars owing to the decomposition of cellulose by the fungus. At the same time, in the solution containing no nitrogen compound neither the growth of the fungus nor the reduction of FEHLING's solution occurred. These experiments were repeated three times and quite similar results were obtained. From these results of the experiments it was concluded that *Piricularia Oryzae* decomposes cellulose and utilizes it as carbon source.

On the whole it was concluded that among these carbohydrates, maltose, soluble starch and glucose were excellently suitable sources of carbon for *Piricularia Oryzae.*

2. Cultural experiments on higher alcohols

It is generally accepted that higher alcohols are more or less suitable carbon sources for fungi. In the present experiments,

glycerine [$CH_2(OH)CH(OH)CH_2OH$] and mannite [$CH_2(OH)CH(OH)CH(OH)CH(OH)CH(OH)CH_2OH$] were used as carbon sources in concentrations of 2%, 1%, 0.5%, 0.25% and 0.125%. The experimental methods were the same as in the preceding experiments concerning sugars. The results of cultures for 30 days are given in the following Table 9. The dry weights of the developed mycelium shown in the table represent average of three cultures in each series of two experiments.

TABLE 9. Results of the cultural experiments on glycerine and mannite as carbon sources

Carbon source	%tage	pH		Dry weight of colony (mg.)		Color of colony
		Initial	Final		average	
Glycerine	2	6.1	6.7 6.7	14.2 13.6	13.90	Smoke gray or light grayish olive
	1	6.1	6.6 6.6	5.6 5.4	5.50	Ditto
	0.5	6.1	6.4 6.5	3.9 4.2	4.05	Light grayish olive
	0.25	6.1	6.3 6.3	3.5 3.5	3.50	Light grayish olive or white
	0.125	6.1	6.2 6.3	2.2 2.3	2.25	Ditto
Mannite	2	6.1	6.7 6.6	12.8 11.9	12.35	Mouse gray
	1	6.1	6.4 6.4	5.8 6.1	5.95	Ditto
	0.5	6.1	6.4 6.4	6.3 6.1	6.20	Mouse gray or pallid gray
	0.25	6.1	6.3 6.3	2.8 2.2	2.50	Ditto
	0.125	6.1	6.3 6.3	2.2 2.0	2.10	Mouse gray

In every concentration of glycerine or mannite adopted in the present experiments, the fungus grew fairly well, and the mycelial

development became better with an increase of the concentration of these compounds up to 2%, the maximum in the present scope of experiment. The fungus colonies were round in shape and of light color. In most cases, the development of aerial mycelium took place, it being especially remarkable in the solutions containing 2% and 1% glycerine, and 2% mannite. The spore production was by no means recognized.

3. Cultural experiments on organic acids

Organic acids are widely found in the cells of stems, leaves or fruits of various plants, in free state or in compound forms. The cell sap of plant tissues generally shows more or less acid reaction, owing generally to the presence of organic acids.

In culture solutions, however, free organic acids unavoidably increase the acidity, and in this connection they retard the growth of fungi notwithstanding their fundamentally nutritive nature for fungi. It is interesting to study the relation of fundamental nutritiousness and reactional noxiousness of organic acids upon the mycelial development of *Piricularia Oryzae*.

In the present experiments the following organic acids were examined as carbon sources in the cultures of the fungus.

Formic acid	$H \cdot COOH$
Acetic acid	$CH_3 \cdot COOH$
Oxalic acid	$COOH \cdot COOH$
Succinic acid	$COOH \cdot CH_2 \cdot CH_2 \cdot COOH$
Fumaric acid	$COOH \cdot CH : CH \cdot COOH$
Lactic acid	$CH_3 \cdot CHOH \cdot COOH$
Malic acid	$COOH \cdot CH_2 \cdot CHOH \cdot COOH$
Citric acid	$CH_2COOH \cdot C(OH)(COOH) \cdot CH_2COOH \cdot H_2O$

These organic acids were added to the standard mineral salts solution as the carbon source in concentrations of 1%, 0.5%, 0.1%, 0.05% and 0.01%. Fifty cc. portions of each of the cultural solutions containing every organic acid in various concentrations were taken in ERLENMEYER's flasks and sterilized in a KOCH's steam sterilizer. The cultures were started by inoculation with a bit of mycelium and were incubated in a thermostat at 28°C.

After 30 days' incubation, the growth of the fungus was examined; average of three cultures for each and every organic acid are shown in the following Table 10.

TABLE 10. Results of cultural experiments on organic acids

Organic acid	Concentration (%)	pH of the cult. solution		Fungus growth
		Initial	Final	
Formic acid	1	*	*	no growth
	0.5	*	*	,,
	0.1	3.1	3.1	,,
	0.05	3.4	3.4	,,
	0.01	4.5	4.3	,,
Acetic acid	1	*	3.2	no growth
	0.5	3.4	3.4	,,
	0.1	4.0	4.0	,,
	0.05	4.4	4.3	,,
	0.01	5.3	6.4	moderate
Oxalic acid	1	*	*	no growth
	0.5	*	*	,,
	0.1	*	*	,,
	0.05	3.0	3.0	,,
	0.01	5.2	6.3	slight
Succinic acid	1	*	3.0	no growth
	0.5	3.0	3.2	,,
	0.1	3.9	3.8	,,
	0.05	4.3	4.6	slight
	0.01	5.4	5.9	a little
Fumaric acid	1	*	*	no growth
	0.5	*	*	,,
	0.1	3.0	3.0	,,
	0.05	3.3	3.4	,,
	0.01	5.2	5.7	slight
Lactic acid	1	*	*	no growth
	0.5	*	*	,,
	0.1	3.3	3.4	,,
	0.05	3.6	3.6	,,
	0.01	4.2	4.1	,,

TABLE 10. (*Continued*)

Organic acid	Concentration (%)	pH of the cult. solution		Fungen growth
		Initial	Final	
Malic acid	1	*	*	no growth
	0.5	3.0	3.0	,,
	0.1	3.4	3.4	,,
	0.05	3.9	4.8	slight
	0.01	5.2	6.3	a little
Citric acid	1	*	*	no growth
	0.5	*	3.0	,,
	0.1	3.5	3.5	,,
	0.05	4.0	4.7	slight
	0.01	5.7	6.1	a little

* H-ion concentration of solution was higher than pH 3.0.

In these cultures the development of the fungus was markedly retarded, and in the solutions containing formic acid or lactic acid it was utterly checked. In other cases very poor growths of the fungus were recognized at the least or next least concentrations of the organic acid. Among these a comparatively good mycelial growth was observed in the culture solution containing 0.01% acetic acid, in which case the fungus colony was about 15 mm. in diameter and smoke gray in color. In the culture solution containing 0.01% malic acid, 0.01% citric acid, 0.01% succinic acid and 0.01% oxalic acid respectively the developments of the fungus were slight and the mycelial colonies were meagre and white in color.

The retardation or poor growth of the fungus in the cultural solutions containing these organic acids should possibly be attributed to the unfavourable influences of the high acidity of the solution. Having this point in mind the writers undertook the next cultural experiments with nutrient solutions containing similar organic acids and being regulated in the hydrogen ion concentrations.

The standard mineral salt solution was concentrated by reducing the amount of redistilled water to 600 cc. instead of the usual 1000 cc. By additions of regulated amounts of alkali solution and redistilled water to the concentrated standard solution, the hydrogen ion concentrations of the solutions were adjusted without affecting substantially the concentrations of the nutrient constituents.

Culture solutions containing each organic acid in various concentrations were prepared by adding varying amounts of organic acids to 120 cc. of the above mentioned standard solution. The amount of organic acids to be added to 120 cc. of the standard solution to make the desired concentrations of the organic acids are as follows:

Concentrations of organic acids after added alkali solution and water to make the whole volume 200 cc. (%)	Amounts of organic acids to be added to 120 cc. of standard solution (g.)
1	2.0
0.5	1.0
0.1	0.2
0.05	0.1
0.01	0.02

Thirty cc. portions of these solutions were taken in each of four ERLENMEYER's flasks, and sterilized in a KOCH's sterilizer for 30 minutes twice with one day's interval. One of these four flasks was used for the determination of the initial hydrogen ion concentration of the solution, and the other three flasks were used for cultures.

The initial hydrogen ion concentration of the culture solutions was regulated to the approximate optimum for the development of the fungus by adding sodium hydroxide solutions and redistilled water, as shown in Table 11. Sodium hydroxide solution and redistilled water were previously sterilized and the mixing procedures were carried out under a strict germ free condition. Every culture was started by an inoculation with a bit of mycelium and incubated in a thermostat at 28°C. for 30 days. The results are shown in the Table 12.

These cultures were repeated two times, from August to September and from January to February. The dry weights shown in the tables represent an average of three cultures in each of these two experiments. The degree of fungus growth was substantially determined according to the size of the colony. The signs in the tables have the following significations: — no growth, ± slight growth, + a little growth, the colony of ca. 5 mm. diameter, ++ colony of ca. 10 mm. diameter, and +++ colony of ca. 15 mm. diameter.

TABLE 11. Showing the measures of adjustments of H-ion concentration of the culture solution containing organic acid in various concentrations

Organic acid		Standard solution (cc.)	NaOH solution		Vol. of redist. water added to make 50 cc. (cc.)	pH
Kinds	conc. (%)		conc.	vol. (cc.)		
Formic acid	1	30	N/2	20.0	0	6.0—6.1
	0.5	30	N/2	10.6	9.4	6.3—6.4
	0.1	30	N/4	4.2	15.8	6.2—6.3
	0.05	30	N/4	2.3	17.7	6.2—6.3
	0.01	30	N/20	2.5	17.5	6.2—6.3
Acetic acid	1	30	N/2	17.0	3.0	6.3—6.4
	0.5	30	N/4	18.2	1.8	6.3—6.4
	0.1	30	N/4	3.4	16.6	6.2—6.3
	0.05	30	N/20	9.0	11.0	6.2—6.3
	0.01	30	N/20	2.0	18.0	6.2—6.3
Oxalic acid	1	30	N/2	16.3	3.7	6.3—6.8
	0.5	30	N/4	15.5	4.5	6.0—6.2
	0.1	30	N/4	3.0	17.5	6.1—6.4
	0.05	30	N/20	7.9	12.1	6.3—6.4
	0.01	30	N/20	1.7	18.3	6.3—6.4
Succinic acid	1	30	N/2	16.3	3.7	6.2—6.3
	0.5	30	N/4	16.5	3.5	6.2—6.4
	0.1	30	N/4	3.3	16.7	6.4—6.5
	0.05	30	N/20	7.8	12.2	6.2—6.3
	0.01	30	N/20	1.75	18.25	6.0—6.4
Fumaric acid	1	30	N/2	17.5	2.5	6.4—6.5
	0.5	30	N/4	17.5	2.5	6.3—6.4
	0.1	30	N/4	3.2	16.8	6.1—6.3
	0.05	30	N/20	8.5	11.5	6.3—6.4
	0.01	30	N/20	1.7	18.3	6.2—6.3
Lactic acid	1	30	N/2	10.3	9.7	6.2—6.3
	0.5	30	N/4	11.25	8.75	6.2—6.3
	0.1	30	N/4	2.1	17.9	6.0—6.3
	0.05	30	N/20	7.2	12.8	6.2—6.3
	0.01	30	N/20	2.7	17.3	6.1—6.2

TABLE 11. (*Continued*)

Organic acid		Standard solution (cc.)	NaOH solution		Vol. of redist. water added. to make 50 cc. (cc.)	pH
Kinds	conc. (%)		conc.	vol. cc.		
Malic acid	1	30	N/2	15.5	4.5	6.5—6.6
	0.5	30	N/4	15.25	4.75	6.4—6.5
	0.1	30	N/20	15.2	4.8	6.3—6.5
	0.05	30	N/20	7.7	12.3	6.3—6.5
	0.01	30	N/20	1.6	18.4	6.3—6.4
Citric acid	1	30	N/2	14.0	6.0	6.1—6.4
	0.5	30	N/4	13.5	6.5	6.1—6.3
	0.1	30	N/20	13.5	6.5	6.3—6.4
	0.05	30	N/20	6.7	13.3	6.3—6.4
	0.01	30	N/20	1.35	18.65	6.1—6.3

TABLE 12. Development of the fungus in the culture solutions containing organic acids in various concentrations and with regulated H-ion concentrations

Organic acid	Conc. (%)	H-ion conc. of the culture solution		Growth of the fungus		
		Initial	Final	Degree	Dry wt. (mg.)	Color of colony
Formic acid	1	6.0—6.1	6.0 6.2	— —	— —	
	0.5	6.3—6.4	6.9 6.9	+ +	* *	White
	0.1	6.2—6.3	6.9 7.0	+± +±	* *	White or drab gray
	0.05	6 2—6.3	6.7 6.7	+± +±	* *	White
	0.01	6.2—6.3	6.3 6.3	± ±	* *	White
Acetic acid	1	6.3—6.4	6.3 6.4	— —	— —	
	0.5	6.3—6.4	6.2 6.5	— ±	— *	White
	0.1	6.2—6.3	6.4 6.3	± ±	* *	White
	0.05	6.2—6.3	6.5 6.5	+± +±	0.7 0.9	White
	0.01	6.2—6.3	6.7 6.5	++ +±	1.2 1.0	White

TABLE 12. (*Continued*)

Organic acid	Conc. (%)	H-ion conc. of the culture solution		Growth of the fungus		
		Initial	Final	Degree	Dry wt. (mg.)	Color of colony
Fumaric acid	1	6.4—6.5	6.6 7.0	++ +++	0.9 1.1	Light grayish olive
	0.5	6.3—6.4	7.6 7.5	++ +++	1.6 1.7	Smoke gray
	0.1	6.1—6.3	6.7 6.9	+++ +++	1.5 1.6	Light grayish olive
	0.05	6.3—6.4	6.3 6.6	+ +	* *	White or pale smoke gray
	0.01	6.2—6.3	7.0 6.8	+ +	* *	White or pale smoke gray
Lactic acid	1	6.2—6.3	5.8 5.9	— —	— —	
	0.5	6.2—6.3	5.8 5.8	— —	— —	
	0.1	6.0—6.3	5.9 6.6	± ±±	1.2 1.3	White or smoke gray
	0.05	6.2—6.3	6.1 7.0	± ±	* *	do.
	0.01	6.1—6.2	6.6 6.1	± ±	* *	White
Oxalic acid	1	6.3—6.8	8.4 8.1	± ±	* *	White
	0.5	6.0—6.2	7.9 8.4	± ±	* *	White
	0.1	6.1—6.4	7.0 6.9	± ±	* *	White
	0.05	6.3—6.4	7.7 7.9	± ±	* *	White
	0.01	6.3—6.4	6.8 6.4	± ±	* *	White
Succinic acid	1	6.0—6.4	6.5 6.5	± ±	* *	White
	0.5	6.2—6.4	7.0 6.6	+++ ++	1.5 1.2	Pale olive gray or white
	0.1	6.4—6.5	6.6 6.5	± ±	1.1 1.0	Olivaceous black
	0.05	6.2—6.3	6.8 6.6	± +	0.8 0.7	Olivaceous black
	0.01	6.0—6.4	6.4 6.9	+ ±	0.7 0.9	White or drab gray

TABLE 12. (*Continued*)

Organic acid	Conc. (%)	H-ion conc. of the culture solution		Growth of the fungus		
		Initial	Final	Degree	Dry wt. (mg.)	Color of colony
Malic acid	1	6.5—6.6	7.7 8.2	± +	* *	White
	0.5	6.4 · 6.5	7.0 8.4	+ ++	0.7 0.9	White or smoke gray
	0.1	6.3—6.5	7.0 6.9	+± +±	1.0 0.8	Smoke gray
	0.05	6.3—6.5	7.4 7.3	+ +	* *	White
	0.01	6.3—6.4	6.4 6.6	± +	* *	White
Citric acid	1	6.1—6.4	6.6 6.6	± ±	* *	White
	0.5	6.1—6.3	6.3 6.3	± ±	* *	White or deep grayish olive
	0.1	6.3—6.4	6.4 6.6	+ ++	0.8 1.0	White or light grayish olive
	0.05	6.3—6.4	6.5 6.6	+ +±	* *	Deep grayish olive or white
	0.01	6.1—6.3	6.5 6.6	+ +	* *	do.

* The dry weight of fungus mat was less than 0.7 mg.

From these results it was concluded that when the initial hydrogen ion concentration of the culture solutions containing organic acids was regulated to near the optimum for the growth of the fungus, the organic acids served as the carbon source and were utilized to some extent by the fungus more or less causing its mycelial development. In general, however, organic acids are by no means very suitable carbon sources for *Piricularia Aryzae*, judging from the poor developments of the fungus in the present experiments, in which even the best developed colony was small in size and its dry weight was less than 2 mg.

Among these organic acids examined, fumaric acid was the best as the source of carbon for nutrition of the fungus. In the solutions containing fumaric acid, the fungus grew fairly well and the mycelial colonies were comparatively deep in color. Succinic acid was the next best, with lactic, malic, citric and acetic acid following in order. Oxalic acid and formic acid were unfavourable

for the growth of the fungus and the mycelial colonies developed in the culture solutions containing these organic acids were less than 0.7 mg. in dry weights.

The concentrations of organic acids which decidedly influenced the growth of the fungus probably resulted from the acid-molecules themselves and not from hydrogen ions. One per cent and 0.5% lactic acid and acetic acid, and 1% formic acid entirely prevented the mycelial development of the fungus. In general, 0.1% seemed to be the favourable concentration of these acids for the mycelial development of the fungus. But, in fumaric acid and succinic acid 0.5% and in acetic acid 0.01% were the favourable concentrations in this regard.

In the cases of liquid cultures the colonies usually sank in the solution, but in some cases of the present cultures concerning organic acids the colonies did not sink and aerial mycelia developed a little on them, in spite of their meagre development. This phenomenon was recognized clearly in the cultures containing citric acid, succinic acid or formic acid.

When the fungus grew fairly well, the hydrogen ion concentrations of the culture solutions were generally decreased, but when the fungus did not grow, the reaction of the solution remained almost unchanged.

4. Conclusion

Looking over the results of these experiments, it may be stated that the carbohydrates and higher alcohols were generally suitable carbon sources in the nutrition of *Piricularia Oryzae,* while the organic acids were in all respects improper. Among these carbon sources, maltose was the most nutritious and soluble starch, glucose, glycerine and mannite following it in order.

In general, in the cultural solutions containing disaccharides, polysaccharides, and higher alcohols the fungus growth became better with increases of concentrations of carbon sources within the limit adopted in the present experiments. In the cases of monosaccharides and organic acids, however, no simple relation was found between the concentration of the carbon sources and the growth of the fungus.

In comparison of dry weights of well developed mycelial colonies, which exceeded 5 mg., the kinds of carbon sources and their concentrations were enumerated in order in Table 13.

TABLE 13. Ordinal enumeration of dry weights of mycelial colonies developed well in relation to the kinds of carbon sources and their concentrations

Order	Carbon source and its concentration		Dry weight (mg.)
1	Maltose	0.25 mol.	33.1
2	Soluble starch	5.00 %	31.0
3	Soluble starch	2.00 %	19.9
4	Maltose	0.70 mol.	19.0
5	Maltose	0.30 mol.	16.4
6	Glucose	0.05 mol.	15.7
7	Soluble starch	0.50 %	15.1
8	Glycerine	2.00 %	13.9
9	Glucose	0.10 mol.	12.7
10	Mannite	2.00 %	12.4
11	Glucose	0.30 mol.	9.0
12	Soluble starch	0.25 %	9.0
13	Sucrose	0.25 mol.	6.8
14	Maltose	0.05 mol.	6.3
15	Mannite	0.50 %	6.2
16	Mannite	1.00 %	5.9
17	Lactose	0.25 mol.	5.9
18	Soluble starch	0.125 %	5.8
19	Sucrose	0.15 mol.	5.7
20	Galactose	0.25 mol.	5.5
21	Glycerine	1.00 %	5.5
22	Lactose	0.35 mol.	5.3
23	Sucrose	0.35 mol.	5.2

VI. Cultural studies concerning nitrogen sources

The nitrogen compounds are generally indispensable in the nutrition of fungi. Fungi can utilize nitrogen from either inorganic or organic compounds, and in particular cases some of them assimilate free nitrogen from the air.

The present experiments were carried out to study the nitrogenous nutrition of *Piricularia Oryzae* in detail regarding various kinds of organic and inorganic nitrogen compounds.

The kinds of nitrogenous compounds used as nitrogen sources in the present cultural studies were as follows:

Inorganic nitrogen compounds	
Potassium nitrate	KNO_3
Sodium nitrate	$NaNO_3$
Calcium nitrate	$Ca(NO_3)_2$
Ammonium nitrate	NH_4NO_3
Ammonium sulphate	$(NH_4)_2SO_4$
Ammonium phosphate (dibasic)	$(NH_4)_2HPO_4$
Potassium nitrite	KNO_2
Sodium nitrite	$NaNO_2$
Organic nitrogen compounds	
Peptone	
Urea	$CO(NH_2)_2$
Acetamide	$CH_3 \cdot CO \cdot NH_2$
Glycocol	$CH_2 \cdot NH_2 \cdot COOH$
Asparagine	$COOH \cdot CH_2 \cdot CHNH_2 \cdot CONH_2$
Glutamic acid	$COOH \cdot CH_2 \cdot CH_2CHNH_2 \cdot COOH$
Ammonium salts of organic acids	
Ammonium acetate	$C_2H_3O_2NH_4$
Ammonium oxalate	$C_2O_4(NH_4)_2 \cdot H_2O$
Ammonium tartarate	$C_4H_4O_6(NH_4)_2 \cdot 4H_2O$

The standard solution was prepared by excluding potassium nitrate and adding 3% sucrose in the mineral salts solution used in the preceding experiments. Each nitrogen compound mentioned above was added to the standard solution in the concentrations of 2%, 1%, 0.5%, 0.25%, and 0.125% to prepare the culture solutions.

Thirty cc. of the culture solutions containing each of these nitrogen sources in various concentrations were taken in ERLENMEYER's flasks and sterilized in a KOCH's steam sterilizer for 30 minutes twice with one day's interval. Every culture in triplicate was started by an inoculation with a bit of mycelium, and incubated for 30 days in a thermostat at 28°C.

The experiments were repeated twice, from September to October and from December to January. The results of these cultures are given in Table 14. In the table, the dry weights of the fungus colonies represent averages of 3 cultures in each of these two experiments, and the signs −, ± and + mean no, slight, and a little growth of the fungus, respectively.

TABLE 14. Mycelial development of *P. Oryzae* in culture solutions containing different nitrogen compounds

Nitrogen source	Concentration (%)	H-ion conc. of culture-sol.		Dry weight of fungus mat		Color of fungus mat
		Initial	Final		Average (mg.)	
KNO_3	2	6.2	6.2 5.9	+ +		White or olivaceous black
	1	6.3	5.9 5.6	1.2 1.0	1.10	White or olivaceous black
	0.5	6.3	5.8 5.8	2.7 2.8	2.75	Olivaceous black or pale olive
	0.25	6.3	5.7 5.7	4.3 5.1	4.70	Dark grayish olive
	0.125	6.3	5.8 5.8	3.3 3.5	3.40	Dark grayish olive
$NaNO_3$	2	6.1	6.0 5.9	+ +		White or light olive
	1	6.1	5.9 5.9	2.1 1.9	2.00	Olive or light olive
	0.5	6.2	5.7 5.3	3.0 3.2	3.10	Olivaceous black or deep grayish olive
	0.25	6.2	5.5 5.2	8.4 9.0	8.70	Olivaceous black or deep grayish olive
	0.125	6.3	5.5 5.5	7.3 8.5	7.90	Olivaceous black or deep grayish olive

TABLE 14. (*Continued*)

Nitrogen source	Concentration (%)	H-ion conc. of culture-sol.		Dry weight of fungus mat (mg.)		Color of fungus mat
		Initial	Final		Average	
$Ca(NO_3)_2$	2	5.2	5.0 4.9	— —		
	1	5.3	5.2 5.1	1.2 0.0	0.60	Light olive or white
	0.5	5.4	5.1 5.3	1.7 1.1	1.40	Olivaceous black
	0.25	5.5	5.0 4.6	2.8 2.5	2.65	Olivaceous black or light olive gray
	0.125	5.7	5.2 5.4	2.1 1.9	2.00	Olivaceous black
NH_4NO_3	2	5.9	5.7 5.2	+ —		
	1	6.0	5.7 4.5	1.0 0.8	0.90	White or light brownish olive
	0.5	6.0	5.6 4.4	1.5 1.7	1.60	Light grayish olive or olivaceous black
	0.25	6.0	5.1 5.0	2.4 1.1	1.75	Grayish or olivaceous black
	0.125	6.0	4.6 4.4	2.8 3.3	3.05	Deep grayish olive or olivaceous black
$(NH_4)_2HPO_4$	2	7.5	7.2 7.1	— —		
	1	7.4	7.1 7.0	± ±		Sepia or white
	0.5	7.3	6.9 6.9	± —		Wood brown or white
	0.25	7.2	6.8 6.6	1.4 1.3	1.35	Wood brown
	0.125	7.0	6.5 6.3	2.3 2.1	2.20	Olivaceous black
$(NH_4)_2SO_4$	2	5.8	5.1 4.4	1.7 2.0	1.85	Olivaceous black or white
	1	5.9	4.5 4.4	2.2 2.4	2.30	Olivaceous black or grayish olive
	0.5	6.0	5.1 4.7	2.7 2.5	2.60	Deep grayish olive
	0.25	6.1	5.5 5.5	1.4 1.0	1.20	Deep grayish olive
	0.125	6.1	4.7 4.1	3.9 4.1	4.00	Deep grayish olive

TABLE 14. (*Continued*)

Nitrogen source	Concentration (%)	H-ion conc. of culture-sol.		Dry weight of fungus mat		Color of fungus mat
		Initial	Final		Average (mg.)	
KNO_2	2	7.0	7.5 7.2	— —		
	1	6.8	7.0 7.0	— —		
	0.5	6.5	6.7 6.5	— —		
	0.25	6.3	6.4 6.3	— —		
	0.125	6.3	6.3 6.3	± +		Brownish olive
$NaNO_2$	2	6.7	7.0 6.9	— —		
	1	6.5	6.8 6.8	— —		
	0.5	6.4	6.7 6.6	— —		
	0.25	6.3	6.6 6.5	— —		
	0.125	6.3	6.5 6.4	± ±		White
Urea	2	8.4	8.2 8.0	— —		
	1	8.2	8.0 8.0	± —		White
	0.5	7.8	7.8 7.7	± —		White
	0.25	7.4	7.5 7.6	± +		White or brownish gray
	0.125	7.1	7.4 7.5	± ±		White
Peptone	2	6.6	5.0 5.4	165.6 190.0	177.8	Cream or light grayish olive
	1	6.6	5.3 5.2	151.1 119.0	135.05	Cream or light grayish olive
	0.5	6.6	5.1 4.8	68.6 77.1	72.85	Light grayish olive or pale olive-buff
	0.25	6.5	4.6 4.4	57.3 54.0	55.65	Light grayish olive or dark grayish olive
	0.125	6.4	4.5 4.0	44.1 43.8	43.95	Ditto

TABLE 14. (*Continued*)

Nitrogen source	Concentration (%)	H-ion conc. of culture-sol. Initial	H-ion conc. of culture-sol. Final	Dry weight of fungus mat	Dry weight of fungus mat Average	Color of fungus mat
Asparagine	2	5.5	5.5 5.2	4.9 5.3	5.10	Olivaceous black
	1	5.7	5.9 5.4	5.5 6.0	5.75	Olivaceous black
	0.5	5.9	5.8 6.1	8.2 7.5	7.85	Olivaceous black
	0.25	6.1	6.1 5.9	4.1 3.6	3.85	Olivaceous black
	0.125	6.2	6.3 6.4	3.3 3.6	3.45	Olivaceous black
Glutamic acid			no growth			
Acetamide	2	4.6	4.7 4.6	— —		
	1	4.8	4.7 4.7	— —		
	0.5	5.2	5.7 5.9	2.4 2.7	2.55	White or smoke gray
	0.25	5.6	5.4 5.5	4.2 4.8	4.50	Grayish olive
	0.125	6.2	4.9 4.7	6.0 6.4	6.20	Light grayish olive or dark grayish olive
Glycocol	2	6.2	5.9 5.8	2.8 3.1	2.95	Grayish olive or yellowish olive
	1	6.2	5 9 5.7	4.5 4.6	4.55	Olivaceous black or yellowish olive
	0.5	6.4	5.8 5.3	3.8 4.4	4.10	Light grayish olive or deep olive
	0.25	6.3	5.9 5.8	3.0 3.2	3.10	Olivaceous black or deep olive
	0.125	6.4	5.0 5.7	2.9 4.0	3.45	Olivaceous black or deep olive
Ammonium oxalate	2	6.4	6.0 5.7	+ 1.8	0.90	Grayish olive or dark olive-buff
	1	6.6	5.9 6.1	1.9 2.4	2.15	Olive brown
	0.5	6.6	5.8 6.3	2.7 3.0	2.85	Pale smoke gray or dark olive
	0.25	6.4	5.7 6.1	4.9 5.1	5.00	Dark olive
	0.125	6.3	6.0 5.9	3.6 4.4	4.00	Olivaceous black or deep grayish olive

TABEL 14. (*Continued*)

Nitrogen source	Concentration (%)	H-ion conc. of culture-sol.		Dry weight of fungus mat		Color of fungus mat
		Initial	Final	(mg.)	Average	
Ammonium acetate	2	6.2	6.3 6.2	— —		
	1	6.1	6.3 6.3	— —		
	0.5	6.1	6.3 6.3	— —		
	0.25	6.1	6.3 6.1	— ±		White
	0.125	6.1	6.2 6.0	± +		White
Ammonium tartarate	2	6.5	6.0 5.7	+ 3.5	1.75	Light grayish olive
	1	6.3	6.0 5.9	2.1 3.8	2.95	Olivaceous black or deep olive
	0.5	6.3	5.9 5.9	2.8 3.5	3.15	Olivaceous black
	0.25	6.3	5.7 5.4	3.7 4.0	3.85	Olivaceous black or yellowish olive
	0.125	6.4	6.0 5.9	4.1 4.4	4.25	Olivaceous black

After 30 days' incubation, the fungus grew more or less in most culture solution in round mycelial masses. The aerial mycelium developed a little only in the culture solution containing peptone, and in the other cultures it was never observed. The spore production was not observed at all in any culture.

Among the inorganic nitrogen compounds, the sodium nitrate was the best nutrient for the mycelial development of the fungus, and potassium nitrate, ammonium sulphate, ammonium nitrate, calcium nitrate and dibasic ammonium phosphate followed it in succession. No marked difference of the nutritive value was recognized between nitrate and ammonium, but in detailed comparisons the nitrates seemed to be generally a little more suitable than the ammoniums. Nitrite (NO_2) is generally considered to be more or less poisonous for a large majority of fungi. Nevertheless, the preseint fungus grew slightly in the culture solutions containing either sodium nitrite or potassium nitrite in the concen-

tration of 0.125%. In the solutions containing more nitrites, however, no development occurred. From these results it would be concluded that in such low concentration of nitrite in the culture solution, *Piricularia Oryzae* not only can stand its poisonous influence but utilizes this compound, though slightly, as a nitrogen source.

Among organic nitrogen compounds, peptone was matchlessly nutritious for the fungus, and the mycelial developments in the culture solutions containing various amounts of peptone were extraordinarily vigorous. Asparagine, acetamide and glycocol were anyway good nitrogen sources. Urea is generally considered to be a good nitrogen source for fungi, but in the case of this fungus the mycelial development was not favoured by supply of this compound contrary to the writers' expectation.

Among the ammonium salts of organic acids, ammonium oxalate and ammonium tartarate were proved to be moderately suitable nutrient for the mycelial development of the fungus. In the case of ammonium acetate, however, the fungus developed only slightly at its lower concentrations in the culture solution. It might be due to the unfavourable influence of the acetate radical of the compound.

Looking over the results obtained in the cultural experiments of nitrogen sources, the growth of the fungus on peptone was matchlessly vigorous and it should be regarded as an exceptional case.

Among the compounds other than peptone, sodium nitrate and asparagine were the most suitable for the nitrogenous nutrition of the fungus, with acetamide, ammonium oxalate, potassium nitrate, glycocol, ammonium tartarate, ammonium sulphate and ammonium nitrate following them in succession, while calcium nitrate, dibasic ammonium phosphate, urea and ammonium acetate were by no means suitable nutrients as the sources of nitrogen for the fungus.

The concentrations of nitrogen compounds in the culture solutions influenced the growth of the fungus. In most cases inorganic nitrogen compounds checked or retarded the fungus growth at concentrations higher than 1%, excepting the case of ammonium sulphate which allowed a moderate mycelial development of the fungus even at 2% concentration. At lower concentrations of these salts the fungus grew gradually better. On the contrary, in the solutions containing organic nitrogen compounds or ammonium salts

of organic acids, the concentrations of these compounds, in general, did not much affect the fungus growth. Acetamide, however, entirely checked the fungus growth at concentrations higher than 1% in the culture solution and the best growth was attained at the lowest concentration i.e., 0.125%. In the case of peptone, the better fungus growth was secured at its higher concentrations. In the cases of asparagine and glycocol the best growth were attained at 0.5% and 1% concentrations respectively.

On the whole it should be understood that the most favourable concentrations of the nitrogen sources for the development of the fungus were different according to the kinds of the compounds.

On the other hand, the fungus growth was affected by the initial hydrogen ion concentrations of the culture solutions containing each of the nitrogen compounds. When the initial hydrogen ion concentrations of the solutions were maintained within the suitable ranges, namely pH 6.0–6.5, the fungus growth was good in general, otherwise it was more or less retarded as observed in the cases of calcium nitrate, dibasic ammonium phosphate and urea.

In these experiments, the fungus did not grow at all in the solutions containing glutamic acid as a nitrogen source owing possibly to the comparatively high acidity of the culture solutions derived by dissociation of this amino-acid. The initial hydrogen ion concentrations of the solutions containing glutamic acid are recorded in the following table.

TABLE 15. Hydrogen ion concentrations of the culture solutions containing varying amounts of glutamic acid

Concentration of glutamic acid	pH of the culture solution
2 %	3.3
1 %	3.4
0.5 %	3.5
0.25 %	3.7
0.125 %	3.9

These hydrogen ion concentrations of the solution were substantially too high for the mycelial development of *Piricularia*

Oryzae, so an attempt to correct them to the approximate optimum for the fungus growth was made by adding alkali solutions.

The concentrated standard solution was prepared by decreasing the amount of redistilled water to 600 cc. instead of the ordinary 1000 cc. Certain amounts of glutamic acid, sodium hydroxide solution and redistilled water were added to 30 cc. of the concentrated standard solution, and 50 cc. culture solution at pH 6.2–6.3 in the hydrogen ion concentration and containing a definite amount of glutamic acid were prepared. Amounts of alkali solutions and redistilled water to be added to 30 cc. of concentrated standard solution to make the whole volume of the culture solution 50 cc. in each flask, and the hydrogen ion concentration of every culture solution are shown in the following table.

TABLE 16. Preparation of culture solutions containing various amounts of glutamic acid and holding the regulated hydrogen ion concentration

Percentage of glutamic acid (%)	Volume of standard solution (cc.)	NaOH solution		Volume of water added to make the whole volume 50cc. (cc.)	pH
		Concentration	Volume (cc.)		
2	30	N/2	13.2	6.8	6.2—6.3
1	30	N/4	13.3	6.7	6.3
0.5	30	N/4	6.5	13.35	6.2—6.3
0.25	30	N/20	16.6	3.4	6.2—6.3
0.125	30	N/20	8.3	11.7	6.3

TABLE 17. Growths of the fungus in the culture solutions with content of various amounts of glutamic acid and with hydrogen ion concentrations regulated to pH 6.2—6.3

Percentage of glutamic acid	pH		Dry weight of colony (mg.)	Color of colony
	Initial	Final		
2	6.2—6.3	5.9—6.0	5.03	Dark olive
1	6.3	5.8—5.9	7.20	Deep olive
0.5	6.2—6.3	6.0	4.03	Olivaceous black
0.25	6 2—6.3	6.1—6.2	3.90	,,
0.125	6.3	6.2—6.3	3.40	,,

The cultures were started by inoculation with a bit of mycelium and were incubated in a thermostat at 28°C. for 30 days. The results are shown in Table 17.

The dry weight of colony represented averages of three cultures. In 1% glutamic acid cultures the central part of the deep olive colored colonies was olivaceous black in color.

From these results, it was easily understood that glutamic acid is a suitable source of nitrogen in the nutrition of the fungus. When the hydrogen ion concentration of the culture solution containing glutamic acid had been left as it stands the fungus could not develop as shown in the preceding experiments, but in the present cases, the hydrogen ion concentrations having been properly regulated the fungus grew fairly well at any concentration of this amino acid. The mycelial colonies developed in the solutions were semispherical in shape and deep in color, and the dry weights of the colonies grown well were pretty large. However, neither aerial mycelium nor spore was produced.

The most favourable concentration of glutamic acid in the culture solution was 1% for the growth of the fungus, which was similar to the case of glycocol. Change of hydrogen ion concentration of the solutions due to the development of the fungus was not remarkable.

Conclusion

The organic nitrogen compounds were generally more suitable than the inorganic ones as nitrogen sources in the nutrition of this fungus. Peptone was extraordinarily nutritious, and it should be regarded as a particular nutrient for the present fungus. The amino-acids and amides with the exception of urea were favourable sources. Among the inorganic nitrogen compounds sodium nitrate proved to be the most avairable nutrient for the fungus. In general, good growths of the fungus were attained in the culture solutions containing organic nitrogen compounds in higher concentrations, while in the cases of inorganic ones the fungus grew better at their lower concentrations.

The hydrogen ion concentrations of the culture solutions used in the present experiment were increased in general by good developments of the fungus.

The relations between the dry weights of the developed mycelium, and the kinds and concentrations of nitrogen compounds are simply enumerated in the following table.

TABLE 18. Ordinal enumeration of nitrogen sources in relation to their nutritiousness for the mycelial development of *Piricularia Oryzae*

Order	Nitrogen source and its concentration (%)		Dry weight (mg.)
1	Peptone	2	117.80
2	,,	1	113.05
3	,,	0.5	72.85
4	,,	0.25	55.65
5	,,	0.125	43.95
6	Sodium nitrate	0.25	8.70
7	,,	0.125	7.90
8	Asparagine	0.5	7.85
9	Glutamic acid	1	7.20
10	Acetamide	0.125	6.20
11	Asparagine	1	5.75
12	,,	2	5.10
13	Glutamic acid	2	5.03
14	Ammonium oxalate	0.25	5.00
15	Potassium nitrate	0.25	4.70
16	Glycocol	1	4.55
17	Acetamide	0.25	4.50
18	Glycocol	0.5	4.10
19	Glutamic acid	0.5	4.03
20	Ammonium sulphate	0.125	4.00
21	Ammonium oxalate	0.125	4.00

The results of glutamic acid cultures were obtained at the emended hydrogen ion concentration of pH 6.2—6.3.

VII. The effects of ferric chloride and copper sulphate upon the mycelial development of the fungus in the nutrient solution

It is an interesting fact that the influences of heavy metals upon the growth of fungi are sometimes markedly variable from stimulating to poisonous according to their concentrations in a nutrient solution. A number of studies have been carried out by many investigators in this connection. Concerning the present fungus in 1930–1931 T. ABE studied the influences of copper sulphate and iron sulphate upon the growth of its mycelial colony. He used potato decoction and its agar medium containing 1% sucrose for culturing the fungus, and concluded that in certain low concentrations either copper sulphate or iron sulphate stimulated the growth of the fungus.

In the present experiments, the writers tried to examine the effects of ferric chloride and copper sulphate upon the mycelial development of the fungus in synthetic culture solutions. The constitution of the standard nutrient solution used in the present experiments was as follows: potassium nitrate 2.0 g., monopotassium phosphate 0.5 g., dipotassium phosphate 0.5 g., magnesium sulphate 0.5 g., calcium chloride 0.1 g., sucrose 30 g. and redistilled water 1000 cc.

This standard solution was relatively concentrated by reducing the amount of redistilled water to 600 cc. instead of the usually 1000 cc., and 108 cc. of this concentrated solution was taken in each flask to which various amounts of 1/10 to 1/1000 mol. solutions of ferric chloride or copper sulphate and certain amounts of redistilled water were added in order to make up the whole volume of the culture solution in a flask to 120 cc.

The details of the preparation of culture solutions containing various amounts of ferric chloride or copper sulphate are shown in the following table.

As the control the culture solution containing no heavy metal salt was prepared.

Thirty cc. portions of these nutrient solutions containing ferric chloride or copper sulphate in various concentrations were taken in each of four ERLENMEYER's flasks, and then sterilized in a KOCH's steam sterilizer for 30 minutes twice with one day's interval. One

TABLE 19. Make up of culture solutions containing ferric chloride or copper sulphate in various concentrations

Concentration of $FeCl_3$ or $CuSO_4$ in culture solutions (mol.)	Volume of standard solution (cc.)	Solution of $FeCl_3$ or $CuSO_4$		Amounts of water added to make the whole volume up to 120cc. (cc.)
		Conc. (mol.)	Amount (cc.)	
1/800	108	1/10	1.5	10.5
1/1000	,,	,,	1.2	10.8
1/1200	,,	1/100	10.0	2.0
1/1600	,,	,,	7.5	4.5
1/2000	,,	,,	6.0	6.0
1/4000	,,	,,	3.0	9.0
1/6000	,,	,,	2.0	10.0
1/8000	,,	,,	1.5	10.5
1/10000	,,	,,	1.2	10.8
1/20000	,,	1/1000	6.0	6.0
1/40000	,,	,,	3.0	9.0
1/60000	,,	,,	2.0	10.0
1/80000	,,	,,	1.5	10.5
1/100000	,,	,,	1.2	10.8
Control	,,	—	—	12.0

of these four flasks was used to measure the initial hydrogen ion concentrations of the nutrient solutions, and other three were used for cultures. The cultures were carried out in a thermostat at 28°C. for thirty days.

1. Experiments on the influence of ferric chloride

The experiments concerning ferric chloride were carried out in concentrations of 1/1000, 1/1200, 1/1600, 1/2000, 1/4000, 1/6000, 1/8000, 1/10000, 1/20000, 1/40000, 1/80000 and 1/100000 mol.

The results of the cultures are shown in Table 20. In the table, the sign * shows the hydrogen ion concentration higher than pH 3.0, and the signs −, ±, and + mean no, slight, and a little growth respectively.

TABLE 20. The mycelial development of the fungus in nutrient solutions containing ferric chloride in various concentrations

Concentration of $FeCl_3$ (mol.)	H-ion concentration		Dry weight of colony (mg.)	Color of colony
	Initial (pH)	Final (pH)		
		3.0	–	
1/1000	*	3.0	–	
		3.0	–	
		3.2	–	
1/1200	3.4	3.2	–	
		3.2	–	
		3.9	–	
1/1600	4.6	4.0	–	
		4.0	1.6	Pale olive buff
		5.1	±	
1/2000	5.6	5.2	6.6	Olivaceous black
		5 4	2.2	Deep olive
		5.6	±	
1/4000	5.7–5.8	5.5	3.7	Deep olive
		4.8	6.8	Pale olive buff
		5.9	+	Olivaceous black
1/6000	5.9	5.7	+	,,
		5.9	±	,,
		6.0	±	Olivaceous black
1/8000	6.0–6.1	5.9	±	,,
		5.9	±	,,
		6.0	2.6	Olivaceous black
1/10000	6.1	5.9	2.1	,,
		6.0	±	,,
		5.7	5.1	Olivaceous black
1/20000	6.1–6.2	5.7	5.4	,,
		5.7	4.8	,,
		6.1	4.3	Olivaceous black
1/40000	6.2	6.1	3.9	,,
		6.2	4.4	,,
		6.1	2.9	Deep grayish olive
1/60000	6.2–6.3	5.9	3.6	Light grayish olive
		5.9	4.0	,,
		5.9	3.2	Deep grayish olive
1/80000	6.3	6.2	3.8	,,
		5.9	3.8	,,
		6.0	5.1	Olivaceous black
1/100000	6.3	6.1	4.4	Deep grayish olive
		5.9	3.7	,,
		6.0	4.8	Dark olive
Control	6.3–6.4	6.0	3.0	Deep grayigh olive
		6.0	3.5	,,

From these results, it may be presumed that the fungus seem to require ferric chloride in low concentrations, as 1/20000–1/100000 mol. in the culture solution. Especially the fungus grew well in the nutrient solutions containing this iron salt in a concentration of 1/20000 mol. This might be the reason why better growth of the fungus was attained in the culture solution containing a little ferric chloride in the cultural experiments described above under the subject of the standard nutrient solution in chapter IV.

On the other hand, the fungus did not grow at all at high concentrations of 1/1000 and 1/1200 mol. of this salt. Indeterminate growths of it were observed in the nutrient solutions containing ferric chloride in the concentrations of 1/2000 mol., 1/4000 mol., 1/6000 mol., 1/8000 mol. and 1/10000 mol. In some cases of the 1/2000 mol. and 1/4000 mol. concentrations, the fungus growth seemed to be stimulated by ferric chloride, but in other cases it was plainly retarded. The hydrogen ion concentrations of the nutrient solutions were markedly increased by addition of ferric chloride in high concentrations. Accordingly in the nutrient solutions containing ferric chloride in higher concentrations, the mycelial growth of the fungus might be affected not only by the metal ion but also by the excess of the hydrogen ion concentration.

2. Experiments on the influence of copper sulphate

The experiments concerning copper sulphate were carried out in concentrations of 1/800, 1/1000, 1/2000, 1/4000, 1/6000, 1/8000, 1/20000, 1/40000, 1/60000, 1/80000 and 1/100000 mol.

The results of these cultures are shown in Table 21. In the table, the signs −, ±, and + mean no, slight and a little growth respectively.

From these results, it was concluded that the mycelial development of the fungus was entirely prevented or partially retarded in the nutrient solutions containing copper sulphate in concentrations of 1/800 mol. to 1/8000 mol. In the cases of concentrations of copper sulphate being higher than 1/1000 mol., the fungus growth was entirely checked, while in some cases of the concentrations of 1/1000, 1/4000, 1/6000 1/8000 mol. the fungus grew a little. On the other hand, the mycelial development of the fungus seemed to be stimulated more or less by the existence of copper sulphate in concentrations of 1/40000 mol. to 1/100000 mol., especially of 1/60000 mol.

TABLE 21. The mycelial development of the fungus in nutrient solutions containing copper sulphate in various concentrations

Concentration of $CuSO_4$ (mol.)	H-ion concentration		Dry weight of colony (mg.)	Color of colony
	Initial (pH)	Final (pH)		
1/800	5.8	5.3	—	
		5.3	—	
		5.3	—	
1/1000	5.8	5.9	±	
		5.8	±	
		5.9	+	
1/2000	5.9	5.9	—	
		5.9	—	
		5.9	—	
1/4000	6.0	5.9	±	
		5.9	—	
		5.9	—	
1/6000	6.1	5.9	—	
		6.0	—	
		5.9	+	
1/8000	6.1	5.9	±	
		5.9	±	
		5.9	+	
1/20000	6.2	5.9	5.6	Deep olive
		6.0	+	
		6.0	+	
1/40000	6.2	6.0	2.7	Deep olive
		6.0	5.4	Light grayish olive
		5.7	6.1	Deep olive
1/60000	6.2	5.8	7.9	Deep olive
		5.7	9.2	,,
		5.8	8.4	,,
1/80000	6.2	5.8	6.5	Deep olive
		5.8	4.6	,,
		5.7	4.8	,,
1/100000	6.2	5.6	8.0	Deep olive
		5.7	7.4	,,
		5.7	5.1	,,
Control	6.3	6.2	3.8	Dark olive
		6.2	4.0	Dark grayish olive
		6.0	5.5	

3. Conclusion

The results of these two experiments indicated either stimulative or retarding influence of ferric chloride and copper sulphate on the

mycelial development of the fungus according to their concentrations in the culture solution. In the nutrient solutions containing more than 1/1600 mol. ferric chloride or more than 1/1000 mol. copper sulphate, the fungus growth was quite prevented. But, in the nutrient solutions containing less than 1/20000 mol. ferric chloride or less than 1/60000 mol. copper sulphate, the mycelial development of the fungus was plainly stimulated.

VIII. General summary

1. The present studies have been carried out to clear up fundamental nutritional behaviors of *Piricularia Oryzae* CAV. in synthetic culture solutions.

2. In an attempt to set up a formula of a suitable synthetic nutrient solution for the culture of the fungus, seven different combinations of several kinds of nutrients were examined by cultural experiments. The following formula of nutrient solution for the culture of *Piricularia Oryzae* is recommended:

Potassium nitrate	2.0 g.
Monopotassium phosphate	0.5 g.
Dipotassium phosphate	0.5 g.
Magnesium sulphate	0.5 g.
Calcium chloride	0.1 g.
Ferric chloride	trace
Sucrose	30.0 g.
Redistilled water	1000 cc.

This was used as the standard nutrient solution for the culture of the fungus throughout the present studies.

3. In the studies of carbon sources, various amounts of sugars: viz., glucose, fructose, galactose, maltose, lactose, sucrose; polysaccharides: viz., soluble starch, cellulose, inulin; higher alcohols: viz., glycerine, mannite, and organic acids: viz., formic acid, acetic acid, fumaric acid, citric acid, lactic acid, succinic acid and malic acid were respectively used instead of sucrose in the standard nutrient solution.

From the results obtained in the cultural studies, it was concluded that the carbohydrates and the higher alcohols were generally suitable for the nutrition of *Piricularia Oryzae* as carbon sources,

while the organic acids were altogether improper. Among those compounds, maltose was the best with soluble starch, glucose, glycerine and mannite following it in succession in relation to the carbon nutrition of the fungus.

4. In the cultural studies concerning the nitrogenous nutrition of the fungus, various amounts of inorganic nitrogen salts: viz., potassium nitrate, sodium nitrate, calcium nitrate, ammonium nitrate, dibasic ammonium phosphate, ammonium sulphate, potassium nitrite, sodium nitrite; organic nitrogen compounds: viz., peptone, urea, acetamide, asparagine, glycocol, glutamic acid; and ammonium salts of organic acids: viz., ammonium acetate, ammonium tartarate, and ammonium oxalate were respectively used instead of potassium nitrate in the standard nutrient solution. In these cultures, peptone was proved to be extraordinarily nutritious, while sodium nitrate, asparagine, glutamic acid and acetamide followed it in order.

Nitrites are generally considered to be poisonous to fungi, but in the present case of *Piricularia Oryzae* a slight mycelial development took place in the nutrient solutions containing 0.125% of sodium nitrite or potassium nitrite as nitrogen source. However, at the concentration of the nitrites higher than 0.25% no mycelial growth was observed.

5. The mycelial development of *Piricularia Oryzae* in the nutrient solution containing ferric chloride or copper sulphate in various concentrations was examined in order to study the influence of these metal salts.

The growth of the fungus was stimulated in culture solutions containing less ferric chloride than 1/2000 mol. and less copper sulphate than 1/60000 mol., but it was retarded partially by higher concentrations beyond these points. Concentrations of more than 1/1600 mol. of ferric chloride and 1/1000 mol. of copper sulphate entirely checked the development of the fungus.

IX. Bibliography

ABE, T. (1930): On the influence of copper sulphate to the growth of *Piricularia Oryzae*, with special reference to the temperature as an environmental factor. Ann. Phytopath. Soc. Japan, II, 171–196.

——— (1932): On the influence of iron sulphate upon the growth and vitality of *Piricularia Ozyzae*, with special reference to the temperature

as an environmental factor. Forsch. aus dem Gebiet der Pflanzenkrankh. Kaiserlich. Univ. Kyoto, II, 186–201.

ARAKAWA, S. (1934): The influence of sugars on the cellulose decomposition by the soil fungi. Trans. Tottori Soc. Agric. Sci., V, 27–35.

CAMP, A. F. (1923): Citric acid as a source of carbon for certain citrus fruit-destroying fungi. Ann. Mo. Bot. Gard., X, 213–298.

KARRER, J L. and WEBB, R. W. (1920): Titration curves of certain liquid culture media. Ann. Mo. Bot. Gard., VII, 298–305.

KLOTZ, L. J. (1923): Studies in the physiology of the fungi. XVI. Some aspects of nitrogen metabolism in fungi. Ann. Mo. Bot. Gard., X, 299–368.

MIYAKE, K. and ADACHI, M. (1922): Chemische Untersuchungen über die Widerstandsfähigkeit der Reisarten gegen die "Imochi-krankheit". Journ. Biochem. Tokyo, I, 223–239.

NISHIKADO, Y. (1917): Studies on the Rice Blast fungus. I. Ber. Ohara Inst. Landw. Forsch., I, 171–218.

——— (1926): Studies on the "Imochi" disease. Rep. Path. Fung. Insect. Imp. Dept. Agric. & Forest., XV.

OHTSUKI, T. (1936): Untersuchungen über die Nitritverwendung und die Nitratreduktion bei Schimmelpilzen. Jap. Journ. Bot., VIII, 269–293.

PIEPER, E. J., HUMPHREY, C. J., and ACREE, S. F. (1917): Synthetic culture media for wood-destroying fungi. Phytopath., VII, 214–220.

REYNOLDS, E. S. (1926): Nutritional studies on *Fusarium Lini*. Plant Physiol., I, 151–164.

RIDGWAY, R. (1912): Colour standard and nomenclature.

SAKAMURA, T. and YOSHIMURA, F. (1930): Über die Bedeutung der H-ionenkonzentration und wichtige Rolle einiger Schwermetallsalze bei den Kugelzellbildung der Aspergillen. Journ. Fac. Sci., Hokkaido Imp. Univ., Ser. V, 317–331.

SAKAMURA, T. (1936): Über einige für Kultur von Aspergillen notwendigen Schwermetalle und das Befreiungsverfahren der Nährlösung von ihren Spuren. Journ. Fac. Sci., Hokkaido Imp. Univ., Ser. V, 99–116.

SETO, F. (1936): Comparison of the cellulose-decomposition by plant-pathogenic fungi. Ann. Phytopath. Soc. Japan, V, 308–316.

SUEDA, H. (1928): The studies on the rice blast fungus. Rep. Dept. Agric. Gov. Res. Inst. Formosa, XXXVI, 1–130.

SUEMATSU, N. (1916): On the artificial culture of *Dactylaria parasitans* CAV. I, II, and III. Tokyo Bot. Mag. XXX, 97–99, 119–123 & 196–201.

——— (1919): On the artificial culture of *Helminthosporium Oryzae*. Tokyo Bot. Mag., XXXIII, 291–297.

TANAKA, I. (1931): Studien über die Ernährung der höheren Pflanzen in den organischen Verbindungen. Jap. Journ. Bot., V, 323–350.

TOCHINAI, Y. (1920): Studies on the food-relations of *Fusarium lini*. Ann. Phytopath. Soc. Japan, III, 1–12.

TOCHINAI, Y. and *Shimamura*, M. (1932): Studies on the physiological specialization in *Piricularia Oryzae* BR. et CAV. Ann. Phytopath. Soc. Japan, II, 414–441.

YOSHIMURA, F. (1936): The action of copper and manganese upon the formation and color of conidium of some species of *Aspergillus*. Journ. Fac. Sci., Hokkaido Imp. Univ. Ser. V, 117–139.

YOUNG, H. C. and RENNETT, C. W. (1922): Growth of some parasitic fungi in synthetic culture media. Amer. Journ. Bot., IX, 459–469.