Comparative Studies on the Physiology of *Fusarium Lini* and *Colletotrichum Lini.*

By

Yoshihiko Tochinai

Botanical Institute, College of Agriculture,
Hokkaido Imperial University.

CONTENTS

[Jour. Coll. Agric., Hokk id Imp. Univ. Vol. XIV. Pt. 4. Nov. 1925.]

I. Introduction.

Wilt-disease, anthracnose and rust-disease are the most serious menaces to the cultivation of flax plants. Among them the wilt-disease and anthracnose attack the flax most virulently at its seedling stage, and their symptoms are somewhat alike at first sight.

In Japan and America the wilt-disease has often annihilated whole crops, but in Europe, where the rotation of crops is being strictly adhered to, this destructive disease has never become an important problem. The causal fungus of the flax wilt-disease was first found in Japan and a few years later also in America. Numerous reports have been published on the subject by HIRATSUKA (*48, 49*), BOLLEY (*9–13*), W. H. TISDALE (*103, 104*), L. R. JONES and W. B. TISDALE (*58*), and the present author (*105–108*).

The anthracnose of flax was first reported in America by BOLLEY (*12*) early in this century, but the minute phytopathological investigation of it remained neglected for many years. It seems to have been confounded with the wilt-disease owing to the apparent resemblance of the symptoms in seedlings. But recently, the anthracnose of flax has attracted the attention of numerous phytopathologists, and reports have been published by SCHOEVERS (*98*), WESTERDIJK (*116, 117*), PETHYBRIDGE and LAFFERTY (*86*), HEMMI (*45*), SAWADA (*95*), HIURA (*50*), and the author of this paper (*105*).

As both diseases attack the flax plants most seriously in their seedling stage and present similar symptoms, comparative studies of these diseases are important and interesting from the phytopathological as well as the mycological standpoints. In the author's previous paper (*105*) the symptoms of the two diseases were compared.

In the present thesis, the author has dwelt on some physiological characters of the causal fungi and also on some phytopathological explanations concerning them.

These studies have been executed in the Botanical Institute of the Hokkaido Imperial University in Sapporo, under the direction of Prof. Dr. KINGO MIYABE.

The writer wishes to express here his heartiest thanks to Prof. Dr. K. MIYABE for his valuable suggestions and great interest shown in the work through the whole course of this investigation. He is also deeply indebted to Prof. Dr. S. ITO for kind advice and encouragement received during the process of the investigation. Thanks are also

due to Prof. Dr. T. SAKAMURA and other colleagues for their friendly favours, and to Dr. N. HIRATSUKA for the use of his library. Acknowledgements are also due to Prof. Dr. K. MIYAKE and other gentlemen in the Chemical Institute of the University for their kind help on the chemical side of the present investigations, and to Mr. K. SAKURAI, Assistant Expert of the Government of the South Sea Islands, for his faithful help while he was an Assistant in our Institute.

II. Historical Review of the Studies on the Wilt-Disease and Anthracnose of Flax in Their Relations to the Causal Fungi.

1. Fusarium Lini BOLLEY.

Fusarium Lini causes the wilt-disease of flax. This disease must have existed in Europe and elsewhere for centuries before the discovery of its causal organism at the end of the nineteenth century in Japan.

In 1892, K. MIYABE first found that a species of *Fusarium* is concerned in the wilt-disease of flax, and under his direction N. HIRATSUKA (*48*) investigated this disease. He confirmed the assumption that the causal organism of the wilt-disease of flax is a species of *Fusarium*, and explained the principle of the rotation of crops with long intervals in the cultivation of flax adopted by the cultivators in Europe (*48*). In America, H. L. BOLLEY (*9*) discovered, in 1901, quite independently of the researches by MIYABE and HIRATSUKA, the causal fungus of the wilt-disease of flax and named it *Fusarium Lini*. Before them, O. LUGGAR (*69*) carried out an investigation on the wilt-disease of flax. He did not succeed in finding its causal organism.

Fusarium Lini attacks the flax plant at any stage of growth, but the greatest damage is done to the seedlings. The young flax plants are easily annihilated by the attack of this fungus, causing them to wither or fall down very rapidly. Grown plants, offering more resistance, never show such a rapid death.

Fusarium Lini is a facultative parasite. It can grow on organic matters in soil for many years, producing conidia and chlamydospores, and attacking the flax plants when grown on the same soil. In another way, the fungus disseminates itself adhering to the surface of flax seeds, or according to HIURA (*50*), the hyphae penetrate into the seedcoats and remain there in a dormant state, and attack the seedlings at the

time of germination.

According to the author's experiments, the germ-tubes of the conidia or the hyphae attack the flax plants either by penetrating through their epidermis or passing through their stomatal slits. According to W. H. TISDALE (*103*), most cases of infection occur on the root hairs of flax.

2. *Colletotrichum Lini* (WESTERDIJK).

Colletotrichum Lini causes the anthracnose of flax. The anthracnose of flax began to attract the attention of phytopathologists at the beginning of this century. It had been overlooked by flax cultivators because the symptoms of this disease can not be distinguished from those of the wilt-disease without a careful examination.

This disease and its causal fungus were first noticed by T. F. MANNS. He made an extensive research upon "Flax sick soil and flax seed" under Prof. BOLLEY of the North Dakota Agricultural College, from 1901 to 1903, and separated a species of *Colletotrichum* as a parasite of flax together with many other species of parasitic as well as saprophytic fungi. He named it *Colletotrichum Lini*. But for some reason his thesis unfortunately has never been published. By his courtesy, I was able to peruse this valuable paper, which was kindly sent to me at my request.[1)]

In 1903, BOLLEY (*12*) reported very briefly on a species of *Colletotrichum* parasitic on flax. Again in 1910, he (*13*) reported on this disease, and named the causal fungus *Colletotrichum Lini*, without giving any specific description, however, and again reporting two years later, in 1912, he described it as "Flax Canker" (*14*).

In 1915, SCHOEVERS (*98*) reported on the occurrence of a species of *Colletotrichum* parasitic on flax in Holland. He found also a species of *Gloeosporium* on the seed-ball of flax, and thought of some possible relation between these two kinds of fungi.

In the same year, WESTERDIJK (*116*) described the causal fungus of this flax disease in Holland, and gave it the name *Gloeosporium Lini*. She reported on it again in 1918 (*117*).

In 1918, PETHYBRIDGE and LAFFERTY (*86*) published an important paper on the investigation of this disease in Ireland. They named the causal organism *Colletotrichum linicolum*, and gave a full description of

1) In this connection my heartiest thanks are due to Dr. T. F. MANNS for permitting me to avail myself of his unpublished thesis.

it. They gave the reason for having treated this fungus as a new species by stating that, "It may be identical with the species already described (but not named) by SCHOEVERS, and it is just possible that it may be BOLLEY'S *Colletotrichum Lini*. Since, however, no description of this latter species has been published, the name cannot be regarded as valid." PETHYBRIDGE and LAFFERTY dwelt rather extensively upon the transmission of their fungus. According to them, the hyphae of the fungus penetrate into the epidermal cells of the seed-coat of flax, and remain there in a dormant state, attacking the seedlings after germination.

In 1922, SCHILLING (*96*) reported on the occurrence of anthracnose of flax in Germany. He adopted the name *Gloeosporium Lini* WESTERDIJK, and discussed the unreasonableness of separating the genus *Colletotrichum* from *Gloeosporium* by placing too much weight on the presence or absence of the setae. He treated *Colletotrichum linicolum*, PETHYB. et LAFF. as a synonym of *Gloeosporium Lini* WESTERDIJK, regarding them to be identical.

In Japan, this disease was first noticed in 1918 by S. ITO and K. KATSUFUJI. T. HEMMI identified the causal fungus as *Colletotrichum linicolum* PETHYB. et LAFF., and he (*45*) spoke on the relation between this fungus and *Colletotrichum Lini* BOLLEY, having the opinion that, as PETHYBRIDGE and LAFFERTY had stated, this fungus is very probably the same as the causal organism of the so-called "Flax Canker," *Colletotrichum Lini* BOLLEY. But the identification of the two fungi is difficult, because we have no full description of the latter.

From T. F. MANNS' manuscript, however, the author was able to establish the absolute identity of *Colletotrichum Lini* BOLLEY, *Gloeosporium Lini* WESTERDIJK, and *Colletotrichum linicolum* PETHYB. et LAFF.

MANNS' description of this fungus in his unpublished thesis presented to the Faculty of the North Dakota Agricultural College for the Master's degree in 1903 is as follows:—"Vegetative hyphae abundantly septate; in deep tissue of host almost hyaline, near surface light to dark brown or sooty, 3 to 10 microns in diameter, average 3.5 microns. Acervules scattered, slightly erumpent; spores sessile on a compact matrix, surrounded by or interspersed with bristles (setae). Conidia biguttulate, hyaline, slightly curved, allantoid, 15 to 20 microns by 2 to 4.5 microns. Chlamydospores olive to brown, spherical to oval, 10 to 12 microns by 10 to 15 microns. This fungus is parasitic on flax

causing in seedlings a typical "damping-off," and in more mature stages weakening and browning of the plants, with a resulting shrivelling of seed at maturity. It is associated with *Fusarium Lini* in causing flax sickness in the northwest flax districts of the United States, and is especially common in flax grown in European countries, as evidenced by specimens of sick flax; also the spores are readily carried upon flax seed. No perfect fruiting stage has been met or produced in cultures."

BOLLEY'S *Colletotrichum Lini* is without any doubt the same as the one described and named by MANNS. BOLLEY has never given any description of this fungus. But fortunately, the author was able to identify the fungus found in Japan, Ireland, Holland and Germany with *Colletotrichum Lini* of BOLLEY with the help of the full description of the fungus given by MANNS in his unpublished thesis.

Thus different names have been given to this fungus by several authors. MANNS' *Colletotrichum Lini* has never been published, and BOLLEY's *Colletotrichum Lini* is a *nomen nudum*, WESTERDIJK named it *Gloeosporium Lini* in 1915, taking the genus in a broad sense. PETHYBRIDGE and LAFFERTY named this fungus *Colletotrichum linicolum* in 1918, apparently without knowing the work of WESTERDIJK.

As the author cannot agree with the proposal to abolish the genus *Colletotrichum* and to include it in *Gloeosporium*, he should like to call this fungus *Colletotrichum Lini* (WESTERDIJK). As a consequence, the names *Colletotrichum Lini* BOLLEY, *Gloeosporium Lini* WESTERDIJK and *Colletotrichum linicolum* PETHYBRIDGE et LAFFERTY should be treated as its synonyms.

III. Cultural Characters of the Causal Fungi.

1. Cultural studies on the carbon sources.

The carbon sources play an important rôle in the nutrition of fungi, for they appropriate the carbon demand from higher organic carbon compounds. Many authors carried out investigations on the nutrition of various fungi with carbon compounds (NAEGELI (*79*), WEHMER (*115*), NIKITINSKY (*81*), LUTZ (*70*), HEMMI (*46*, *47*), KÜSTER (*46*), YOUNG and BENNET (*120*), RICHARDS (*93*), etc.) There is, however, a marked specific variation in the affinity of fungi to carbon compounds.

The author (*106*) has studied the nutritive values of carbohydrates, higher alcohols, organic acids and phenol derivatives as carbon sources

for the growth of *Fusarium Lini*, and found that the carbohydrates and mannite were suitable, while glycerin and organic acids were generally unsuitable. Phenol derivatives seemed to be poisonous to the fungus. Apparently no experiment on this line with *Colletotrichum Lini* has been undertaken by any author.

The author has carried out the present studies with a view to examine the nutritive value of various carbohydrates and higher alcohols as carbon sources in the culture of *Colletotrichum Lini* with parallel experiments on *Fusarium Lini*.

a. Carbohydrates.

The carbohydrates are the best source of carbon for fungi. The sugars and polysaccharides are in general easily used by fungi as their food.

In the present cultural studies, there have been used nine compounds of these carbohydrates, viz. d-glucose, l-fructose, d-galactose, sucrose, maltose, lactose, soluble starch, inuline and glycogen.

As the standard nutrient solution, a synthetic solution of the following formula was used:

Peptone	10.00 grams,
Mono-kalium phosphate	0.50 ,,
Magnesium sulphate	0.25 ,,
Redistilled water	1000.00 *cc.*

Solutions containing 2 per cent of each of the above carbohydrates were prepared by adding six grams of carbohydrates to 300 c.c. of the standard nutrient solution. It may be better to compare the nutritive value of any chemical substances in the equimolecular, isothermal or isotonic conditions. As it is somewhat difficult to do so in the case of carbohydrates and about the same content of carbon atoms occurs in the same quantity of carbohydrates, the weight percentage is used in the present study. Fifty c.c. portions of these solutions were placed in each of six ERLENMEYER'S flasks of 200 c.c. capacity. They were twice sterilized for about one hour in KOCH'S steam sterilizer with one day's interval. These cultural solutions were estimated to be all about pH 5.1 in their hydrogen-ion concentration. Then they were inoculated with small and approximately equal quantities of the mycelium of the fungi, and cultivated in a thermostat at 26°C. The cultures for every fungus were carried out in triplicate. After a fortnight, the developed mycelia

were gathered on pieces of filter paper of known weight, and washed with water several times. Then, after the greater part of the water had been evaporated in a vacuum desiccator, they were placed in a calcium chloride desiccator for two weeks, and weighed.

The results of fourteen days' culture are shown in the following tables:

Table I. Showing the mycelial development of *Fusarium Lini* in cultural solutions containing different carbohydrates.

Kinds of carbohydrates	Av. wt. of grown mycelium in gr.	Remarks
Glucose	0.325	Matty growth, with moderately developed aerial mycelia.
Fructose	0.419	Thick matty growth, with sparsely developed aerial mycelia.
Galactose	0.392	Matty growth, with sparsely developed aerial mycelia.
Sucrose	0.408	Matty growth, with sparsely developed aerial mycelia.
Maltose	0.725	Very thick matty growth, with a few aerial mycelia.
Lactose	0.385	Compact matty growth, with vigorously grown aerial mycelia.
Soluble starch	0.350	Matty growth, with vigorously grown aerial mycelia.
Inuline	0.382	Matty growth, with no aerial mycelium.
Glycogen	0.333	Matty growth, with moderately developed aerial mycelia; the submerged mycelia show dark colour.

Table II. Showing the mycelial development of *Colletotrichum Lini* in cultural solutions containing different carbohydrates.

Kinds of carbohydrates	Av. wt. of grown mycelium in gr.	Remarks
Glucose	0.127	Mycelial masses submerged, white, mixed with very light salmon coloured mycelium.
Fructose	0.118	Mycelial masses submerged, white, mixed with light salmon coloured mycelium.
Galactose	0.152	Mycelial masses submerged, white, mixed with very light salmon coloured mycelium.

Sucrose	0.112	Mycelial masses submerged, white-coloured mycelium.
Maltose	0.345	Matty growth, light salmon coloured but no aerial mycelium.
Lactose	0.272	Matty growth, very strongly salmon coloured.
Soluble starch	0.262	Matty growth, less strongly salmon coloured.
Inuline	0.077	Small mycelial masses submerged, white, with dark coloured mycelium.
Glycogen	0.242	Matty growth, white, with strongly salmon and dark-coloured mycelium.

In these cultural experiments, in every case both fungi showed visible growth two days after inoculation. The growth was generally good, but the development of *Fusarium Lini* was far more rapid and vigorous than that of *Colletotrichum Lini*. The former generally produced aerial mycelium except in the cultural solution containing inuline, while the latter showed no aerial growth. In the former the mycelia were generally white in colour, except in the cultural solution containing glycogen, in which dark coloured mycelial growths appeared, while in the latter, submerged mycelial growths always showed fresh salmon colour; moreover, in the cultural solutions containing inuline and glycogen, these mycelial growths were accompanied by dark coloured portions.

As shown in the above tables, the mycelial growths of the fungi primarily differ with the kinds of carbohydrates, without any influence of the hydrogen-ion concentration, for the latter has the same value in all cultural media. On the whole, the sugars showed no marked difference in their nutritive relation for both fungi, while the polysaccharides, however, differed markedly from each other in this respect.

Among mono- and disaccharides, glucose was comparatively less suitable, galactose and lactose were intermediate, and sucrose proved comparatively good while maltose was the best for the nutrition of *Fusarium Lini*. For *Colletotrichum Lini*, sucrose and glucose were comparatively less suitable, galactose and lactose were intermediate, while maltose was the best. Fructose was very nutritious for the former fungus, but it was inferior in the nutrition of the latter. Amongst polysaccharides, soluble starch and glycogen were comparatively less suitable for *Fusarium Lini*, while they were relatively good for *Colletotrichum Lini*, and inuline was suitable for *Fusarium Lini*, while it was unsuitable for *Colletotrichum Lini*. It is a very interesting fact that fructose and inuline stand in a perfectly contrary nutritive relation to these fungi.

b. Higher alcohols.

Higher alcohols are closely related to sugars. Accordingly, they are generally nutritious for fungi. In the present studies, glycerin and mannite have been used to an extent of 2 per cent as in the previous experiments with carbohydrates. The formula of the standard solution, the preparation of nutrient media, and the other cultural conditions were also similar to the previous experiments.

The results of fourteen days' culture in a thermostat at 26°C. are shown in the following tables:

Table III. Showing the mycelial growth of *Fusarium Lini* in the cultural solutions containing higher alcohols as carbon sources.

Kinds of higher alcohols	Av. wt. of grown mycelium in gr.	Remarks
Glycerin	0.250	Thin matty growth.
Mannite	0.527	Thick matty growth.
Control (Sucrose 2%)	0.400	Moderate matty growth.

Table IV. Showing the mycelial growth of *Colletotrichum Lini* in the cultural solutions containing higher alcohols as carbon sources.

Kinds of higher alcohols	Av. wt. of grown mycelium in gr.	Remarks
Glycerin	0.038	Salmon-coloured small mycelial masses.
Mannite	0.039	Salmon-coloured small mycelial masses.
Control (Sucrose2%)	0.112	Well-developed, salmon-coloured mycelial masses.

In these results, the mycelial development of *Colletotrichum Lini* in solutions containing glycerin and mannite were almost alike in their weights, but the apparent growth in the solution containing mannite seemed somewhat better than that in the solution containing glycerin. Compared with the control, these higher alcohols used as carbon sources, were inferior to sucrose in their nutritive value for the mycelial development of this fungus.

For *Fusarium Lini*, mannite was a very suitable carbon source, and the growth of the mycelium in the solution containing mannite was even better than in the control solution containing sucrose. In the present case, glycerin was far inferior to mannite and sucrose. Glycerin seems to be generally less nutritive for the mycelial development of several fungi (*120*).

c. **Conclusions from the cultural studies on the carbon sources.**

1. The carbohydrates were generally suitable for the nutrition of both fungi as carbon sources, but it was noticeable that there are marked differences among their nutritive values.

2. The best growth of the fungi in both cases was attained in the cultural solution containing maltose.

Glucose was comparatively less suitable, galactose, and lactose were intermediate, and sucrose and fructose proved comparatively good for the nutrition of *Fusarium Lini*, while glucose and sucrose were comparatively less suitable, galactose and lactose proved relatively good for that of *Colletotrichum Lini*. Soluble starch and glycogen were comparatively less suitable for *Fusarium Lini*, while they were relatively good for *Colletotrichum Lini*. Inuline was suitable for *Fusarium Lini*, while it was unsuitable for *Colletotrichum Lini*.

3. For *Fusarium Lini*, glycerin was not suitable, but mannite was very nutritious, while for *Colletotrichum Lini*, neither compound showed marked difference in nutritive value; both being comparatively unsuitable as a carbon source for this fungus.

4. *Colletotrichum Lini* was usually far more feeble than *Fusarium Lini* in mycelial development in all the compounds which were used as carbon sources.

2. Cultural studies on the nitrogen sources.

The fungus appropriates the nitrogen demand either from inorganic or organic nitrogen compounds. In nature, the fungi generally feed on organic nitrogen compounds, with the exception of nitrogen-fixing species, but the fungi which have saprophytic habits are easily cultured with inorganic nitrogen salts on artificial cultural media. Organic nitrogen compounds generally serve in the nutrition of fungi not only as the nitrogen source, but also as the carbon source.

As a nitrogen source for the nutrition of fungi, various organic and inorganic nitrogen compounds have been used by many authors

(NAEGELI (*79*), NIKITINSKY (*81*), KÜSTER (*64*), MAYER (*75*), PFEFFER (*87*), CURRIE (*26*), RAULIN (*92*), USCHINSKY (*110*), etc.). The author (*106*) used ammonium phosphate, ammonium sulphate, ammonium nitrate, sodium nitrate, sodium nitrite, urea and peptone as nitrogen sources in the previous studies on the food relation of *Fusarium Lini*. But in none of many investigations has this point been tried with *Colletotrichum Lini*.

The author has carried out the present cultural studies on the nitrogen source of *Fusarium Lini* and *Colletotrichum Lini* with the inorganic nitrogen compounds of ammonium form, nitrate form, and nitrite form, and with such the organic nitrogen compounds as proteins, peptone, and amino-acids, in order to examine in what nutritive relation they stand to these fungi as nitrogen sources.

As the standard nutrient solution the author has used a synthetic solution of the following formula:

Sucrose	20.00 grams
Mono-kalium phosphate	0.50 „
Magnesium sulphate	0.25 „
Redistilled water	1000.00 c.c.

The cultural solutions were prepared by addition to the standard nutrient solution of each nitrogen compound in such amounts as to give equivalence of nitrogen in each case. The fungi were cultured under similar conditions as described in the preceding studies on the carbon sources. The hydrogen-ion concentration of each nutrient solution was determined at the beginning and end of the experiment, and relative growth of the fungi in these cultural solutions was determined by the average weight of the grown mycelium of three cultures each.

a. Inorganic nitrogen compounds.

The comparison of the nutritive values of the ammonium group and nitrate group with the help of cultural experiments is somewhat difficult, and the results thus obtained cannot be said to be perfectly reliable, for as NH_4- and NO_3-ions differ from each other in their charge, the combination of their salts with a similar atom or atom group can never be expected. In order to diminish the error of the experiment caused by the influence of the different atoms or atom groups combined with NH_4 and NO_3, it would be necessary to use the salts of NH_4 and NO_3 with the atom or atom group which is possibly indifferent to the

nutrition of fungi being neither favorable nor unfavorable.

The author has used the following kinds of nitrogen salts in the present cultural studies:

Ammonium chloride as ammonium source,
Sodium nitrate as nitrate source,
Sodium nitrite as nitrite source,
Ammonium nitrate as the control.

Ammonium nitrate, was taken to the extent of one per cent, and the other compounds were taken to hold the nitrogen equivalent to the former compound, *i. e.* 1.34% of ammonium chloride, 2.13% of sodium nitrate and 1.73% of sodium nitrite. The results of a fortnight's culture in a thermostat at 26°C. are shown in the following tables:

Table V. Showing the mycelial development of *Fusarium Lini* in the cultural solutions containing different inorganic nitrogen salts.

Kinds of nitrogen salts	pH value of media Initial	Final	Av. wt. of grown mycelium in gr.	Remarks
Ammonium nitrate	5.2	5.8	0.096	Loose mycelial growth.
Ammonium chloride	5.1	2.6	0.041	,,
Sodium nitrate	5.3	5.6	0.018	Slight growth.
Sodium nitrite	5.2	5.2	0	No growth.

Table VI. Showing the mycelial development of *Colletotrichum Lini* in the cultural solutions containing different inorganic nitrogen salts.

Kinds of nitrogen salts	pH value of media Initial	Final	Av. wt. of grown mycelium in gr.	Remarks
Ammonium nitrate	5.2	3.0	0.049	Loose mycelial growth, slightly salmon-coloured.
Ammonium chloride	5.1	2.8	0.044	,,
Sodium nitrate	5.3	5.6	0.019	Slight growth, salmon-coloured.
Sodium nitrite	5.2	5.2	0	No growth.

The best mycelial development for both fungi was attained in the control cultural solution containing ammonium nitrate. Comparing the growth of the fungi in the cultural solutions containing ammonium chloride and sodium nitrate, it was clearly better in the former than in the latter. The hydrogen-ion concentrations of these two solutions were markedly altered by the growth of the fungi, although the initial pH values of these solutions were about the same. That is to say the hydrogen-ion concentration was increased in the solution containing ammonium chloride, while it was decreased in the solution containing ammonium nitrate. This diversity seems to be the result of the differences in the relation of absorption or utilization of ions, namely NH_4-ion in the former, and NO_3-ion in the latter solution. Such reaction changes of these media could have some influence upon the growth of these fungi. As a whole, however, NH_4 seems to be more suitable than NO_3 as a nitrogen source for the fungi. The development of *Colletotrichum Lini* was somewhat better than that of *Fusarium Lini* in the cultural solutions containing ammonium chloride or sodium nitrate, but it was the reverse in the solution containing ammonium nitrate. The mycelial growth of these fungi in the solutions containing ammonium nitrate was better than that in the solutions containing ammonium chloride or sodium nitrate. Especially, this relation was remarkable in the case of *Fusarium Lini*. The hydrogen-ion concentration of the solution containing ammonium nitrate was changed in opposite directions by these fungi, that is it was somewhat decreased by *Fusarium Lini*, while increased by *Colletotrichum Lini*. This may be due to the facts that *Colletotrichum Lini* primarily and principally utilizes NH_4-ion, but *Fusarium Lini* rapidly utilizes NO_3-ion, though it can take NH_4- and NO_3-ion simultaneously. This speciality seems to be a reason for the better growth of *Fusarium Lini* compared with that of *Colletotrichum Lini* in the solution containing ammonium nitrate.

KÜSTER (*64*) holds the opinion that, nitrites are never as poisonous as they have been considered for a long time, and it has already become known that many fungi appropriate the nitrogen demands from nitrite. But in the present case, nitrite seems to be injurious to the growth of both fungi and no sign of mycelial development was ever found in the solution containing sodium nitrite.

b. Amino-acids.

As proteins form the principal portion of cell contents, the amino-acids, which are the constitutional unit of protein (*33*), have an important bearing upon the vital functions of organisms, and on the nutrition of the heterotrophic organisms (27). In nature, the parasites assimilate several kinds of amino-acids or proteins. It is interesting to know in what relation the various kinds of amino-acids stand to the nutrition of fungi.

In the present cultural studies, the following four kinds of amino-acids as nitrogen sources were tested in single and mixed supplies:

Glycocoll.[1]
Leucine.[2]
Glutamic acid.[3]
Asparagine.[1]

Asparagine, as control, was taken to an extent of one per cent, and the other amino-acids were taken to hold the nitrogen equivalence to the asparagine, *i. e.* 1.14% of glycocoll, 2.23% of glutamic acid and 1.99% of leucine. The cultural solution for the mixed use of amino-acids was prepared by mixing equal quantities of the cultural solutions containing asparagine, glutamic acid and glycocoll. These cultural solutions varied in their hydrogen-ion con centrations and as those of the solutions containing glutamic acid, asparagine and the three kinds of amino-acids are too high for the growth of the fungi in question, they were adjusted by addition of minimal quantities of conc. NaOH solution within a limit not injurious to the growth of the fungi. The initial and adjusted pH values of the cultural solutions containing these amino-acids and the quantities of the NaOH solution used for the adjustment are shown in the following table.

---

1) Glycocoll and asparagine were of ordinary commodity.

2) Leucine was kindly supplied by Mr. TAMACHI.

3) Glutamic acid was prepared by the following method:

A certain quantity of epicurean powder (Ajinomoto) is dissolved in 3 to 4 parts of water, and decoloured by animal charcoal, and filtered. Add 5% HCl gently to the filtrate untill a turbidity caused by the crystals of glutamic acid appears. By moderate agitation the turbidity increased rather rapidly. The crystals are collected on a filter, and purified by means of recrystallization from water several times.

Table VII. Showing the initial and adjusted hydrogen-ion concentrations and the quantities of conc. NaOH solution used for the adjustment.

Kind of the nitrogen source	pH values Initial	Adjusted	Quantities of conc. NaOH solution
Glycocoll	5.6	5.6	0
Leucine	5.4	5.4	0
Glutamic acid	3.2	5.3	1.6 c.c.
Glycocoll, glutamic acid, asparagine	3.4	5.2	0.7 c.c.
Asparagine	4.4	5.2	2 drops

The results of a fortnight's culture in a thermostat at 26°C. are shown in the following tables:

Table VIII. Showing the mycelial growth of *Fusarium Lini* in the cultural solutions containing various amino-acids.

Kinds of amino-acids	pH values Initial	Final	Av. wt. of grown mycelium in gr.	Remarks
Glycocoll	5.6	8.8	0.248	Thick matty growth.
Leucine	5.4	7.8	0.418	Very thick matty growth.
Glutamic acid	5.3	8.4	0.239	Thick matty growth.
Glycocoll, glutamic acid, asparagine	5.2	9.2	0.290	Thick matty growth.
Asparagine	5.2	7.6	0.204	Thick matty growth.

Table IX. Showing the mycelial development of *Colletotrichum Lini* in the cultural solutions containing various amino-acids.

Kinds of amino-acids	pH values Initial	Final	Av. wt. of grown mycelium in gr.	Remarks
Glycocoll	5.6	4.4	0.174	Well developed, strongly salmon coloured mycelial masses.
Leucine	5.4	4.2	0.156	Well developed, salmon coloured mycelial masses
Glutamic acid	5.3	5.0	0.163	Ditto.
Glycocoll, glutamic acid, asparagine	5.2	4.6	0.199	Well developed, strongly Salmon coloured mycelial masses.
Asparagine	5.2	4.8	0.106	Well developed, salmon coloured mycelial masses.

From these results, it has been concluded that all the amino-acids used are suitable for the nutrition of the fungi as nitrogen sources, if the cultural solutions possess the proper hydrogen-ion concentration. But considerable differences of nutritive value appear among them. Asparagine was comparatively less suitable and glycocoll was relatively good for the nutrition of the present fungi. Leucine suited very well for *Fusarium Lini*, while it was not so good for *Colletotrichum Lini*, although it was better than asparagine.

Finally, it was a remarkable phenomenon that the solutions containing amino-acids became alkaline to a considerable degree, when *Fusarium Lini* had luxuriantly developed. I shall dwell upon this subject fully in later pages. In the case of *Colletotrichum Lini*, however, such reaction change in stale cultural solutions has never occurred. The specific contrast in the reactions of the stale cultural solutions in which *Fusarium Lini* or *Colletotrichum Lini* has grown, probably has a deep significance concerning the pathogeny of the flax diseases caused by these fungi.

c. Proteins.

It is interesting to know the nutritive values of some kinds of protein substances as nitrogen sources for the fungi, especially to find out in what nutritive relation animal and plant protein stand owing to the difference in their properties. POTTER (*91*) suggested a relation between the protein contents of plants and their immunity or susceptibility to a parasitic disease. In the present cultures, egg albumin, casein and gelatine for animal protein, and mucin[1] of yam tubers for plant protein have been used.

The media containing albumin were sterilized at low heat several times intermittently, care being taken to prevent the albumin from coagulation. After a week's incubation, those flasks which were free from contamination, were used for the culture. The media containing gelatine were left to congeal. The greater part of casein and mucin remained insoluble in the nutrient solution. After a fortnight's culture, the proteins not yet consumed by the fungi and remaining

1) Preparation of mucin :—Ground tuberous roots of yam are extracted with water, and the extract is filtered from the residue. Acetic acid is added gently to the filtrate with moderate agitation until a turbidity caused by mucin appears. It is collected on a filter, and purified with alcohol and ether (*84*).

in solid condition in the cultural solutions were separated from the mycelia by the following means. In the case of gelatine, it was dissolved by warming, and strained through filter paper before it congealed. In the case of casein, it was dissolved by making the solution alkaline with an addition of sodium hydroxyde solution. In the case of mucin, careful washing with sufficient water was resorted to in order to eliminate it. Then the mycelia were gathered on filter paper and their weights were determined.

In the present culture work, each of the above proteins was added to the standard solution to an extent of one per cent, for the nitrogen contents of each protein are almost equal. The results of a fortnight's culture in a thermostat at 26°C. are shown in the following tables:

Table X. Showing the mycelial development of *Fusarium Lini* in cultural solutions containing various proteins.

Kinds of proteins	pH values of media	Av. wt. of grown mycelium in gr.	Remarks
Albumin	6.0	0.138	Thin mycelial growth.
Casein	5.0	0.282	Moderate matty growth.
Gelatine	5.4	0.263	Moderate matty growth.
Mucin	5.0	0.266	Moderate matty growth.
Peptone	5.4	0.407	Thick matty growth.

Table XI. Showing the mycelial development of *Colletotrichum Lini* in cultural solutions containing various proteins.

Kinds of proteins	pH values of media	Av. wt. of grown mycelium in gr.	Remarks
Albumin	6.0	+	Sparse white mycelial growth.
Casein	5.0	0.086	Strongly salmon-coloured, moderately grown mycelial masses.
Gelatine	5.4	0.047	Small white mycelial masses.
Mucin	5.0	0.089	Well-grown mycelial masses having salmon and blackish or purplish-brown coloured spots.
Peptone	5.4	0.150	Well developed salmon-coloured mycelial masses.

The proteins are generally suited to the nutrition of the fungi. It is noteworthy that the growth of the fungi in the cultural solution containing albumin as the nitrogen source was far inferior to that in the cultures containing the other proteins. Especially the mycelial growth of *Colletotrichum Lini* was so weak that its weight could not be exactly determined. The best mycelial development of *Colletotrichum Lini* was attained in the culture containing mucin. Mucin was the only kind of phyto-protein used in this experimental series. The development of the fungi in the cultures containing peptone, the control, was far more vigorous than that in the cultures containing the various proteins. The fungi seem not to be able to assimilate the proteins so readily as peptone, for the chemical combination of the constituent amino-acids of the proteins is stable and characteristic, and the decomposition of them by the enzymes of the fungi is not so easy as in the case of peptone. Gelatine has been known as an unsuitable protein for the nutrition of animals as it lacks some kinds of the constituent amino-acids (*89*). But the nutritive value of gelatine to the fungi was markedly different from its value to animals.

d. Conclusions from the cultural studies on the nitrogen sources.

1. Organic nitrogen compounds were generally more suitable than inorganic nitrogen salts as nitrogen sources for the fungi.

2. Among the inorganic nitrogen salts the nitrogen of the ammonium form was assimilated more easily than that of the nitrate form, and the nitrite was unsuitable for the fungi.

3. Amino-acids were good nutrients for the fungi. The mixed use of various amino-acids of the proper kind might be an ideal nitrogen source for the fungi.

4. The proteins were in general suitable nitrogen sources for the growth of the fungi, but due to the stable and characteristic combination of the constituent amino-acids, they were not so readily assimilable as peptone.

3. Influence of the acids upon the fungi.

It is interesting from a phytopathological as well as physiological standpoint to study the influence of the acids on the growth of the subject fungi. The immunity and susceptiblity of plants to parasites are, as has

been proved in many cases, dependent upon the organic acids contained in the cells of host plants. On the other hand, the organic acids serve fungi as a carbon source when they were supplied as proper salts. On this point many experiments have been carried out with the free organic acids or their ammonium salts and amino-acids (PASTEUR (see *63*, *34*), NAEGELI (*79*), WARBURG (*111*), WEHMER (*115*), BASSALIK (*6*), LOEW (*67*), LOEB (*66*), CZAPEK (*28*), etc.). The author himself (*106*) has studied the nutritive relations of malic, succinic, maleic, fumaric, and citric acid to *Fusarium Lini*, and found that the difference of the molecular constructions or of the stereochemical structures has more or less relation to the nutrition of the fungus. On this point, there is no experiment for *Colletotrichum Lini*.

In the present investigations, the author has examined firstly, the influence of the hydrogen-ion concentration in a preliminary meaning secondly, the influence of the various organic acids; thirdly, the influence of various concentrations of tannic acid; fourthly, the influence of various concentrations of citric acid upon the mycelial development of the fungi.

a. Influence of the hydrogen-ion concentration of the cultural solution on the mycelial development of the fungi.

According to WEBB (*114*) who gave a review of the literature on the subject up to 1919, there existed even then rather extensive studies on the relation of the hydrogen-ion concentration of culture media to the life of micro-organisms. This problem has been investigated from various sides by many authors (ITANO (*55*), CURRIE (*26*), GILLESPIE (*35*), MEACHAM (*76*), ITANO and NEILL (*56*), GUSTON (*39*), MATSUMOTO (72), KARRER (*59*), HOPKINS (*51*, *52*), MAC INNES (*71*), etc.). Recently, SIDERIS (*94*) studied the change in the hydrogen-ion concentration of culture media of various nutrient substances at different initial hydrogen-ion concentrations during the culture of *Fusarium cromyophthoron*. To the particular hydrogen-ion concentration toward which the final reaction was pointing the term "isometabolic point" has been assigned, and it is said that the initial reaction of the cultures at the hydrogen-ion concentration of the isometabolic point was maintained more or less constantly during the growth of the organism.

In the present studies the relation of the hydrogen-ion concentration to the mycelial growth of the fungi was determined as a preliminary

measure in order to gain a standard for the cultural studies of the fungi. The hydrogen-ion concentrations of the cultural solutions were measured with the potentiometer or the colorimetric methods (*20*,77).

The formula of the standard nutrient solution is as follows:

Asparagine	10.00 grams
Mono-kalium phosphate	0.50 „
Magnesium sulphate	0.25 „
Sucrose	20.00 „
Redistilled water	1000.00 c.c.

Regulated quantities of 1/100 or 1/10 mole solutions of hydrochloric acid or sodium hydroxide were added to this standard nutrient solution in order to prepare the cultural solutions of different hydrogen-ion concentrations. Fifty c.c. of the cultural solutions varying in their hydrogen-ion concentrations were placed in ERLENMEYER's flasks of about 200 c.c. capacity, and sterilized twice in a KOCH's steam sterilizer for 30 minutes with a 24 hours' interval. Then the hydrogen-ion concentration was measured.

The culture was carried out in a thermostat at 26°C. The mycelial growth of the fungi was determined by comparison of the average weights of three cultures, after a fortnight's incubation, and the hydrogen-ion concentrations of the stale solutions were also determined. The results are shown in the following tables:

Table XII. Showing the mycelial growth of *Fusarium Lini* in varying hydrogen-ion concentrations.

pH value of cultural solutions		Average weight of grown mycelium in gram.
Initial	Final	
2.6	4.9	0.085
2.7	4.7	0.081
3.1	4.6	0.171
3.3	4.8	0.146
3.7	6.6	0.152
4.0	5.4	0.147
4.6	6.2	0.166
5.1	6.2	0.200
5.3	6.3	0.145

pH value of cultural solutions		Average weight of grown mycelium in gram.
Initial	Final	
5.7	6.8	0.144
5.8	6.2	0.142
6.0	6.4	0.165
6.1	6.7	0.140
6.7	6.2	0.144
6.8	6.3	0.118
7.0	6.3	0.113
7.2	6.4	0.111
7.8	6.6	0.127
8.0	7.8	0.152
8.5	7.8	0.136
8.9	8.0	0.154
9.9	7.7	0.153

Table XIII. Showing the mycelial growth of *Colletotrichum Lini* in varying hydrogen-ion concentrations.

pH value of cultural solutions		Average weight of grown mycelium in gram.
Initial	Final	
2.6	2.9	Trace
2.7	3.2	Trace
3.1	3.6	0.020
3.3	3.8	0.027
3.7	4.5	0.057
4.0	4.2	0.054
4.6	4.4	0.055
5.0	4.2	0.061
5.1	4.0	0.052
5.3	4.2	0.045
5.7	4.3	0.065
5.8	4.3	0.047
6.0	4.1	0.051
6.1	4.0	0.083

6.7	4.8	0.039
6.8	4.8	0.055
7.0	4.8	0.059
7.2	4.8	0.063
7.8	5.0	0.034
8.0	7.6	0.054
8.5	7.8	0.036
8.9	8.2	0.037
9.9	8.8	0.031

From these results it is evident that both fungi are capable of growing in cultural solutions varying through a wide range of hydrogen-ion concentrations. Especially *Fusarium Lini* shows much resistance to both high and low hydrogen-ion concentrations.

The optimum hydrogen-ion concentration for *Fusarium Lini* seems to be near pH 5.0. A hydrogen-ion concentration as high as pH 2.6 retarded the growth of the fungus only a little, and as low a hydrogen-ion concentration as pH 9.9 had almost no marked influence upon the growth of the fungus. Near the neutral point (pH 7.0) the growth of the fungus suffered a slight setback.

Colletotrichum Lini, however, seems to be far more sensitive to high or low hydrogen-ion concentrations than *Fusarium Lini*, and acidities of the cultural solution higher than pH 3.0 almost inhibited the mycelial development of the fungus, and a hydrogen-ion concentration lower than pH 8.5 evidently retarded its growth. The optimum hydrogen-ion concentration for the fungus seems to be near pH 6.0.

The hydrogen-ion concentration of the cultural solution, however, changes rather rapidly owing to the growth of the fungi, due to the disproportional consumption of the elements by them. Therefore the prepared hydrogen-ion concentrations influenced the growth of the fungi only at the start of their development. The reaction-changes of the stale solutions differ in both fungi. In the case of *Fusarium Lini* the cultural solution changes to alkaline or acidic respectively, if its initial hydrogen-ion concentration is higher or lower than about pH 6.5. The demarcation value of the initial hydrogen-ion concentration is about pH 4.5 in *Colletotrichum Lini*. These critical points of hydrogen-ion

concentration may correspond to the so-called "isometabolic point" of SIDERIS (*94*).

b. Effect of the various organic acids on the fungi.

It is evident that the dissociated hydrogen-ions in the cultural medium have an important effect upon the micro-organisms. Likewise, the dissociated acid radicals and undissociated acid molecules are of considerable effect on the cells of the organism, especially in the organic acids, and the molecular construction of the organic acid also deeply affects the cells of the organism.

HEMMI (*46*) reported on the influence of malic, citric and tartaric acid upon the hyphal development of various species or strains of *Gloeosporium* and *Colletotrichum*.

The author has carried out the present studies to learn in what nutritive relationship the fungi stand to an equal gram molecule of various organic acids in the nutrient solution.

The following kinds of organic acid were used:

Fatty acids,

1. Formic acid.
2. Acetic acid.
3. Propionic acid.
4. Butyric acid.

Carboxylic acids derived from aromatic hydrocarbons,

5. Benzoic acid.
6. Phthalic acid.

Hydroxy-carboxylic acids derived from aromatic hydrocarbons,

7. Salicylic acid.
8. Gallic acid.

Carboxylic acids derived from glycolls,

9. Oxalic acid.
10. Malonic acid.
11. Succinic acid.
12. Fumaric acid.

Hydroxy-carboxylic acids derived from glycolls,

13. Lactic acid.
14. Malic acid.
15. Tartaric acid.
16. Citric acid.

The formula of the standard nutrient solution was as follows:

Asparagine	1.00 gram
Ammonium nitrate	1.00 „
Mono-kalium phosphate	0.50 „
Magnesium sulphate	0.25 „
Sucrose	20.00 „
Redistilled water	1000.00 c.c.

The cultural solution was prepared by adding each organic acid to an extent of 1/150 mole to the standard nutrient solution. The cultural solution was twice sterilized for 30 minutes in a KOCH'S steam sterilizer with one day's interval. The cultures were inoculated with small and approximately equal masses of the mycelium of the fungi.

The growth of the fungi was computed by determining the average weight of the grown mycelium after a fortnight's cultivation. The results of the cultures are shown in the following tables:

Table XIV. Showing the influence of organic acids upon the mycelial growth of *Fusarium Lini.*

Kinds of organic acid	pH value of media	Av. wt. of grown mycelium in gr.	Remarks
Formic acid	3.4	0.146	Matty growth.
Acetic acid	3.6	0.086	Thin matty growth.
Propionic acid	3.6	+	Inoculated mycelial mass increased in size a little.
Butyric acid	3 8	0	No sign of growth.
Benzoic acid	3.2	0	No sign of growth.
Phthalic acid	2.9	0.021	Loose mycelial growth.
Salicylic acid	2.6	0	No sign of growth.
Gallic acid	3.4	0.138	Matty growth.
Oxalic acid	2.2	+	Inoculated mycelial mass increased in size a little.
Malonic acid	2.6	0.054	Loose mycelial growth.
Succinic acid	3.2	0.135	Matty growth.
Fumaric acid	2.8	0.050	Moderately developed mycelial masses.
Lactic acid	3.0	0.153	Matty growth.
Malic acid	3.0	0.134	Thin matty growth.
Tartaric acid	3.0	0.114	Matty growth.
Citric acid	3.0	0.132	Matty growth.
Control (no acid)	5.1	0.174	Matty growth.

Table XV. Showing the influence of organic acids upon the mycelial growth of *Colletotrichum Lini*.

Kinds of organic acid	pH value of media	Av. wt. of grown mycelium in gr.	Remarks.
Formic acid	3.4	0.051	Loose mycelial growth.
Acetic acid	3.6	0.042	Ditto.
Propionic acid	3.6	0	No sign of growth.
Butyric acid	3.8	0	Ditto.
Benzoic acid	3.2	0	Ditto.
Phthalic acid	2.9	+	Inoculated mycelial mass increased in size a little.
Salicylic acid	2.6	0	No sign of growth.
Gallic acid	3.4	0.015	Small mycelial masses.
Oxalic acid	2.2	+	Inoculated mycelial mass increased in size slightly.
Malonic acid	2.6	0.017	Small mycelial masses.
Succinic acid	3.2	0.053	Salmon-coloured loose mycelial growth.
Fumaric acid	2.8	0.023	Small mycelial masses.
Lactic acid	3.0	0.043	Salmon-coloured loose mycelial growth.
Malic acid	3.0	0.033	Small mycelial masses.
Tartaric acid	3.0	0.022	Salmon-coloured small mycelial masses.
Citric acid	3.0	0.044	Ditto.
Control (no acid)	5.1	0.063	Salmon-coloured moderately grown mycelial masses.

The relation between the proper action and the hydrogen-ion concentration of the cultural solution containing the organic acids examined seems to be rather complicated in the present studies. In the case of higher hydrogen-ion concentration in the equimolecular solution, the deleterious effect of the organic acid seems to be caused by the dissociated hydrogen-ion primarily. For example, oxalic acid for *Fusarium Lini*, and phthalic, salicylic, oxalic, malonic and fumaric acids for *Colletotrichum Lini* are in this case. However, the actions of the organic acids did not seem always to correspond to the hydrogen-ion concentrations of the cultural solution due to their dissociation, for there are some differences among these organic acids.

In the cultural solution containing formic acid the growth of the fungi was fairly good. Acetic acid was clearly more unfavorable

to both fungi. In the cultural solution containing propionic acid *Fusarium Lini* showed a trace of hyphal growth, but *Colletotrichum Lini* showed no sign of development. Butyric acid, however, inhibited utterly the growth of both fungi, although the hydrogen-ion concentration of the cultural solution containing that acid was the lowest of all the fatty acids examined.

The acids derived from aromatic hydrocarbons generally retarded the growth of the fungi very badly, but gallic acid was an exception among them, especially for *Fusarium Lini*. It gave a far lower hydrogen-ion concentration than the other acids of this kind.

Carboxylic acids derived from glycolls generally gave high hydrogen-ion concentrations to the nutrient solution, but their influence upon the hyphal growth of the fungi was not so severe as in the case of the acids derived from aromatic hydrocarbons. Among them, succinic acid gave an exceptionally low hydrogen-ion concentration to the nutrient solution, and naturally, the fungi showed a comparatively good development.

The hydroxy-carboxylic acids were generally mild in their action upon the fungi. The entrance of the OH-group into the molecule of organic acids seemed to be favorable for the fungi, as LOEB (*66*) had found in the case of sea-urchins.

c. Effect of various concentrations of tannic acid on the fungi.

On the effect of tannic acid upon fungi, a number of interesting reports have been published by various authors. PFEFFER (*88*), COOK (*24*), and others attributed a protective meaning to the production of tannic acid in plant bodies. KNUDSON (*61*), CLINTON (*22*) and HEMMI (*41*) have reported that the tannins in low percentages were utilized by the fungi, although they retarded their growth in high concentrations. MATSUMOTO (*73*) found that tannic acid retarded the hyphal growth of *Rhizoctonia Solani* even at a relatively low degree of concentration, but in media containing sucrose it appeared to stimulate the growth at an extremely low concentration of the acid. He also reported that tannic acid inhibited the diastic action of the fungus, but not the action of invertase. The author (*108*) has previously reported that tannic acid seemed never to be assimilated by *Fusarium Lini*, and that it retarded the mycelial development of the fungus in proportion to its concentration, although the fungus was able to survive in a com-

paratively high concentration of the acid for a fairly long time, being forced to form chlamydospores.

The nature of the nutrient solutions to which tannic acid is added, seems to influence the effect of the acid on the growth of fungi. But the kinds of culture media suited to the experiment with tannic acid are limited, for the tannic acid generally coagulates the colloids and precipitates many of the organic substances. The use of such nutrient compounds which will combine with the tannic acid must be avoided in the culture media, for such chemical changes in the media may cause unfavourable complications.

In the present studies, a nutrient solution of the following formula has been used:

Ammonium nitrate	1.00 gram
Mono-kalium phosphate	0.50 „
Magnesium sulphate	0.25 „
Sucrose	20.00 „
Redistilled water	1000.00 c.c.

The cultural solutions were prepared by the addition of regulated amounts of Japanese pharmacopoeial tannic acid to the standard nutrient solution.

The cultural conditions were just the same as in the previous studies. The results of a fortnight's culture are shown in the following table:

Table XVI. Showing the influence of tannic acid upon the growth of the fungi.

Fusarium Lini				*Colletotrichum Lini*
Percentage of tannic acid	pH value of media	Av. wt. of grown mycelium in gr.	Remarks	Remarks
0.1	3.8	0.0639	Thin mycelial growth.	No growth.
0.2	3.6	0.0427	Ditto.	Ditto.
0.3	3.5	0.0257	Loose mycelial growth.	Ditto.
0.4	3.4	0.0260	Ditto.	Ditto.
0.5	3.2	0.0153	Small brown mycelial masses.	Ditto.
0.6	3.2	0.0150	Ditto.	Ditto.
0.7	3.0	0.0117	Ditto.	Ditto.
0 (Control)	5.0	0.1373	Thin matty growth.	Moderately grown mycelial masses: average weight is 0.077 gram.

Colletotrichum Lini was completely checked in its growth even with the lowest concentration of tannic acid in this series, although their hydrogen-ion concentration was not beyond the growing range of the fungus, whereas in the control cultures the fungus grew into well developed mycelial masses, which showed the characteristic salmon colour and attained an average weight of 0.077 grams. Then the growth of the fungus was complementarily examined in 0.05% and 0.01% concentrations of the tannic acid. In the cultures containing 0.01% of tannic acid, the fungus developed moderately well, but in the cultures containing 0.05% of the acid it showed only a trace of growth, the size of the inoculated mycelial mass having increased only slightly.

In these experiments the tannic acid effected a retarding action on the growth of the fungi. Especially *Colletotrichum Lini* was very sensitive to the existence of tannic acid in the nutrient solution. The maximum concentration of the acid for the growth of the fungus seemed to be about 0.05%, and a 0.01% concentration of the acid was enough to check the growth. On the other hand, *Fusarium Lini* grew a little even when the highest percentage of the acid in this series was used, but comparing it with the control culture, the existence of tannic acid in the cultural solution was apparently unfavorable to the fungus. Moreover, in the author's previous studies (*108*), as slight a presence of the acid as 0.01% evidently retarded the growth of the fungus, although even so high a concentration of the acid as 1.3% could not utterly inhibit its growth, and the germination of the inoculated conidia.

Therefore, it may be concluded that *Fusarium Lini* stands in an interesting relation to tannic acid, inasmuch as the mycelial growth of the fungus is fairly retarded by even a slight presence of the acid in the cultural media, although it shows a remarkable endurance to high concentrations of the acid, while *Colletotrichum Lini* is very sensitive and non-resistant to tannic acid, as even 0.1% of it in the culture media utterly checks the mycelial growth of the fungus.

HEMMI (*46*) reported an interesting fact on the toxity of tannic acid upon the various strains and species of *Gloeosporium* and *Colletotrichum*. He examined over 30 strains of these fungi, isolated from various host plants, concerning their relation to tannic acid of a concentration of 0.1, 0.5 and 1.0%. In this case, the sensibility of the examined strains of *Gloeosporium* and *Colletotrichum* to tannic acid varied in many ways, some of them being strongly resistant to the

increase of the acid concentration, while others were very sensitive and showed no growth even in the lowest percentage of the acid. The relation of the latter strains to tannic acid seems to be somewhat analogous to that of *Colletotrichum Lini*.

It is of the greatest importance to take particular care that the tannic acid is of the very best material. So far the preparation of pure tannic acid has been considered to be a very difficult thing, and the ordinary commodities are never chemically pure. On that point COOK and WILSON (*25*) gave a valuable account of the chemical and biological nature of tannic acid, having compared four kinds of plant extract containing tannin with the commercial tannic acid made by Merck. According to them those tannins differed from each other in their stimulating and poisoning actions on the organism. Moreover, when the same kind of tannic acid was used in the culture media placed under similar conditions, the difference of the culture media affected the results of the experiment. This can be explained by the effect of nutrition of the culture media on the growth of fungi or the chemical effect of the substances contained in the culture media on the acid. Therefore the results of the cultural studies of tannic acid can not be easily compared with those of other experiments.

d. Effect of various concentrations of citric acid on the fungi.

An acid medium being generally favorable to most fungi, the culture media are often acidulated by addition of some organic acids. For this purpose citric, lactic and tartaric acid were commonly used in the culture media. APPEL and WOLLENWEBER (*3*) used a potato-sap agar medium containing 1% of citric acid in their studies for a monograph on the genus *Fusarium*.

Concerning the toxic influence of citric acid HEMMI (*42*) reported that *Gloeosporium Aracearum* was retarded in its mycelial growth by an excess of citric acid, and that the conidia and short mycelia produced from the resultant chlamydospores of various sizes aggregated in small masses. According to him such a chlamydospore formation has a protective purpose. Comparing the influence of several organic acids upon the growth of many strains of *Gloeosporium*, including *Colletotrichum*, HEMMI (*46*) concluded that, by a limited addition of citric, malic or tartaric acid many *Gloeosporia* are very often influenced

favorably in their growth, while the addition of higher concentrations affects them poisonously. THIEL and WEISS (*102*) reporting on the effect of citric acid upon the germination of the teleutospores of *Puccinia graminis*, thought it highly probable that the germination of the teleutospores might have been obtained much earlier had this treatment been discovered earlier in the season. In the author's previous studies (*108*), the mycelial growth of *Fusarium Lini* was stimulated by a slight quantity of citric acid in the culture media, but the higher concentrations of the acid retarded the hyphal growth and the production of conidia, while the formation of chlamydospores was accelerated within certain limits. The effect of citric acid seemed to be dependent upon the kind of nutrient medium. In a synthetic nutrient solution which was prepared by modifying PFEFFER's formula (*87*), a 1.5% concentration of the acid greatly retarded the growth of the fungus, and a 2% concentration utterly checked it; in a potato decoction agar medium, even a 3% concentration of the acid never inhibited the hyphal growth and the production of conidia, while a 5% concentration of the acid checked the hyphal growth and the production of conidia, but not the formation of chlamydospores.

In the present studies, a nutrient solution of the following formula has been used:

Peptone	10.00 grams
Mono-kalium phosphate	0.50 ,,
Magnesium sulphate	0.25 ,,
Sucrose	20.00 ,,
Redistilled water	1000.00 c.c.

Regulated amounts of citric acid were added to this nutrient solution to an extent varying from 0.1% to 0.7%. The cultural conditions were just the same as in the preceding studies. After a fortnight the cultures were graded by comparison of the average weights of the grown mycelium and the appearance of the growth. The results are shown in the following tables:

Table XXV. Showing the influence of citric acid upon the growth of *Fusarium Lini.*

Percentage of citric acid	pH value of media	Av. wt. of grown mycelium in gr.	Remarks
0.1	4.2	0.412	Very thick matty growth, with vigorously grown aerial mycelium.
0.2	4.1	0.408	Very thick matty growth, with moderately grown aerial mycelium.
0.3	4.0	0.319	Thick matty growth, with less aerial mycelium.
0.4	4.0	0.288	Thick matty growth, with little aerial mycelium.
0.5	3.8	0.258	Matty growth, with no aerial mycelium.
0.6	3.8	0.248	Moderate matty growth, with no aerial mycelium.
0.7	3.6	0.210	Ditto.
Control (no acid)	5.4	0.412	Very thick matty growth, with little aerial mycelium.

Table XXVI. Showing the influence of citric acid upon the growth of *Colletotrichum Lini.*

Percentage of citric acid	pH value of media	Av. wt. of grown mycelium in gr.	Remarks
0.1	4.2	0.091	Moderate matty growth, tinged with salmon colour.
0.2	4.1	0.055	Thin matty growth, tinged with salmon colour.
0.3	4.0	0.014	Moderately-grown white mycelial masses.
0.4	4.0	0.006	Weakly-developed white mycelial masses.
0.5	3.8	0	No sign of growth.
0.6	3.8	0	Ditto.
0.7	3.6	0	Ditto.
Control (no acid)	5.4	0.150	Matty growth, tinged with salmon colour.

In the present experiments, the influence of citric acid upon the mycelial growth was mild in the case of *Fusarium Lini*, and somewhat severe in the case of *Colletotrichum Lini*. In the case of *Fusarium Lini*, concentrations lower than 0.3% did not show an unfavorable effect upon the mycelial growth, nor did the presence of the acid ever accelerate it. When the concentration increased above 0.4% the mycelial growth of the fungus was gradually retarded, and in the highest concentration of this series the weight of the grown mycelium decreased

to half of that of the control culture. The development of the aerial hyphae was stimulated by a slight amount of the acid, and again retarded when the concentrations increased.

In the case of *Colletotrichum Lini*, the increase of the acid concentration effected a marked retardation upon the mycelial growth. Concentrations above 0.5% completely checked the growth of the fungus, although the hydrogen-ion concentration was not high.

The stimulating effect of citric acid upon the growth of fungi has often been observed by numerous investigators. Such an effect may be due to the acidification of the medium by the dissociation of the acid. In the present case, however, the standard nutrient solution is prepared in such a way that it has a comparatively favorable or at least a tolerable hydrogen-ion concentration for the fungi in question, and therefore the addition of citric acid, perhaps, serves no better end than to be indifferent.

e. Conclusions concering the influence of acid on the fungi.

1. *Fusarium Lini* was found to be capable of growing in a wider range of the hydrogen-ion concentration of the cultural solution than *Colletotrichum Lini*, the former being less sensitive to both high and low hydrogen-ion concentrations than the latter.

2. The optimum hydrogen-ion concentration of the nutrient solution in the present case was about pH 5 for *Fusarium Lini*, and about pH 6 for *Colletotrichum Lini*.

3. *Fusarium Lini* decreased the hydrogen-ion concentration of the cultural solution when its initial pH value was smaller than about 6.5, but when it was greater than that value the hydrogen-ion concentration was changed towards the contrary direction. The demarcation value of the initial hydrogen-ion concentration was about pH 4.5 in the case of *Colletotrichum Lini*.

4. The toxic actions of the organic acids did not seem always to correspond to the hydrogen-ion concentrations of the cultural solution due to their dissociation.

5. The entering of the hydroxy group into the molecule of an organic acid, as a rule, seems to weaken its toxic aciton.

6. Tannic acid retarded the growth of the fungi even if only a small quantity was contained in the cultural solution.

7. *Colletotrichum Lini* being very sensitive to tannic acid, its growth was greatly retarded in a nutrient solution containing 0.05% of the acid, and it was entirely checked at its 0.1% concentration. *Fusarium Lini* was more or less resistant even to considerably higher concentrations of the acid, the maximum concentration for its growth being higher than 1.3%.

8. Citric acid influenced the growth of the fungi far more mildly than tannic acid.

9. In the case of *Fusarium Lini*, the concentrations of citric acid lower than 0.2% showed almost no retardation on the growth of the fungus, but above 0.3% the unfavorable effect of the acid slowly increased according to the increase of the concentration. According to the author's previous studies (*108*), the maximum concentration of the acid for the growth of the fungus probably is about 5%.

10. In the case of *Colletotrichum Lini*, however, the injurious effect of the citric acid rapidly advanced with its increased concentration, and the growth of the fungus was utterly checked at 0.5%.

4. Relation of Temperature to the Fungi.

Investigations on the temperature relation of fungi are interesting as well as important not only from the physiological, but also from the phytopathological point of view. The germination of spores and the growth of hyphae being dependent on temperature conditions, the breaking out of parasitic diseases is usually controlled by the climate. BUTLER (*19*) gave an interesting account concerning the grain smut of jowar in India as a case of disease-escaping. As the climate in the Ganges plain at the seed-time of jowar is too high for the germination of the spores of *Sphacelotheca Sorghi* adhering to the seeds, the host plant escapes from the infection of the parasite. He also explained the case of the tikka-disease of the groundnut, caused by *Cercospora personata*, saying that this disease appears in the warmer temperatures of August and September. The damage is controlled by cultivating early ripening strains of the crop. Such facts suggest the importance of investigating the temperature relations of fungi with a view to plant-protection.

In the present studies, the author has carried out experiments, firstly, concerning the influence of varying temperatures upon the mycelial development of the fungi in order to determine the minimum, optimum

and maximum temperatures for their growth; secondly, on the power of resistance of the fungi to high temperatures in order to determine the thermal death points in connection with the time factors; and thirdly, on the effects of low temperatures upon the fungi.

a. Relation between temperature and growth of the fungi.

On the relation of temperature to the growth of *Fusarium* and *Gloeosporium*, several papers have been published.

The species of *Fusarium*, as a rule, have been considered to require a comparatively high temperature for their vigorous growth and virulent attack. L. R. JONES (*57*) said that the damping off of coniferous seedlings was facilitated by high temparature. WOLLENWEBER (*119*) also said that the wilt diseases, caused by *Fusarium*, occur severely in warm climates. WOLF (*118*) reported on the wilt disease of pansy, caused by *Fusarium Violae*, saying that the disease was found only in July, and then only when the beds in which the plants were growing had been heavily covered with fresh horse manure, both of which facts suggested a dependence of the fungus on high temperature. H. B. HUMPHREY (*53*) reported on the relation of certain species of *Fusarium* to tomato blight disease, and said that *Fusarium orthoceras* and *F. oxysporum* caused the tomato blight in high temperature, the optimum temperature for the growth of the fungi being about 30°C. GILMAN (*36*) studied the relation of temperature to the cabbage yellow disease caused by *Fusarium conglutinans*, and reported in 1914 that the occurrence of this disease increased according to the rise in temperature. Two years later, in 1916, he (*37*) reported again on this problem saying that a high temperature was favorable to the mycelial growth of the fungus, and the germination of the conidia whcih occurred within only three hours at 33°C. The cabbage seedlings were attacked most severely at 28°C. to 30°C., but even at low temperatures (as 10° to 12°C.) the conidia could germinate. TISDALE (*104*), who studied the relation of temperature to the growth and infecting power of *Fusarium Lini*, reported that the minimum, optimum and maximum temperatures for the growth of the fungus on potato agar medium were 10° to 11°C., 26° to 28°C. and 35°C. respectively, and the critical temperature for the infection by the fungus was from 14° to 16°C. In 1922, L. R. JONES and W. B. TISDALE (*58*) stated in their paper on the influence of soil temperature

upon the development of flax wilt, that the optimum temperature for its development was 24° to 28°C., and the minimum and maximum temperatures were 14°C. and 38°C. respectively. CLAYTON (*21*), who studied the relation of temperature to the wilt disease of tomato caused by *Fusarium lycopersici* reported that the temperature conditions of soil and air most favorable for the disease, as determined in tanks, are a soil temperature of about 27°C., and an air temperature, after the fungus has established itself in thes tem, of about 28°C. The author (*108*) has found the minimum, optimum and maximum temperatures for the growth of *Fusarium Lini* to be from 10° to 12°C., 30°C. and from 36° to 37°C. respectively.

On the temperature relations of *Gloeosporium* and nearly-related fungi causing the anthracnose of plants, several studies from phyto-pathological and physiological standpoints have been published by SCHNEIDER-ORELLI (*97*), KRÜGER (*62*), AMES (*2*), EDGERTON (*30*), BROOKS and COOLEY (*16*), STEVENS (*101*), HEMMI (*42, 43, 44*), LAURITZEN (*65*) and others. In 1920 HEMMI (*46*) published extensive and important studies on the morphology and physiology of the Japanese *Gloeosporium* giving in his paper a detailed list of the literature relating to the subject. He carried out two series of experiments with different nutrient solutions with a view to establish the temperature relations of 48 strains of anthracnose-causing fungi. He distinguished three special groups of *Gloeosporia* with regard to their relations to high temperatures. In this connection he divided the *Gloeosporia* into a thermo-tolerant and a thermo-intolerant group according to whether they showed active or no growth at all at a temperature of about 35°C. The strains, which showed weak growth or only a trace of it at that critical temperature, he treated as a mesophilous group. In his case, the optimum and minimum temperatures for the growth of the fungi varied from 21° to 28°C. in the former, and from 5° to 10°C. in the latter, and some of them showed a slight sign of mycelial growth even at 3.5°C.

In the present studies, the author has used for the culture of *Fusarium Lini* a synthetic solution of the following formula:

Peptone	10.00 grams
Ammonium nitrate	1.00 "
Mono-kalium phosphate	0.50 "
Magnesium sulphate	0.25 "
Sucrose	20.00 "

Redistilled water	1000.00 c.c.

The nutrient solution was sterilized about an hour in KOCH'S steam sterilizer. After removal of some precipitates by filtration, the filtrate, 50 c.c. each, was placed in ERLENMEYER'S flasks of about 250 c.c. capacity, and sterilized again for an hour. They were inoculated with a small mass of the mycelium, and placed in thermostat at regulated temperatures. After a fortnight, the growths at the varying temperatures were compared with each other by taking the average weights of grown mycelium in three flasks for each temperature.

For *Colletotrichum Lini*, a corn meal agar medium of the following formula was used:

Corn meal	15 grams
Peptone	5 "
Sucrose	10 "
Agar	15 "
Distilled water	1000 c.c.

Portions of the liquified medium, 20 c.c. each, were placed in PETRI-dishes of 4 inches diameter, and sterilized for two hours. Then the plates were inoculated in the centre with a small mass of the mycelium, and cultured at regulated temperatures. After 12 days culture, the growth of the fungus at the various temperatures was ascertained by taking the average diameter of the developed mycelial masses in three cultures for each temperature.

The results are shown in the following tables and figures:

Table XIX. Showing the mycelial growth of *Fusarium Lini* in various temperatures.

Temperatures	Av. wt. of grown mycelium in gr.	Remarks
5°— 6°C.	0	No sign of growth.
10°—11°C.	+	Slight growth.
13°—14°C.	0.062	Weakly developed mycelial masses.
17°—18°C.	0.156	Well developed mycelial masses.
21°—22°C.	0.276	Moderate matty growth.
24.5°—25°C.	0.405	Thick matty growth.
26°—26.5°C.	0.440	Thick matty growth.
28.5°—29.5°C.	0.536	Very thick matty growth.
34°—35°C.	0.101	Loose mycelial growth.
37°C.	+	Very small mycelial masses.
40°C	0	No sign of growth.

Table XX. Showing the mycelial growth of *Colletotrichum Lini* in various temperatures.

Temperatures	Av. diam. of mycelial masses in c.m.	Remarks
5°— 6°C.	0	No sign of growth.
10°—11°C.	+	Slight growth.
12°—13°C.	1.6	White small mycelial mass.
17°—18°C.	5.3	Center of mycelial mass showed salmon colour.
21°—22°C.	7.5	Center of mycelial mass showed salmon colour, and conidia were produced.
25°—26°C.	8.1	Diameter of mycelial mass rapidly increased.
28°—28.5°C.	7.4	Increase of diameter somewhat delayed.
29.5°—30°C.	6.0	Increase of diameter more delayed.
33°—34°C.	2.9	The growth was retarded.
36°C.	0	No sign of growth, and the mycelium was killed within a week.

In the present case, the mycelial development of *Fusarium Lini* seemed to begin at about 10°C., but at that temperature the growth was very weak and slow, only a trifling increase being observed in the size of the small mycelial mass used in inoculation together with a slight swelling of the conidia without any germination. In higher temperatures than that, the development of the mycelium became more and more vigorous proportionately to the rise of the temperature up to the optimum point at 28.5° to 29.5°C. Above that temperature the mycelial growth weakened rather rapidly, so that at 37°C. it showed only a slight growth. At 40°C. the growth was completely checked.

These results were almost the same as those of W. H. TISDALE (*104*) and accorded well with the author's previous studies (*108*) on the same fungus.

It may be concluded that for the mycelial growth of *Fusarium Lini*, the minimum temperature is at about 10°C., the optimum lies at from 28° to 30°C., or nearly at 30°C., and the maximum is at about 37°C.

In *Colletotrichum Lini*, mycelial development began to take place at 10° to 11°C., although only to a very slight extent. Above that temperature, the growth of the fungus increased in its vigour and rapidity proportionately to the rise of the temperature up to its optimum point at 25° to 26°C. Then it decreased gradually at an inverse ratio to the rise of temperature up to 30°C., beyond which the growth was

Fig. I. Showing the growth-curve of *Fusarium Lini* with respect to various temperatures.

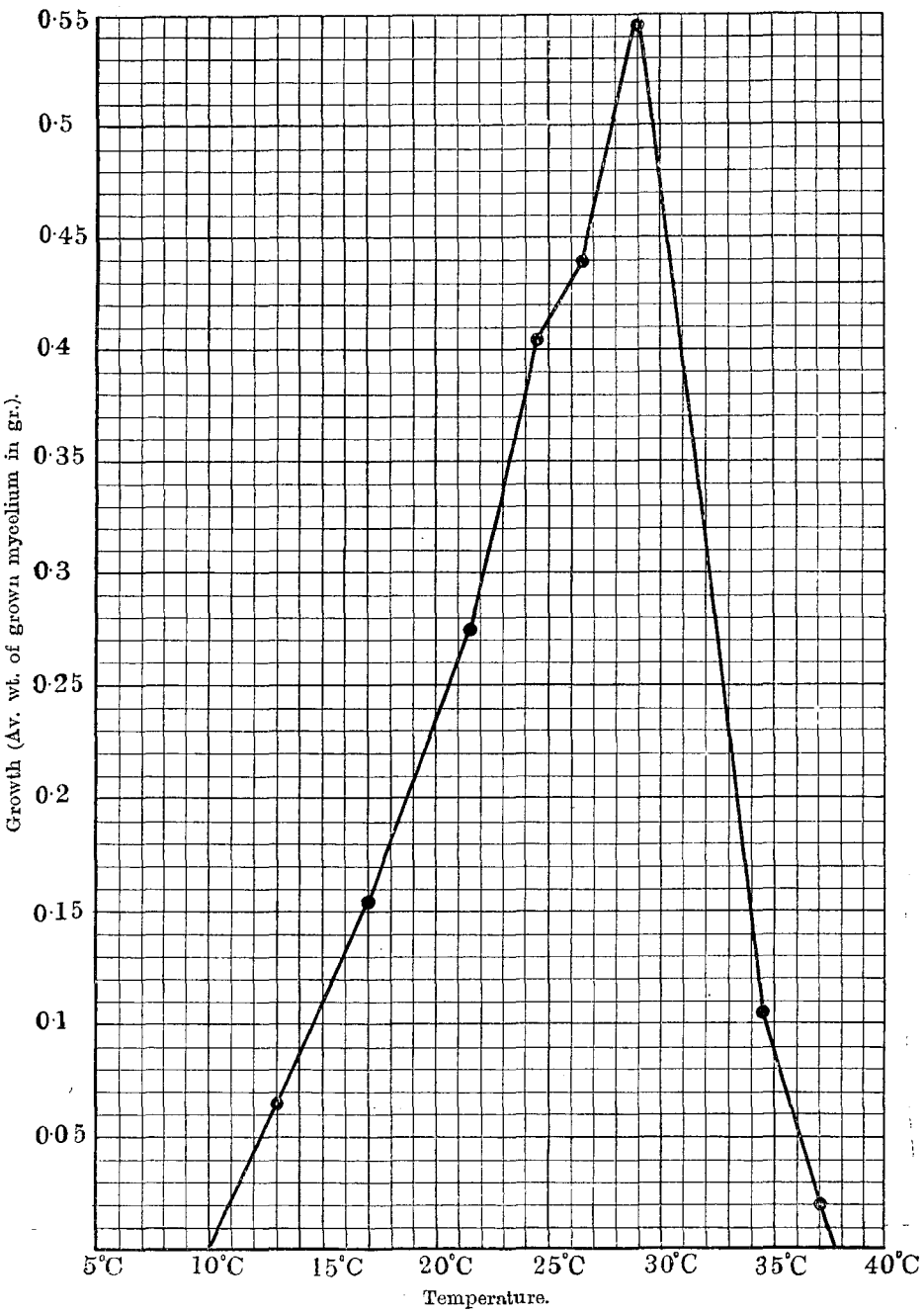
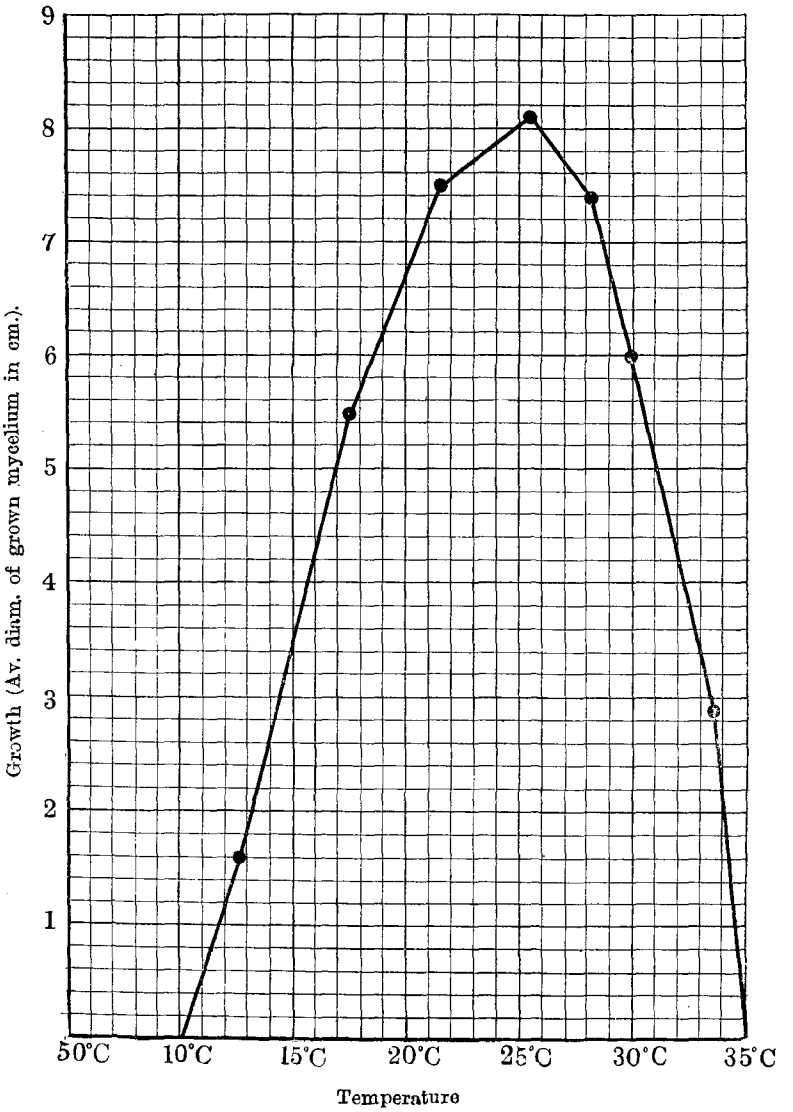


Fig. II. Showing the growth-curve of *Colletotrichum Lini* with respect to various temperatures.



rapidly retarded, and at 36°C. it was utterly checked. Further, incubation at that temperature for one week killed the fungus, though it was able to hold out for five days.

From these results, it may be concluded that the minimum, optimum and maximum temperatures for the mycelial growth of *Colletotrichum Lini* are from 10° to 11°C., ca. 25°C. and nearly 35°C. respectively. According to the classification proposed by HEMMI (*46*), *Colletotrichum Lini* corresponds to the mesophilous group of *Gloeosporium*.

Comparing their relations to temperature, *Fusarium Lini* and *Colletotrichum Lini* seem to be analogous as far as the minimum temperature for the mycelial growth of the fungi is concerned. But both the optimum and maximum temperatures are markedly higher in the former species than in the latter, so that it may be said that the adaptable range of temperature is wider in *Fusarium Lini* than in *Colletotrichum Lini*.

b. Effect of high temperatures on the fungi.

The effect of high temperatures upon the vitality of fungi has been studied most frequently in the case of smut fungi with a view to seed-disinfection. In this connection, ATANASOFF and JOHNSON (*4*) gave an extensive review of literature. NAUMOV (*80*) reported that the mycelium of *Gibberella Saubinetii*, present in the interior of cereal kernels, was killed or considerably weakened by dry heat at 60°C., for wheat, barley and oats, and at 65°C. for rye, with periods ranging from 24 hours to 3 days. O'BRIEN (*83*) examined the resistance of the hyphae and conidia of *Aspergillus flavus, Botrytis vulgaris, Rhizopus nigricans, Sterigmatocystis nigra* and *Penicillium glaucum* to high temperatures. HEMMI (*46*) studied the resistance of the conidia of various kinds of *Gloeosporium* to high temperatures, and reported that the conidia of *Gloeosporium* in moist condition were killed within ten minutes by temperatures above 57°C. At 50°C. it required more time to kill them. Temperatures lower than 45°C. were not effective for their sterilization with the exception of some species. The author (*108*) investigated the resistance of the mycelium of *Fusarium Lini* to high temperatures, as well as the effects of high temperatures upon the germination of its conidia, and gained the following results: At 50°C. the fungus was not killed within 3 hours, but at 60°C. two-thirds of the examined cultures were killed within 3 hours, and in either

temperature a treatment of more than 4 hours was enough to kill the fungus; the conidia, however, lost their germinating power after 2 hours at 50°C., and about half of the examined conidia were found to be shrivelled up within an hour. Recently, the author himself and ENOMOTO (*109*), investigating the dry heat sterilization of flax-seeds for the prevention of flax anthracnose, reported that the mycelium of *Colletotrichum Lini* in the tissues of flax-seed was fatally affected by a heating at 70°C. for 30 minutes; the germinating power of the flax-seed was scarcely harmed at that temperature within an hour.

In the present studies, the same nutrient solution as in the preceding experiment on temperature was used. Test tubes containing 10 c.c. of the nutrient solution were inoculated with small pieces of the mycelium of the fungi, and kept during periods varying from 10 minutes to 8 hours at various constant temperatures in a lidded water-tank having a thermo-regulator and electric agitator. After the treatment the test tubes were removed to thermostat at 26°C. for examination of the vitality of the mycelia used in inoculation. The results are shown in the following tables:

Table XXI. Showing the effects of high temperatures on the vitality of *Fusarium Lini*.

Temperature	Living (+) or dead (−) mycelium											
	Control	10m.	20m.	30m.	1h.	2h.	3h.	4h.	5h.	6h.	7h.	8h.
45°C.	+	+	+	+	+	+	+	+	+	+	+	+
50°C.	+	+	+	+	+	+	+	+	+	−	−	−
55°C.	+	+	+	+	+	+	+	±	−	−	−	−
60°C.	+	+	+	+	+	+	±	−	−	−	−	−

Table XXII. Showing the effects of high temperatures on the vitality of *Colletotrichum Lini*.

Temperature	Living (+) or dead (−) mycelium											
	Control	10m.	20m.	30m.	1h.	2h.	3h.	4h.	5h.	6h.	7h.	8h.
45°C.	+	+	+	+	+	+	+	±	−	−	−	−
50°C.	+	+	±	−	−	−	−	−	−	−	−	−
55°C.	+	−	−	−	−	−	−	−	−	−	−	−
60°C.	+	−	−	−	−	−	−	−	−	−	−	−

In the present case, *Fusarium Lini* showed far stronger resistance to high temperatures than *Colletotrichum Lini*.

An eight hours' exposure at 45°C., was never able to lower the vitality of *Fusarium Lini*, but in *Colletotrichum Lini* the fatal effect set in after 4 hours, and after more than 5 hours the mycelium was killed.

At a temperature of 50°C. *Fusarium Lini* was killed after 6 hours, exposure, but *Colletotrichum Lini* lost its vitality within half an hour, and the fatal effect already showed itself after 20 minutes' exposure.

At a temperature of 55°C., in *Fusarium Lini* the fatal effect appeared after 4 hours, and it was killed within 5 hours, but *Colletotrichum Lini* was killed within only 10 minutes.

At a temperature of 60°C., in *Fusarium Lini* the fatal effect appeared after 3 hours, and it was killed within 4 hours, but *Colletotrichum Lini* was killed before 10 minutes had lapsed.

The results in *Fusarium Lini* at 50°C. and 60°C. did not wholly agree with those in the author' previous studies (*108*). This may be due to the difference in the nutrient solutions.

Then it may be concluded that temperatures higher than 50°C. are effective for the sterilization of *Colletotrichum Lini*, but for *Fusarium Lini* even the highest temperature in the present series (60°C.) did not accomplish sterilization in less than 3 hours.

c. The effect of low temperatures on the fungi.

On the effects of low temperatures on fungi, a number of studies have been carried out from the physiological and phytopathological standpoints. It is noteworthy that ERIKSSON (*31*) proposed the "Mycoplasma hypothesis" to explain the epidemical ravages of the yellow rust of cereals in Sweden, where no intermediate host plants for the causal fungus have been found, and where, according to his opinion, the winter climate is so severe that the uredospores of the fungus can hardly be supposed to survive. ISTVANFFY (*54*) examined the effects of low temperatures varying from −25°C. to 8°C. upon *Botrytis cinerea*, *Monilia fructigena* and *Coniothyrium Diplodiella*. EWERT (*32*) studied the wintering of summer-conidia of *Mycosphaerella sentina*, *Pseudopeziza Ribis*, *Fusicladium dendriticum* and *Fusicladium pirinum*, and their resistance to low temperature. He exposed them for 2, 4 and 6 hours to low temperatures varying from about −16° to −4°C. and proved that the examined conidia showed a fairly strong resistance against these low temperatures. In the author's previous paper (*108*), he has reported

that a temperature as low as −21°C did not harm the vitality of *Fusarium Lini*, at least within 24 hours.

In the present studies, the same agar medium as in the preceding experiments on temperature was used. The cultures, having shown vigorous growth in a thermostat at 26°C. for 10 days, were kept 24 hours in thermos-bottles containing a freezing mixture. For this purpose, a mixture of sodium chloride and crushed ice was used. In this way, temperatures as low as −21°C. were reached. The cultures were first cooled at 0°C. and then at −12°C. for some minutes, and at last kept in the thermos-bottles at −21°C. After the treatment, the test tubes were left at room temperature until the frozen culture media melted and became soft. Then a part of the cultures was removed on an agar medium and incubated at 26°C. in a thermostat in order to test whether the fungi were alive or dead.

In the present case, the fungi were treated for 24 hours at temperatures varying from −21° to −18°C. in one thermos-bottle, and −21° to −20°C. in the other.

The results were as follows: the vitality of neither fungus was affected by these low temperatures, and the test cultures showed good development of the mycelia, though the rate of their growth was somewhat inferior to that of the control cultures.

From these results it may be understood that the fungi under consideration possess a fairly strong resistance against low temperatures. Under their natural living conditions in Hokkaido, such low temperatures as to effect their sterilization can never be expected. The frost death point (*74*, *78*) of these fungi should be at a far lower temperature.

d. Conclusions from the investigations on the temperature relation.

1. The adaptive range of temperature is wider in *Fusarium Lini* than in *Colletotrichum Lini*.

2. The minimum, optimum and maximum temperatures for the mycelial growth of *Fusarium Lini* were ca. 10°C., ca. 30°C., and ca. 37°C. respectively.

3. The minimum, optimum and maximum temperatures for the myclial growth of *Colletotrichum Lini* were ca. 10°C., ca. 25°C., and ca. 35°C. respectively.

4. Against high temperatures, *Fusarium Lini* was far more resistant than *Colletotrichum Lini*.

5. *Fusarium Lini* was killed at 50°C. within 6 hours, at 55°C. within 5 hours, at 60°C. within 3 hours, but at 45°C. no fatal effect resulted even after 8 hours' exposure.

a. *Colletotrichum Lini* was killed at 45°C. within 5 hours, at 50°C. within half an hour, and at 55° and 60°C. within only 10 minutes.

7. Temperatures above 55°C. in moist condition may practically serve as an effective means of sterilization for *Colletotrichum Lini,* but not so for *Fusarium Lini.*

8. Temperatures as low as from −21° to −20°C. did not kill the fungi under consideration, at least within 24 hours.

9. The low temperatures recorded in Hokkaido do not seem to provide an effective means of sterilization of the two fungi in question.

IV. Gas-production and Pathogeny of Wilting.

The chemical process involved in the metabolism of nutrient substances by the growth of fungi directly affects their growth, as well as the symptoms of the disease of the host plant in the case of parasites. The intermediate or final products of metabolism sometimes accelerate and sometimes retard the development of disease. In nature, the relation between fungi and their host plants differs in many ways. In one case, as in obligate parasites, the action of the fungus is extremely mild and rather symbiotic (*113*), while in other cases, as in the facultative parasites, it is very severe and destructive. The symptoms, too, of the diseases are very varied. In some cases the tissues of the host plants are partially killed by parasites, causing various characteristic spots, and in the other cases the whole plant bodies are brought immediately to death without the appearance of any conspicuous spots. Such diverse phenomena may be caused by the metabolism products of the parasites, besides the direct effects brought about by the invasion of mycelia.

1. Gas-production.

Carbohydrates and amino-acids are the most important nutrient substances for fungi. Accordingly, it is of great interest to know in what way they are decomposed or assimilated.

Fermentation or gas-production is one of the most remarkable phenomena in the decomposition of carbohydrates. The fermentation of carbohydrates by fungi has been studied mostly from the standpoint of technical mycology, but scarcely from that of phytopathology.

The author has carried out the present studies using nine kinds of carbohydrates in order to investigate the power of gas-production by *Fusarium Lini* and *Colletotrichum Lini*. The kinds of carbohydrates and the formula of the nutrient solution were the same as in the foregoing cultural studies of the carbohydrates; as a nitrogen source either peptone or asparagine was used.

Twenty-five cubic centimeter portions of each cultural solution containing one of the nine kinds of carbohydrates to the extent of two per cent of the standard solution, were placed in EINHORN'S fermentation-tubes. They were sterilized intermittently in KOCH'S steam sterilizer, and inoculated with a bit of the mycelium.

After three days' incubation at 26°C. in a thermostat, the inoculated mycelium grew into a mass which occupied in most cases the diameter of the tube of a fermentation-tube. The mycelial mass was pushed into the long arm of the tube with a sterilized platinum needle, and the volume of the gas produced was observed every day for about a month.

In the present experiments, gas-production took place in the case of *Fusarium Lini*, but never in the case of *Colletotrichum Lini*. The gas produced by the former is shown in the tables XXIII and XXIV.

In these experiments, the most remarkable fact was that *Colletotrichum Lini* never showed gas-production in the decomposition of the carbohydrates, while in the case of *Fusarium Lini* production of considerable volumes of gas took place in most cases. It was also interesting to note that the volume of the gas and the speed of its production were closely connected with the kind of carbohydrates and the substance used as a nitrogen source.

The author (*107*) has previously investigated the gas-production of eight kinds of carbohydrates, except glycogen, by *Fusarium Lini* in a cultural solution which contained one per cent of ammonium nitrate as a nitrogen source. Comparing the results of these previous and present experiments, it becomes clear that the volume of the gas produced by the decomposition of each of the carbohydrates changes according to the kind of the nitrogen source, as shown in table XXV.

Table XXIII. Showing the gas-production from carbohydrates by *Fusarium Lini* when asparagine was used as a nitrogen source.

Kinds of carbohydrates	Average gas volumes (in c.c.) produced within										Maximum Gas volumes
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	10 days	14 days	21 days	
Glucose	2.5	4.0	5.0	6.0	6.8	7.0	7.0	7.0	7.5	9.0	9.0
Fructose	1.5	3.0	5.0	6.5	8.0	10.0	11.0	17.5	27.4	33.5	33.5
Galactose	Trace	0.3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sucrose	1.0	2.0	3.0	4.0	5.0	6.0	6.0	8.0	10.0	13.5	14.0 (28 days)
Maltose	0.5	1.0	1.5	2.0	2.5	2.7	3.0	4.8	10.5	15.3	24.5 (35 days)
Lactose	0	0	0	0	0	0	0	0	0	0	0
Soluble starch	Trace	Trace	0.9	1.5	2.0	2.8	3.5	4.8	6.5	8.8	8.8
Inuline	Trace	Trace	0.4	0.5	0.6	1.0	1.4	1.8	3.0	3.5	4.0
Glycogen	Trace	Trace	Trace	Trace	Trace	Trace	Trace	10.0	21.3	23.3	23.3

Table XXIV. Showing the gas-production from carbohydrates by *Fusarium Lini* when peptone was used as a nitrogen source.

Kinds of carbohydrates	Average gas volumes (in c.c.) produced within									Maximum Gas volumes
	1 day	2 days	3 days	4 days	6 days	7 days	10 days	14 days	21 days	
Glucose	0.2	3.8	9.5	13.5	22.0	24.5	31.8	32.6	32.9	32.9
Fructose	Trace	2.0	7.3	10.4	17.5	20.5	28.8	32.0	34.3	34.3
Galactose	Trace	0.2	4.6	8.9	16.5	20.0	27.0	31.0	31.4	36.4 (28 days)
Sucrose	Trace	0.9	6.8	11.8	21.0	23.6	29.8	32.1	32.4	42.0 (28 days)
Maltose	0	0	5.3	8.0	10.3	14.0	18.5	23.5	28.5	28.5
Lactose	0	0	0	0	0	0	0	0	0	0
Soluble starch	Trace	0.5	2.3	2.8	5.5	7.5	16.0	19.0	20.0	22.2
Inuline	0	0	Trace	Trace	2.0	3.6	4.6	5.0	5.0	7.0
Glycogen	Trace	0.3	1.0	2.0	2.3	2.6	10.3	15.3	17.2	18.2

The gas volumes above 10 c.c. were measured by replacing the gas with the nutrient solution which was displaced by the gas produced and occupied the bulb of the short arm of the fermentation tube. Correctness of this measurement was proved by comparison with some parallel experiments with a fermentation tube of 30 c.c. capacity.

Table XXV. Showing the average gas volumes (in c.c.) produced by *Fusarium Lini* at the end of 3 weeks in cultural solutions containing different nitrogen sources.

Kinds of carbohydrates	Kinds of nitrogen source		
	Ammonium nitrate	Asparagine	Peptone
Glucose	12.0	9.0	32.9
Fructose	7.2	33.5	34.3
Galactose	6.2	0.5	31.4
Sucrose	6.2	13.5	32.4
Maltose	5.4	15.3	28.5
Lactose	0	0	0
Soluble starch	12.0	8.8	20.0
Inuline	11.0	3.5	5.0
Glycogen	1	23.3	17.2

As shown in the above table, the gas-production due to the decomposition of the carbohydrates is variously affected by the compounds used as a nitrogen source. When ammonium nitrate is used as a nitrogen source (*107*), the most vigorous gas-production happened in solutions containing glucose and soluble starch. Inuline comes next, while the other sugars, except lactose, produced gas moderately. In this case, glucose produced gas extra rapidly, and its amount reached the maximum volume within a week.

When asparagine was used as a nitrogen source, the liveliest gas-production was attained in the solution containing fructose. Glycogen was next best, maltose, sucrose, glucose and soluble starch following it in succession. In this case, inuline produced gas poorly and galactose showed only a trace of it, the amount being only 0.5 c.c. Lactose showed no sign of gas-production at all.

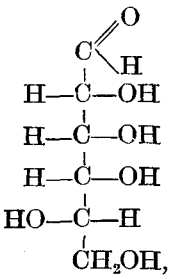
When peptone was taken as a nitrogen source, the monosaccharides and disaccharides generally produced gas well, but lactose showed no sign of gas-production. The polysaccharides were inferior to them in gas-production, especially inuline was the worst of all, except lactose.

It is very interesting that the molecular structures of the carbohydrates profoundly affected the gas-production. But the most conspicuous fact is that there is no close relation between the gas-production and the nutritive value of sugars, especially that the solution containing lactose never showed any sign of the gas-production. Both glucose and galactose produced more or less gas in every case. Lactose must

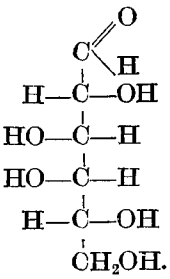
be decomposed into one molecule of glucose and one molecule of galactose with the addition of one molecule of water in hydrolysis. To prove this chemical process, the author, using BERTRAND'S method, measured the difference in the reducing power of cultural solutions which contained lactose, both before the inoculation with the fungus and at a mid-course of cultivation. In this test, the volume of potassium permanganate solution used for titration of the initial cultural solution was 11 c.c., and that for a cultural solution five days old was 13 c.c. Such an increase of the reducing power must be due to the glucose and galactose which are produced by the hydrolysis of lactose. But the gas-production never took place in a cultural solution containing a certain amount of glucose and galactose produced by the hydrolysis of lactose due to the growth of the fungus. Repetition of the experiments on the solution containing lactose never showed any sign of gas-production at all.

The other interesting phenomenon is that in the case of asparagine given as a nitrogen source, the solution containing galactose showed only a trace of gas-production, in spite of the fact that the gas-production was very vigorous in the solution containing fructose, and moderate in the solution containing glucose. Fructose being ketohexose, its molecular construction plainly differs from glucose or galactose which are aldohexose, the molecular construction for the former being $CH_2OH(CHOH)_3$-CO-CH_2OH, and for the latter $CH_2OH(CHOH)_4$-CHO. But galactose differs from glucose only in its stereochemical structure, the hydrogen atoms and hydroxy groups which combine with the asymmetric carbons being opposite in three positions as for

glucose



and for galactose



Notwithstanding this slight difference of molecular structure, there was a marked disparity in their gas-production.

It is also interesting that the gas-production was very weak in cultural solutions containing inuline irrespective of whether asparagine or peptone was used as a nitrogen source, while it was fairly good when

ammonium nitrate was given as a nitrogen source. It was good also in the case of other polysaccharides.

Thus the volume of gas produced by the decomposition of the carbohydrates by *Fusarium Lini* can not be determined in general according to the kinds of the carbohydrates used, for the gas-production seems to be affected by the combined conditions of the carbohydrates and the nitrogen sources.

The speed of the gas-production was graded according to the length of time it took to reach a point which was nearly its maximum, thence forward the increase of the gas volume being small. From this point of view it may be generally stated that the monosaccharides produced gas most rapidly, the disaccharides, except lactose, came next, and the polysaccharides were the slowest of all.

In the cultural solutions which contained asparagine or peptone the pH values of the solutions were increased by the growth of *Fusaruim Lini*. For example, in the cultural solutions containing glucose and maltose, the initial pH value of the former was 4.5, and that of the latter 4.4, which increased to 8.8 and 8.6 respectively after one week's culture of *Fusarium Lini*. The same facts also have been observed in the foregoing cultural studies when amino-acids were present as nitrogen sources. Such a fact, however, has never been noticed in either the foregoing or present experiments with *Colletotrichum Lini*.

Such facts suggest a special complication of the combined chemical process in the decomposition or assimilation of some nutrient substances by fungi. The different physiological properties of *Fusarium Lini* and *Colletotrichum Lini* in the decomposition of carbohydrates and amino-acids seem to have some close relation to the pathogeny of the wilt-disease and anthracnose of flax. This problem will be considered in detail in the next paragraphs.

2. Discussions on the pathogeny of the wilting.

On the pathogeny of the flax-wilt and other *Fusarium*-wilts, a number of views have been advanced. BOLLEY (*10*) said that the wilting of flax results from the plugging of the vessels by the mycelium. TISDALE (*103*), however, did not accept his opinion and concluded that the wilting symptom of flax, due to the invasion of *Fusarium Lini*, is produced by: (1) a partial destruction of the root system which limits the food and water supply of the plant; (2) the use of a part

of the food and water supply of the plant by the fungus; (3) an increase in transpiration and an increase in the growth of the fungus due to a rise in temperature; (4) the possible production of toxic substances by the fungus, which interfere with the normal function of the host protoplasm. GILMAN (*37*) came to the conclusion, that the yellowing of cabbage is due to the slow drain caused by *Fusarium conglutinans* on the water supply combined with the high temperature, which causes an increase both of the growth of the fungus and the transpiration of the plant. HASKELL (*40*), in his studies on the *Fusarium*-wilt of potatoes, and BRANDES (*15*), in his observation on the *Fusarium*-wilt of bananas, have concluded that the death of the plants is caused by toxic substances produced by the fungi. Recently CLAYTON (*21*), who studied the relation of temperature to the *Fusarium*-wilt of the tomato, has accepted the toxin hypothesis.

These opinions, however, do not appear sufficiently appropriate as the pathological explanation of the *Fusarium*-wilt, at least in the case of the flax-wilt. Having examined a great number of wilted flax stems, the author has never found a vessel which was plugged by the mycelium as BOLLEY (*10*) said. TISDALE (*103*) obtained almost similar results to the author's by the observation of the tissues of wilted flax. TISDALE'S (*103*) conclusions about the wilting of flax may be partially true, but they can not serve to account for the rapid wilting of the flax seedlings. For if the wilt be caused by the partial destruction of the root system and the seizure of a part of the food and water supply by the fungus, as stated in his first and second conclusions the symptoms of the wilting must progress gradually. He found that the infection takes place mostly at the root hairs, and this is true according to the author's observations also. The infection may take place usually on some root hairs, leaving the greater part of them unaffected in normal cases, therefore the destruction of the root system, and the seizure of a part of the food and water supply of the host plant by the fungus must advance gradually. For that reason the wilting must take place in gradual progression.

The wilting of flax seedlings caused by the attack of *Fusarium Lini*, however, is in most cases very rapid and lethal, and never gradual and recoverable even for a time. TISDALE'S third conclusion which coincides with GILMAN'S (*37*) explanation of the yellowing of cabbage is a special case in high temperature. The average temperature of

soil and air at the seedling period of flax is far lower than the optimum temperature for the growth of *Fusarium Lini* in Hokkaido, and yet the wilt-disease ravages the flax seedlings most severely. The last conclusion of TISDALE, namely the poisoning of flax by toxic substances produced by the fungus, has been indorsed by several investigators of *Fusarium*-wilts, but the chemical properties of the toxic substances, the mode of their production and the process of the poisoning by them have never been touched.

As to other fungi and bacteria, many studies on their toxic products have been published. DE BARY (*29*), who studied *Sclerotinia Libertiana* concluded that the breaking down of the cell walls was due to enzymes secreted by the fungus. Besides this, he supposed that soluble oxalates may play a role in decomposing the tissue of the host plants. NORDHAUSEN (*82*) agreed with DE BARY, and he assumed that the action of the fungus extract on plant tissues was due to both enzymes and toxins. BEHRENS (7) found that the toxic action of the extracts of *Mucor stolonifer* and *Penicillium Luteum* were never lost by boiling, and from these results he assumed that the toxic substances were neither volatile nor enzymatic. R. E. SMITH (*100*) said that the toxic substance produced by *Botrytis cinerea* is oxalic acid. POTTER (*90*), who studied the white rot of turnip caused by *Pseudomonas destructans*, said that this bacterium produced oxalic acid which acted as a toxin in plasmolyzing and killing the protoplasm, and it might also take a part in dissolving the middle lamella. But E. F. SMITH (*99*) said that free oxalic acid has never been found in the stale product of the cultures of bacteria, and that oxalates, however, were present causing the toxic effects upon the plant cells or tissues.

On the contrary, WARD (*112*) and KISSLING (*60*) expressed a doubt on the toxic action of *Botrytis* due to oxalic acid or oxalates, for the action of the fungus extract disappeared by boiling. They thought that the killing of the cells of the host plant may be caused by the action of the cellulose-dissolving enzymes. WILLIAM BROWN (*17*, *18*) expressed an opinion on this problem, saying that the lethal action of the fungus extract is accompanied by macerating action due to enzymes, and as the lethal action of the fungus extract disappeared by boiling, if the fungus produced some toxic substance, it must be of colloidal nature and never oxalic acid or oxalates.

Under these circumstances, it seems rather questionable to consider

that the fungus secretes a toxic substance. But in such a special and violent case as the wilting of flax seedlings, it is natural for one to consider that the wilting is due to poisoning by some kind of toxin. If it be the case, it is necessary to give a chemical or physiological interpretation of the toxic substance and its action.

From the author's experiments on the decomposition of carbohydrates and the change of reaction of the medium by the growth of the fungus under consideration, he has come, however, to the following conclusions about the pathogeny of rapid wilting of flax seedlings. Their rapid wilting is principally caused by gas-emboli of the xylem tubes due to the gas which is abundantly and rapidly produced by the decomposition of carbohydrates by the fungus, interrupting the ascent of sap, and by the poisoning of the cells by the alkalinity of the sap brought about by the growth of the fungus, the hydrogen-ion concentration of the sap of healthy flax ranging from pH 5.5 to pH 5.7, according to Prof. KOJI MIYAKE[1] and Assistant Prof. M. ADACHI of the Chemical Institute of this University.

It is evident that the monosaccharides, as glucose, and some kinds of nitrogenous compounds, as amino-acids and proteins are present in the body of the flax seedling, and that a comparatively abundant gas-production and increase of the hydroxy-ion concentration is caused by the mycelial development of the fungus, as has been observed in the preceding experiments with the nutrient solution containing peptone.

When the production of gas due to the decomposition of carbohydrates by *Fusarium Lini* occurs in the tissues of the vascular bundles of the flax seedling, a comparatively small quantity of the gas produced would occupy a part of the fine tubes as bubbles, causing gas-emboli of the tubes and interrupting at once the water ascent of the host plant at that point. Though actually the plugging of the xylem tubes by the abundant mycelial growth of the parasite in them is a rare case, the gas-production due to the decomposition of carbohydrates may be caused by a comparatively trifling development of the mycelium in the lumen, and act so strongly as to cause the gas-emboli of the vessel, thereby bringing about the rapid wilting of the

1) The reports on these studies will be published by them in the near future. Thanks are due to Dr. K. Miyake and Assistant Prof. M. Adachi for their kind favours.

seedling. Considering the striking power of *Fusarium Lini* in gas-production by the decomposition of carbohydrates, this argumentation in favour of the acceptance of the gas-emboli of the xylem tubes as the cause of the rapid wilting of the flax seedling seems to be well founded.

Nutrient solutions having higher hydrogen-ion concentration than pH 6.5, increased their pH values and inclined to fairly strong alkalinity as the fungus grew. This has been observed in the previous experiments on the relation of hydrogen-ion concentration of the medium to the growth of the fungus.

Since the hydrogen-ion concentration of the sap of flax in a normal healthy condition is always from pH 5.5 to 5.7, it may be certain that such an increase of the hydroxy-ion concentration as a consequence of the mycelial growth of the parasite unavoidably interferes with the normal function of the cells or brings them to death.

As the infection by *Fusarium Lini* occurs mostly on the root system of flax, the gas-production and the alkalinity of the sap take place in the lower part of the plant, where the gas-emboli of the xylem tubes or the death of the cells will affect the whole plant body, killing it rapidly. One side wilting, often observed in a fairly grown plant, may be explained by the gas-emboli of the vessels and the poisoning of the cells through a partial invasion of the mycelium into the tissues of the central cylinder of that side.

The partial destruction of the root system and the seizure of a part of the water and food supply of the host plant by the fungus, on which TISDALE laid stress as one of the causes of the flax-wilt, may indeed accelerate the wilting. Partial destruction of tissues and seizure of a part of the water and food supply of the host plant by fungi being the usual phenomena occurring in the cases of parasitic diseases in plants, it does not seem acceptable to the writer to consider them particularly as the causes of the rapid wilting of the flax seedlings by the attack of *Fusarium Lini*.

In numerous cases of *Fusarium*-wilt of various plants, TISDALE (*103*), HASKELL (*40*), BRANDES (*15*) and CLAYTON (*21*) supposed that the fungi produce some toxins which cause a fatal effect upon the plant cells. But in the case of the flax wilt-disease, the toxic action seems to be caused by an alkalinity of the sap brought about by the mycelial growth of the fungus and never by a special substance which could

be regarded as a toxin. In other words, the poisoning of the cells of flax in the case of the wilt-disease is not caused by a toxic substance secreted by the parasite, but is caused by the decomposition product of the nutrient substances formed as a result of the growth of the fungus in question.

Colletotrichum Lini has, according to the author's studies, neither produced any gas by the decomposition of carbohydrates, nor increased the pH value of the nutrient solutions so much as to make them alkaline. To the contrary it made them rather acidic. In nature, too, the sap of the affected flax plant may increase its hydrogen-ion concentration.

The wilting of the flax seedlings in the case of anthracnose is caused by the general decay of tissues in stems and leaves, showing a sunken yellowish spot on the former and one with a water soaked appearance on the latter. The decay of tissues may be caused by the direct enzymatic action of the mycelium and by the acids produced due to the decomposition of substances by the growth of the fungus. Especially the partial destruction of the tissues of hypocotyl is fatal to the flax seedling, as it causes the portion above the lesion to be drained to death by the interruption of the water supply. Such symptoms as in the anthracnose of flax being common and normal in plant diseases, a rapid death of the whole plant body without showing any diseased spots as in the case of the *Fusarium*-wilt in flax seedlings is certainly a striking fact. It is very interesting from the phytopathological point of view that the symptoms of the wilt-disease and anthracnose of flax seedlings correspond very well with the physiological character of *Fusarium Lini* and *Colletotrichum Lini* described already in the foregoing pages.

3. Conclusions regarding the gas-production and the pathogeny of the wilting.

1. *Fusarium Lini* showed a lively gas-production due to the decomposition of the carbohydrates, except lactose, while *Colletotrichum Lini* never produced any gas.

2. The volume of the gas produced and the speed of its production were closely related to the kinds of the carbohydrates and the substance used as nitrogen source.

3. When ammonium nitrate was used as a nitrogen source, glucose

and the polysaccharides produced gas in the most lively manner, fructose, galactose, sucrose, and maltose following them in succession.

4. When asparagine was used as a nitrogen source, fructose produced gas most vigorously, glycogen, maltose, sucrose, glucose and inuline following it in succession, while galactose, however, showed only a trace of gas-production.

5. When peptone was used as a nitrogen source, the monosaccharides and disaccharides, except lactose, produced gas vigorously, while the polysaccharides were inferior to them. Inuline especially was relatively poor in its gas-production.

6. Lactose produced no gas in any case, and galactose showed only a trace of it in the nutrient solution containing asparagine as a nitrogen source.

7. To generalize, the speed of the gas-production of the carbohydrates through their decomposition by *Fusarium Lini* was rapid in the case of the monosaccharides, intermediate in the disaccharides, and slow in the polysaccharides.

8. *Fusarium Lini* increased the pH value of the solution by the decomposition of amino-acids, but such a fact has never been observed in the case of *Colletotrichum Lini.*

9. The opinions formerly given concerning the pathogeny of flax-wilt seem to be inadequate to account for the rapid wilting of the seedlings.

10. The rapid wilting without the appearance of any diseased spots in the case of the wilt-disease in flax seedlings seems to be caused by the gas-emboli of the xylem tubes from the decomposition of carbohydrates, and by the poisoning of the cells due to the alkalinity of the cell sap caused by the growth of *Fusarium Lini.*

11. As the infection of *Fusarium Lini* occurs mostly on the root system of flax, the gas-emboli of the xylem tubes and poisoning of the cells would happen in the lower parts of the plant in most cases, causing the wilting of the whole plant body without showing any diseased spots.

12. One side wilting may be explained by the gas-emboli of the xylem tubes and the poisoning of the cells situated on that side, due to a partial invasion by the mycelium.

13. A partial destruction of the root system and the seizure of a part of the water and food supply of the host plant by the fungus

may act as the causes of a progressive wilting, but they seem to be inadequate to consider as the effective causes of the rapid wilting as in the case of the *Fusarium*-wilt of flax seedlings. An increase in transpiration of the host plant and an increase in the growth of the fungus due to a rise in temperature may be a cause of the wilting in special cases in high temperature, but it seems never to be acceptable at least in Hokkaido, where the average temperature of soil and air at the seedling period of flax is far lower than the optimum one for the growth of *Fusarium Lini*.

14. The author is unable to share those views which attribute the injurious action of the parasite to some toxic substances secreted by them. The author is of the opinion that the toxic effects upon the cells of the host plants are due to the decomposition products of the cell components by the enzymatic action of the parasites; in the present case, the death of the cells of flax is caused by the alkalinity of the cell sap due to the decomposition of the amino-acids through the action of the enzymes secreted by *Fusarium Lini*, besides the maceration of cells by the action of carbohydrases.

15. *Colletotrichum Lini* neither produces gas in the process of decomposing the carbohydrates nor increases the pH value of cell sap. The morbid changes of the flax seedlings caused by the attack of this fungus are not so rapid as in the case of the wilt-disease caused by *Fusarium Lini*.

16. The wilting of the flax seedling in the case of anthracnose can be principally accounted for by the partial destruction of the tissues of the hypocotyl causing the interruption of the ascent of sap, by which the seedling is gradually drained to death. The death of the tissues of the leaf and the seizure of a part of the water and food supply of the host plant by the parasite accelerate the progress of the disease.

17. The symptoms of the wilt-disease and anthracnose in flax seedlings correspond with the physiological characters of *Fusarium Lini* and *Colletotrichum Lini* as observed in the decomposing process of the carbohydrates and the change of the reaction of the sap as a growth-medium of the fungi.

V. Summary.

1. Wilt-disease and anthracnose attack the flax most virulently at its seedling stage in Hokkaido. In the present paper, the author has

dwelt upon some physiological characters of the causal fungi and also upon some phytopathological explanations concerning them.

2. The wilt-disease of flax is caused by a well known fungus, *Fusarium Lini* BOLLEY. To the causal fungus of flax-anthracnose different names have been given by several authors, but the author should like to call the fungus *Colletotrichum Lini* (WESTERDIJK).

3. The carbohydrates were generally well suited as carbon sources for the nutrition of the fungi, but there were marked differences in their nutritive value. Maltose was the most nutritious for both fungi. For *Fusarium Lini*, fructose and sucrose proved comparatively suitable, galactose, lactose and inuline were intermediate, soluble starch, glycogen and glucose following them in succession. For *Colletotrichum Lini*, lactose, soluble starch and glycogen were relatively suitable, galactose and glucose following them in succession, fructose and sucrose were less suitable, inuline was unsuitable.

4. As carbon sources the higher alcohols seemed to be nutritious for the fungi. Especially mannite was very nutritious for *Fusarium Lini*.

5. Comparing the nitrogenous nutrition of the fungi with ammonium, nitrate and nitrite, the nitrogen in ammonium form was more easily assimilable than that in nitrate form, while nitrite was entirely unsuitable for both fungi.

6. Amino-acids were generally suited to the fungi as a nitrogen source. The mixed use of several kinds of amino-acids was generally preferable for the nutrition of the fungi.

7. The proteins were generally suitable for the fungi as a nitrogen source, but they were not so easily assimilable as peptone, probably due to the stable construction of the molecules.

8. On the whole, the organic nitrogen compounds were more suitable than the inorganic nitrogen salts, and the best nutrition may be obtained by the use of peptones, polypeptides or a proper combination of several amino-acids.

9. The hydrogen-ion concentration of the cultural solution plays an important rôle in the growth of the fungi. *Fusarium Lini* was capable of growing in a wider range of it than *Colletotrichum Lini*, that is to say, the former was less sensitive to the high and low hydrogen-ion concentrations than the latter. The optimum hydrogen-ion concentration for the former was about pH 5 and for the latter about pH 6.

10. Organic acids greatly affected the growth of the fungi. The toxic actions of the organic acids did not correspond always to the dissociation of the hydrogen-ions.

11. Tannic acid retarded the growth of the fungi even when minute quantities only were present in the cultural solution. *Colletotrichum Lini* being very sensitive to the acids, its growth was greatly retarded in a nutrient solution containing only 0.05% of tannic acid and it was entirely checked at a 0.1% concentration of the acid, while *Fusarium Lini* offered strong resistance, being able to live in quite high concentrations of the acid. The maximum concentration for its growth presumably lies higher than 1.3%.

12. Citric acid influenced the growth of the fungi rather mildly. Especially in the case of *Fusarium Lini* the retarding action of the acid on its growth advanced slowly with the increase of the concentration. However, for *Colletotrichum Lini* it rapidly became fatal, although relatively low concentrations were used. In the cultural solution containing 0.5% of the acid, the mycelial development of this fungus was utterly checked.

13. The temperature relations for the mycelial growth of the fungi were as follows: the minimum temperature for both fungi was nearly 10°C.; the optimum temperature for *Fusarium Lini* was 28.5° to 29.5°C., or nearly 30°C., and for *Colletotrichum Lini*, it was about 25°C.; the maximum temperature for *Fusarium Lini* was about 37°C., and for *Colletotrichum Lini*, it was about 35°C.

14. The resistance of both fungi to high temperatures showed specific difference. *Fusarium Lini* was far more resistant than *Colletotrichum Lini*, for instance, exposing the former to a temperature of 50°C. did not kill it within 4 hours, while the latter was killed within 30 minutes. Again by exposure at 60°C., the former was fatally affected after 3 hours, while the latter was easily killed within 10 minutes.

15. To low temperatures both fungi were fairly strongly resistant. A series of low temperatures varying from −21° to −20°C. for at least 24 hours worked no harm upon the vitality of the fungi.

16. The carbohydrates, with the exception of lactose, produced comparatively large volumes of gas in a short time in their decomposition by *Fusarium Lini*. The vigour of the production of the gas varied according to the kind of carbohydrates and largely depended

upon the kind of nitrogen source. In the case of *Colletotrichum Lini*, however, such a gas-production has never been observed.

17. *Fusarium Lini* increased the pH value of the cultural solution when its initial hydrogen-ion concentration was higher than about pH 6.5, and it was very remarkable in the solution containing organic nitrogen sources as the stale cultural solution remained fairly strongly alkaline. *Colletotrichum Lini*, however, did not increase the pH value of the cultural solution when its initial hydrogen-ion concentration was lower than about pH 4.5, and never alkalized the stale cultural solution. The hydrogen-ion concentration of the cell sap of the healthy, normal flax seedling being from pH 5.5 to 5.7 and containing organic nitrogen compounds, it seems to be sure that *Fusarium Lini* makes the sap of the affected flax seedling more or less alkaline, while *Colletotrichum Lini* never alkalizes the sap, but rather makes it acidic.

18. The production of the gas and the alkalinity of the cell sap being highly probable in the bodies of flax seedlings, when they are attacked by *Fusarium Lini*, the rapid wilting without showing any spots may be explained as being a case of gas-emboli of the xylem tubes due to the gas produced, and the poisoning of the cells due to the alkalinity of the cell sap. On the other hand, in the case of the anthracnose of the flax seedlings, the gradual advance of the characteristic morbid changes and appearance of spots may be explained, as in the ordinary cases of parasitic plant diseases, by the decay of the tissues due to the direct enzymatic action of the mycelium, by the seizure of part of the water and food supply by the causal fungus, *Colletotrichum Lini*, and, moreover, perhaps by the death of the affected cells due to the increase of the hydrogen-ion concentration of the sap resulting from the growth of the fungus.

The physiological behavior of both fungi in their decomposing of the carbohydrates and the change of reaction of the sap as a growth-medium of the fungi, exactly corresponded with the symptoms of the wilt-disease and the anthracnose of the flax seedling.

VI. Literature Cited.

1. ABDERHALDEN, E.:—Lehrbuch der physiologischen Chemie. IV Auflage, I. Teil. 1920.
2. AMES, A.:—The temperature relations of some fungi causing storage rot. Phytopathology, Vol. 5, pp. 11–19. 1915.
3. APPEL, O. and WOLLENWEBER, H. W.:—Grundlagen einer Morphologie der Gattung *Fusarium* (LINK.). Arbeit. kais. biol. Anst. Land- u. Forstw. Bd. 8, pp. 1–207. 1913.
4. ATANASOFF, D. and JOHNSON, A. G.:—Treatment of cereal seeds by dry heat. Jour. Agr. Res. Vol. 18, pp. 379–390. 1920.
5. BARTETZKO, H.:—Untersuchungen über das Erfrieren von Schimmelpilzen. Jahrb. f. wiss. Bot. Bd. 47, pp. 57–98. 1909.
6. BASSALIK, K.:—Ueber die Verarbeitung der Oxalsäure durch *Bacillus extroquens* n. sp. Jahrb. f wiss. Bot. Bd. 53, pp. 255–302. 1914.
7. BEHRENS, J.:—Beiträge zur Kenntnis der Obstfäule. Centralb. f. Bact. Abt. II. Bd. 4, pp. 514–522, 547–553, 577–585, 635–644, 700–706, 739–746, 770–777. 1898.
8. BLACKMAN, V. H. and WELSFORD, E. J.:—Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. Ann. Bot. Vol. 30, pp. 389–398. 1916.
9. BOLLEY, H. L.:—A preliminary note on the cause of flax-sick soil. *Fusarium Lini* sp. nov. Proceedings Twenty-Second Annual Meeting of Society for Promotion of Agricultural Science, pp. 1–4. 1901.
10. BOLLEY, H. L.:—Flax wilt and flax sick soil. North Dakota Agricultural College, Government Agricultural Experiment Station for North Dakota, Bulletin No. 50, pp. 27–57. 1901.
11. BOLLEY, H. L.:—Preliminary efforts to develop a continuous process of seed disinfection by means of formaldehyde vapour. Proceedings Twenty-Third Annual Meeting of Society for Promotion of Agricultural Science. 1902.
12. BOLLEY, H. L.:—Flax and flax seed selection. N. D. Agr. Coll. Governm. Agr. Exp. Stat. for North Dakota, Bull. No. 55, pp. 174–198. 1903.
13. BOLLEY, H. L.:—Seed disinfection and crop production methods and types of machinery needed. N.D. Agr. Coll. Governm. Agr. Exp. Stat. for North Dakota, Bull. No. 87, pp. 131–166. 1910.
14. BOLLEY, H. L.:—Flax canker. North Dakota Agr. Exp. Stat. Press Bull. No. 52, 1912.
15. BRANDES, E. W.:—Banana wilt. Phytopathology, Vol. 9, pp. 339–389. 1919.
16. BROOKS, C. and COOLEY, J. S.:—Temperature relation of apple-rot fungi. Jour. Agr. Res. Vol. 8, pp. 139–163. 1917.
17. BROWN, W.:—Studies in the physiology of parasitism. I. The action of *Botrytis cinerea*. Ann. Bot. Vol. 29, pp. 313–348. 1915.
18. BROWN, W.:—Studies in the physiology of parasitism. III. On the relation between "infection drop" and the underlying host tissue. Ann. Bot. Vol. 30, pp. 399–406. 1916.
19. BUTLER, E. J.:—Immunity and disease in plants. The Agr. Journ. India. Special India Science Congress Number. pp. 10–28. 1918.
20. CLARK, W. M.:—The determination of hydrogen ions. Baltimore. 1922.
21. CLAYTON, E. E.:—The relation of temperature to the *Fusarium*-wilt of the tomato. American Jour. Bot. Vol. 10, pp. 71–88. 1923.

22. CLINTON, G. P.:—Chestnut bark disease. Connecticut Agr. Exp. Stat. Rept. No. 36, p. 539. 1913.
23. COHEN, J. B.:—Practical organic chemistry. London. 1908.
24. COOK, M. T.:—The relation of parasitic fungi to the contents of the cells of the host plants. I. The toxity of tannin. Delaware Agr. Exp. Stat. Bull. No. 91, p. 21. 1911.
25. COOK, M. T. and WILSON, G. W.:—The influence of the host plant on *Endothia parasitica* and related species. Bot. Gaz. Vol. 60, pp. 346-361. 1915.
26. CURRIE, J. N.:—The citric acid fermentation of *Aspergillus niger*. Jour. Biol. Chem. Vol. 31, pp. 15-37. 1917.
27. CZAPEK, F.:—Biochemie der Pflanzen. Bd. II. Berlin. 1905.
28. CZAPEK, F.:—Ueber also eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen. Jena. 1911.
29. DE BARY, A.:—Ueber einige Sclerotinien und Sclerotinienkrankheiten. Bot. Zeitg. Bd. 44, pp. 377-374. 1886.
30. EDGERTON, C. W.:—Effects of temperature on *Glomerella*. Phytopathology, Vol. 5, pp. 247-259. 1915.
31. ERIKSSON, J.:—Ueber das vegetative Leben der Getreiderostpilze. IV. *Puccinia graminis* PERS. in der heranwachsenden Getreidepflanzen. Svenska Vetensk.-Ak. Handlingar 39. Nr. 5. 1905. Review in Pflanzen Krankheiten. Bd. 16, pp. 306-308. 1906.
32. EWERT R.:—Die Ueberwinterung von Sommerkonidien pathogener Ascomyceten und die Wiederstandfähigkeit derselben gegen Kälte. Zeitschr. f. Pflanzenkrankh. Bd. 20, pp. 129-141. 1910.
33. FISCHER, EMIL:—Untersuchungen über Aminosäuren, Polypeptide und Proteine. Berlin. 1906.
34. FRANKLAND, P.:—Pasteur memorial lecture. Jour. of the Chem. Soc. London, Vol. 71, pp. 683-743. 1897.
35. GILLESPIE, L. J.:—The growth of the potato scab organism at various hydrogen-ion concentrations as related to the comparative freedom of acid soils from the potato scab. Phytopathology, Vol. 8, pp. 257-269. 1918.
36. GILMAN, J. C.:—The relation of temperature to the infection of cabbage by *Fusarium conglutinans* WOLLENWEB. Phytopathology, Vol. 4, p. 404. 1914.
37. GILMAN, J. C.:—Cabbage yellows and the relation of temperature to its occurence. Ann. Missouri Bot. Gard. Vol. 2, pp. 25-84. 1916.
38. GRAFE, V.:—Einführung in die Biochemie für Naturhistoriker und Mediziner. Wien. 1913.
39. GUSTON, F. C.:—Comparative studies on respiration XI. The effect of hydrogen-ion concentration on the respiration of *Penicillium chrysogenum*. Journ. of General Physiology, Vol. 2, pp. 617-626. 1920.
40. HASKELL, R. J.:—*Fusarium*-wilt of potato in the Hudson river valley, New York. Phytopathology, Vol. 9, pp. 223-260. 1919.
41. HEMMI, T.:—On a new canker disease of *Prunus yedoensis*, *P. Mume* and other species caused by *Valsa japonica* MIYABE et HEMMI, sp. n. Journ. of the College of Agriculture, Tohoku Imperial University, Sapporo, Japan, Vol. 7, pp. 257-315. 1916.

42. HEMMI, T. :—On the Gloeosporiose of *Caladium*. Transactions of the Sapporo Natural History Society, Vol. 7, pp. 41-70. 1918. (In Japanese).

43. HEMMI, T. :—Effect of temperature on some fungi causing anthracnose of plants. Journal of the Society of Agriculture and Forestry, Sapporo, Japan. Year 10, No. 46, pp. 239-282, No. 47, pp. 389-417. 1918. Year 11, No. 50, pp. 289-337. 1919. (In Japanese).

44. HEMMI, T. :—Effect of temperature on fungi causing bitter-rot of apples. Jour. Soc. Agr. and Forest. Sapporo, Japan. Year 11, No. 52, pp. 12-31. 1920. (In Japanese).

45. HEMMI, T. :—Kurze Mitteilung über drei Fälle von Anthraknose auf Pflanzen. Ann. Phytopath. Soc. of Japan. Vol. 1, pp. 13-21. 1920.

46. HEMMI, T. :—Beiträge zur Kenntnis der Morphologie und Physiologie der Japanischen *Gloeosporien*. Jour. Coll. of Agr., Hokkaido Imperial University, Sapporo, Japan. Vol. 9, pp. 1-159. 1920.

47. HEMMI, T. :—Nachträge zur Kenntnis der *Gloeosporien*. Jour. Coll. Agr., Hokkaido Imp. Univ. Sapporo, Japan, Vol. 9, pp. 305-346. 1921.

48. HIRATSUKA, N. :—Report of the investigation on flax wilt-disease. Resources of Northern Japan, Vol. 48. 1896. (In Japanese).

49. HIRATSUKA, N. :—On the cause of flax wilt-disease and its prevention. Bull. of the Agricultural Society of Hokkaido, Vol. 2, No. 25. 1903. (In Japanese).

50. HIURA, M. :—Studies on flax anthracnose. Jour. Soc. Agr. and Forest. Sapporo, Japan. Year 15, No. 64, pp. 1-23. 1923. (In Japanese).

51. HOPKINS, E. E. :—Hydrogen-ion concentration in its relation to wheat scab. American Jour. Bot. Vol. 9, pp. 159-179. 1922.

52. HOPKINS, E. E. :—The effect of lactic acid on spore production by *Colletotrichum Lindemuthianum*. Phytopathology, Vol. 12, pp. 390-393. 1922.

53. HUMPHREY, H. B. :—Studies on the relation of certain species of *Fusarium* to the tomato blight of the Pacific Northwest. Washington Agr. Exp. Stat. Bull. No. 115, pp. 1-22. 1914.

54. ISTVANFFY, GY. :—Ueber die Lebensfähigkeit der *Botrytis*, *Monilia* and *Coniothyrium* Sporen. Mathematikai è termèszettudomanyi èrtesitö. Vol. 21, pp. 222-235. 1903. Review in Zeitschrift für Pflanzenkrankheiten, Bd. 14, pp. 301-302. 1904.

55. ITANO, A. :—I. The relation of hydrogen-ion concentration of media to the proteolytic activity of *Bacillus subtilis*. II. Proteolysis of *Streptococcus erysipelatis* and *Strept. lacticus* compared under different hydrogen-ion concentration. Massachusetts Agr. Exp. Stat. Bull. No. 167, pp. 139-184. 1916.

56. ITANO, A. and NEIL, J. :—Influence of temperature and hydrogen-ion concentration upon the spore cycle of *Bacillus subtilis*. Jour. Gen. Physiology, Vol. 1, pp. 421-428. 1919.

57. JONES, L. R. :—The damping off of coniferous seedlings. Vt. Agr. Exp. Stat. Rept. No. 20, pp. 342-347. 1908.

58. JONES, L. R. and TISDALE, W. B. :—The influence of soil temperature upon the development of flax wilt. Phytopathology, Vol. 12, pp. 409-413. 1922.

59. KARRER, J. L. :—Studies in the physiology of the fungi XIII. The effect of hydrogen-ion concentration upon the accumulation and activation of amylase produced by certain fungi. Ann. Missouri Bot. Gard. Vol. 8, pp. 63-96. 1921.

60. KISSLING, E.:—Zur Biologie der *Botrytis cinerea*. Hedwigia, Vol. 28, pp. 227–256. 1889.

61. KNUDSON, L.:—Tannic acid fermentation. Jour. Biol. Chem. Vol. 14, pp. 154–184. 1913.

62. KRÜGER, F.:—Beiträge zur Kenntnis einiger *Gloeosporien* I und II. Arbeit. a. d. kais. biol. Anst. f. Land- u. Forstwirtschaft, Bd. 9. pp. 233–323. 1913.

63. KRUSE, W.:—Allgemeine Mikrobiologie. Leipzig. 1900.

64. KÜSTER, E.:—Kultur der Mikroorganismen. Leipzig. 1921.

65. LAURITZEN, J. I.:—Relations of temperature and humidity to infection by certain fungi. Phytopathology, Vol. 9, pp. 7–35. 1919.

66. LOEB, G.:—Chemische Konstitution und physiologische Wirksamkeit der Säuren. Biochem Zeitschr. Bd. 15, pp. 254–271. 1909.

67. LOEW, O.:—Die chemischen Verhältnisse des Bakterienleben. Centralb. f. Bakt. u. Par. Bd. 9 pp. 659–789. 1891.

68. LÖHNIS, F.:—Handbuch der landwirtschaftlichen Bakteriologie. Berlin. 1910.

69. LUGGAR, O.:—Treatise on flax culture. Minnesota Agr. Exp. Stat. Bull. No. 13. 1890.

70. LUTZ, O.:—Ueber den Einfluss gebrauchter Nährlösungen auf Keimung und Entwicklung einiger Schimmelpilze. Ann. Mycol. Vol. 7, pp. 91–133. 1909.

71. MAC INNES, J.:—The growth of the wheat scab organism in relation to hydrogen-ion concentration. Phytopathology, Vol. 12, pp. 290–294. 1922.

72. MATSUMOTO, T.:—Studies in the physiology of the fungi XII. Physiological specialization in *Rhizoctonia Solani* KÜHN. Ann. Missouri Bot. Gard. Vol. 8, pp. 1–62. 1921.

73. MATSUMOTO, T.:—Further studies on physiology of *Rhizoctonia Solani* KÜHN. Bull. Imp. Coll. of Agr. and Forest., Morioka, Japan. No. 5. 1923.

74. MAXIMOW, N. A.:—Experimentelle und kritische untersuchungen über das Gefrieren und Erfrieren der Pflanzen. Jahrb. f. wiss. Bot. Bd. 53, pp. 327–420. 1914.

75. MAYER, A.:—Untersuchungen über die alkoholische Gärung, den Stoffbedarf und den Stoffwechsel der Hefepflanze. Heidelberg. 1879.

76. MEACHAM, M. B.:—Note upon the hydrogen-ion concentration necessary to inhibit the growth of four wood-destroying fungi. Science, Vol. 48, pp. 499–500. 1918.

77. MICHAELIS, L.:—Praktikum der physiologischen Chemie inbesondere der Kolloidchemie für Mediziner und Biologen. Berlin. 1922.

78. MOLISCH, H.:—Untersuchungen über das Erfrieren der Pflanzen. Jena. 1897.

79. NAEGELI, C.:—Ernährung der niederen Pilze durch Kohlenstoff- und Stickstoffverbindungen. Nachtr. z. Seitzung d. math.-phys. Klasse. Bd. 3, pp. 277–367. 1880.

80. NAUMOV, N. A.:—Intoxicating bread. P'iany Khlieb Truy Biuro po Miki Fitopat No. 12, pp. 1–216. 1916. Review by M. Shapovalov in Phytopathology, Vol. 7, pp. 384–386. 1917.

81. NIKITINSKY, J.:—Ueber die Beeinflussung der Entwicklung einiger Schimmelpilze durch ihre Stoffwechselprodukte. Jahrb. f. wiss. Bot. Bd. 40, pp. 1–93. 1904,

82. NORDHAUSEN, M.:—Beiträge zur Biologie parasitärer Pilze. Jahrb. f. wiss. Bot. Bd. 33, pp. 1–46. 1899.

83. O'Brien, A. A.:—Notes on the comparative resistance to high temperatures of the spore and mycelium of certain fungi. Bull. Torrey Bot. Club. Vol. 29, pp. 170–172. 1902.

84. Oshima, K. and Tadokoro, T.:—On the carbohydrate group in Yam mucin. Jour. Coll. Agr., Tohoku Imp. Univ. Sapporo, Vol. 4, pp. 242–249. 1912.

85. Palladin, W.:—Pflanzenphysiologie. Berlin. 1911.

86. Pethybridge, G. H. and Lafferty, H. A.:—A disease of flax seedlings caused by a species of *Colletotrichum*, and transmitted by infected seed. Scientific Proceedings of the Royal Dublin Society, Vol. 15, No. 30, pp. 359–384. 1918.

87. Pfeffer, W.:—Ueber Election organischer Nährstoffe. Jahrb. f. wiss. Bot. Bd. 28, pp. 205–268. 1895.

88. Pfeffer, W.:—Pflanzenphysiologie, Bd. 1. 1897.

89. Plimmer, R. H. A.:—The chemical constitution of the proteins. London. 1913.

90. Potter, M. C.:—On the parasitism of *Pseudomonas destructans*. Proceedings of the Royal Society, Vol. 70, pp. 392–397. 1902.

91. Potter, M. C.:—Bacterial disease of plants. Jour. Agr. Soc. Vol. 4, pp. 323–337. 1912.

92. Raulin, J.:—Études chimiques sur la végétation. Ann. Sc. Nat. Bot. Vol. 2, pp. 93–299. 1887.

93. Richards, H. M.:—Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. f. wiss. Bot. Bd. 30, pp. 665–688. 1897.

94. Sideris, C. P.:—Studies on the behavior of *Fusarium cromyophthoron* in carbohydrates, glucosides, proteins and various decoctions, with a discussion on the "isometabolic point" of substances. Phytopathology, Vol. 15, pp. 129–145. 1925.

95. Sawada, K.:—Materials for the fungi in Formosa, XXII. Reports of the Taiwan Natural History Society, Year 10, pp. 38–44. 1921. (In Japanese).

96. Schilling, E.:—Beobachtung über eine durch *Gloeosuporium Lini* verursachte Flackskrankheit in Deutschland. Faserforschung, Bd. 2, pp. 87–113. 1922.

97. Schneider-Orelli, O.:—Zur Kenntnis des mitteleuropäischen *Gloeosporium fructigenum*. Centralb. f. Bakt. und Infektionskr. Abt. III, Bd. 32, pp. 459–467. 1912.

98. Schövers, T. A. C.:—Voorloopige medeeling over eine nog onbekende, wellicht niet ongeveerlyke van het vlas. Tijdsch. over Plantenziekten, Vol. 21, p. 100. 1915.

99. Smith, E. F.:—Bacteria in relation to plant diseasess, Vol. II. 1911.

100. Smith, R. E.:—The parasitism of *Botrytis cinerea*. Bot. Gaz. Vol. 33, pp. 421–436. 1902.

101. Stevens, N. E.:—Temperatures of the cranberry regions of the U. S. in relation to the growth of certain fungi. Jour. Agr. Res. Vol. 11, pp. 521–529. 1917.

102. Thiel, A. F. and Weiss, F.:—The effect of citric acid on the germination of the teliospores of *Puccinia graminis tritici*. Phytopathology, Vol. 10, pp. 448–452. 1920.

103. Tisdale, W. H.:—Flax wilt: a study of the nature and inheritance of wilt resistance. Jour. Agr. Res. Vol. 11, pp. 573–607. 1917.

104. Tisdale, W. H.:—Relation of temperature to the growth and infection power of *Fusarium Lini*. Phytopathology, Vol. 7, pp. 536–560. 1917.

105. Tochinai, Y.:—On the causes of flax wilt-disease, seed disinfection and the effect of soil heating on the growth of the flax plant. Bull. Agr. Soc. Hokkaido, Vol. 22, No. 1. 1920. (In Japanese).

106. TOCHINAI, Y. :—Studies on the food relation of *Fusarium Lini.* Annals of the Phytopathological Society of Japan, Vol. 1, pp. 22-33. 1920.
107. TOCHINAI, Y. :—On the fermentation of carbohydrates by *Fusarium Lini.* Journal of Plant Protection, Vol. 8, No. 2, pp. 71-78. 1921. (In Japanese).
108. TOCHINAI, Y. :—Studies on the physiology of *Fusarium Lini.* Transactions of the Sapporo Natural History Society, Vol. 8, pp. 19-42. 1921.
109. TOCHINAI, Y. and ENOMOTO, S. :—On the dry heat sterilization of flax-seeds for the prevention of its anthracnose. Jour. Soc. Agr. and Forest., Sapporo, Japan. Year 16, No. 66, pp. 225-234. 1924. (Japanese with English resumé).
110. USCHINSKY. :—Ueber eine eiweissfreie Nährlösung für pathogene Bakterien, nebst einigen Bemerkungen über Tetanusgift. Centralb. f. Bakt. u. Par. Bd. 14, pp. 316-319. 1893.
111. WARBURG, O. :—Ueber die Bedeutung der organischen Säuren für den Lebensprozess der Pflanzen (speziell der sog. Fettpflanzen). Untersuchungen aus dem Botanischen Institut zu Tübingen, Bd. 2, pp. 53-150. 1886.
112. WARD, H. M. :—A lily disease. Ann. Bot. Vol. 2, pp. 319-382. 1889.
113. WARD, H. M. :—Cronian Lecture. On some relations between host and parasite in certain epidemic diseases of plants. Proceed. Royal Soc. London, Vol. 47, pp. 393-443. 1890.
114. WEBB, R. W. :—Studies in the physiology of fungi, X. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. Ann. Missouri Bot Gard. Vol. 6, pp. 201-222. 1919.
115. WEHMER, C. :—Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einigen Pilze. Bot. Zeitg. Bd. 49, pp. 233-638. 1891.
116. WESTERDIJK, J. :—Anthracnose van het vlas. Phytopath. Labor. Willie Commelin Scholten, Jaarverslag 1915, pp. 6-7.
117. WESTERDIJK, J. :—Neueres über Flachskrankheiten. Jahrber. Vereinig. f. angew. Bot. Vol. 16, pp. 4-7. 1918.
118. WOLF, F. A. :—A *Fusarium*-disease of the pansy. Mycologia, Vol. 2, pp. 19-22. 1910.
119. WOLLENWEBER, H. W. :—Pilzparasitäre Welkekrankheiten der Kulturpflanzen. Ber. d. deut. bot. Ges. Bd. 31, pp. 17-34. 1913.
120. YOUNG, H. C. and BENNETT, C. W. :—Growth of some parasitic fungi in synthetic culture media. American Jour. Bot. Vol. 9, pp. 459-469. 1922.