STUDIES ON THE PHYSIOLOGY OF *Fusarium lini*

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亞麻立枯病菌の生理的研究

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Introduction

There are three species of fungus as the causal fungi of the flax-wilt disease, *Asterocystis radicis* De Wild, *Fusarium lini* Bolley and *Collectotricum linicolum* Pethybridge et Lafferty. The most general and destructive wilt disease is caused by *Fusarium lini* Bolley.

The flax-wilt disease caused by *Fusarium* has become an important problem in Japan and America. In Europe, crop rotation has been practised habitually for flax cultivation for centuries, and as a consequence the flax-wilt disease did not present so virulent a form as to attract the attention of farmers and phytopathologists. But in Japan and America, where farmers, without knowing the real cause of the crop rotation adopted by European cultivators as the result of bitter experiences from ancient times on the ravages of this dreadful disease, cultivated it at first without paying any attention to the necessity of rotation of crops. Consequently, the wilt disease appeared in most severe form and even annihilated often in many places the whole crop of the flax field.

In Japan the disease was first noticed at the end of the nineteenth century. Prof. Dr. K. Miyabe discovered in 1892, that a species of *Fusarium* is concerned in the wilt disease of flax. The next year Dr. N. Hiratsuka investigated this disease in our phytopathological institute under the direc-

tion of Prof. Miyabe and affirmed that the flax-wilt disease is caused by *Fusarium*, and to control the disease a long interval of rotation of crops is absolutely necessary. Before this, in America, Prof. O. Luggar carried out a valuable investigation of this disease, but he did not succeed in finding out its causal organism. In 1901, Prof. H. L. Bolley discovered quite independently without knowing the result of the researches by Dr. Miyabe and Dr. Hiratsuka, the causal fungus of the flax-wilt disease, and named it *Fusarium lini*.

In recent years the disease of plants caused by the species of *Fusarium* have assumed great importance from an economic as well as from a scientific standpoint. A large amount of work has been done on the taxonomy and prophylaxy of these fungi and on the diseases they produce, but there have been comparatively few studies in regard to the relations of physiological characters of these fungi. About the physiology of *Fusariun lini* W. H. Tisdale has reported on a temperature study.

I have carried out an investigation on some physiological characters of this fungs for the further investigation of the wilt disease of flax.

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I. General Cultures

1. Apricot-juice agar

Steep 200 grams of seedless dried apricot in 500 cc. of distilled water for 24 hours and filter through filter paper. Melt 30 grams of agar in 500 cc. of distilled water in boiling water bath and precipitate the impurities with white of egg and strain through cotton. Mix these two solutions. Fill 10 cc. of the fluid in to each test tube. Sterilize them in Koch's steam sterilizer for 20 minutes twice with one day's interval. Then incline them and let them set as agar slants.

This medium has acid reaction and is cream yellow colour. The medium was inoculated with the conidia or a bit of the mycelium, and was incubated at 25°C.

The growth of the mycelium is good, but not rapid. The aerial mycelium grows vigorously after few days with white cottony appearance.

The conidia are formed abundantly, and the microconidia are more numerous than macroconidia. In old cultures many chlamidospores appear.

The colour of the medium changes to reddish brown.

It is one of the suitable cultural media for the fungus.

2. Flax decoction hard agar

Young flax plants	50 grams
Agar	4.5 grams
Distilled water	300 cc.

Cook the stems and leaves of flax plants for an hour in a water bath, and straining through filter paper add agar and melt. Then strain through cotton and pour into test tubes. Sterilize them in the Koch's steam sterilizer for an hour. The colour of the medium is pale ochre green.

Inoculate with a bit of the mycelium or conidia, and incubate at 25°C.

Development of the mycelium is fair, and aerial mycelium is present. Macro- and microconidia are produced equally. Chlamydospores are formed very abundantly. The colour of the medium remains unchanged.

3. Cooked flax stems

Take flax seedlings, about 3 inches in height, in the test tubes, and heated them at 100°C. with moisture for an hour. Inoculate them and incubate at 25°C.

Aerial hyphae develop moderately in a cob-web like manner. Production of conidia is very good. On this medium the typical macrotype conidia are produced much more than the microtype conidia. Later chlamydorspores are produced, buried in the tissues.

On the living flax stems this fungus produces sporodochia scattered, but on these cooked stems the development of the mycelium is very rapid, im-

mediately covering all over the surface of the stems and producing the spores on every part in diffuse manner and not in spore clumps.

4. Potato cylinder

It was prepared as in the method of bacterial culture. Inoculate with a bit of the mycelium or conidia, and incubate at 25°C.

The mycelium develops well, and the aerial mycelium appears thick and cottony.

The spore formation is very good. Macroconidia and microconidia are produced almost in equal quantity. Chlamydospores are comparatively rare.

The colour of the medium is at first white, but changes later to ochre yellow.

5. Corn meal agar

This medium was prepared as the Cook's formula.

The mycelium creeps in the substratum, and the aerial mycelium grows poorly in a cob-web like manner.

Conidial formation is poor, and chlamydospores are produced abundantly. The colour of the medium remains unchanged.

6. Bean agar

Boil 200 cc. of ordinary soup bean in 500 cc. of distilled water for half an hour until they become plump, and strain through cloth. Melt 15 grams of agar in 500 cc. of distilled water. Mix these two solutions, and strain through cotton. Place the mixture in test tubes, 10 cc. each, and sterilize for two hours in Koch's steam sterilizer twice with one day's interval.

Inoculate with a bit of mycelium or conidia, and incubate at 25°C.

The mycelium develops fast and vigorously, the white aerial mycelium covering all over the medium in 3 to 4 days after inoculation.

Conidial formation is good, and macroconidia are produced much more than microconidia. Chlamydospores are produced moderately.

The colour of the medium remains unchanged, but a putrid smell is generated.

This is one of the good media for this fungus.

7. Potato decoction hard agar

Boil 500 grams of peeled potatoes in 1000 cc. of distilled water for an hour in a water bath, and strain through filter paper. Add 100 grams of cane sugar and 20 grams of agar to 1000 cc. of this filtrate, melt the agar with boiling and strain through cotton. Distribute this medium in test tubes, about 10 cc. each, and sterilize in Koch's steam sterilizer for two hours twice with one day's interval.

Inoculate with a bit of mycelium or conidia, and incubate at 25°C. Colour of the medium is white and semitransparent.

White cottony aerial mycelium grows thickly, covering all over the surface of the medium. Afterward the mycelium present a light pink colour, especially when the culture is exposed to light.

Conidia are produced abundantly, and microconidia are more numerous than macroconidia. Chlamydospores are produced poorly.

Colour of the medium remains uuchanged.

This medium is suitable for the growth of the mycelium and also for the conidial formation of this fungus.

8. Soil decoction

Steep 1 kilogram of soil in 2000 cc. of distilled water for 24 hours, then boil it for an hour and filter. Thus may be obtained 300 cc. of transparent soil decoction. Take 50 cc. of this decoction in each Erlenmeyer's flask having 200 cc. capacity. Sterilize them in Koch's steam sterilizer for two hours twice with one day's interval. The colour of the fluid is light yellow. Inoculate with a bit of mycelium or conidia, and incubate at 25°C.

Mycelium develops poorly, and produces no aerial mycelium.

Formation of conidia is also poor, but chlamydospores are produced abundantly. The colour of the medium remains unchanged.

The medium of the following formula is generally suitable for the culture of the soil organism.

Soil decoction	1000 cc.
Cane sugar	20 grams.
Potassium biphosphate	0.5 grams.

This solution has weak acid reaction. Development of the mycelium is good. The aerial mycelium is produced vigorously on the surface of the solution.

Formation of the conidia is poor, the chlamydospores are produced also poorly.

On the hard agar medium of these two solutions the fungus develops in a similar manner as in the solution.

9. Miyoshi's soy agar

The formula of this cultural medium as produced by Prof. M. Miyoshi is as follows :—

Japanese soy	20 cc.
Cane sugar	5 grams.
Boiled onion juice	25 cc.
Agar	1.5 grams.
Distilled water	50 cc.

Distribute this medium in test tubes, and sterilize them in Koch's steam sterilizer for an hour and let them set. Inoculate them with a bit of mycelium or conidia, and incubate at 25°C. Colour of the medium is reddish black and semitransparent.

Aerial and creeping mycelium grows vigorously, covering all over the surface of the medium in a short time.

The formation of conidia is fair, the macro- and microconidia are produced equally. Chlamydospores are very rarely formed. Colour of the medium remains unchanged.

10. Synthetic solution

I have used a nutritive solution of the following formula :—

Ammonium nitrate (NH_4NO_3)	10.0 grams
Potassium biphosphate (KH_2PO_4)	5.0 „
Magnesium sulphate ($MgSO_4$)	2.5 „
Cane sugar ($C_6H_{12}O_6$)	50.0 „
Iron chloride (Fe_2Cl_6)	a trace
Distilled water (H_2O)	1000.0 cc.

This solution is colourless and transparent, and has an acid reaction.

Take 50 cc. of this solution in each Erlenmeyer's flask which has 200 cc. capacity. Sterilize the flasks for half an hour in Koch's steam sterilizer twice. When sterilization is too long, this solution change its colour to yellow. Inoculate with a bit of the mycelium or conidia, and incubate at 25°C.

The cottony aerial mycelium grows vigorously, covering all over the surface of the solution. Afterwards the mycelium presents a light pink colour, especially when exposed to light.

Conidial formation is very poor, and chlamydospores are produced moderately in the old cultures.

Colour of the medium changes to yellow gradually.

Lack of iron salt is almost indifferent to the growth of the fungus.

This is a good medium for this fungus, especially suited for the hyphal growth.

11. General cultural characters

From the results of these cultural studies with the ten kinds of cultural medium, the following conclusion may be drawn.

In saprophytic condition *Fusarium lini* seems to be omnivorous, and on the several kinds of cultural media it develops vigorously and produces conidia.

The mycelium has ordinarily aerial habit, and white cottony appearance. On the corn-meal agar medium it develops, however, creeping mycelium. On a diseased flax stem, we find mostly macroconidia, but on the artificial cultural medium the microconidia are generally rather more numerous than macroconidia. The conidia in artificial cultural media are not formed on sporodochia as on the flax stem in natural condition, but they are produced on loose hyphae. Therefore, we could not recognize a pink colour of the spore masses on any artificial cultures, but on a certain kind of cultural medium, for example, on the synthetic solution, the mycelium present a light pink colour, especially when the cultures are exposed to light. This colour, however, is not caused by the spore clumps, but by some pigment formed in the mycelial cells.

In old cultures, the fungus produces brown coloured chlamydospores. Sometimes in some mycelial cells the wall thickens and turns brown by the deposition of a brown pigment. They are known as "Dauelmycel."

I have never found higher fruiting bodies of this fungus on my cultural media, but the results of the further experiments tell us that the fungus has very strong resistance to the unfavorable outer conditions, especially the chlamydospores may be said to be almost absolutely insensitive to any imaginable natural bad conditions. In an unfavourable condition, the mycelium forms chlamydospores or "Dauelmycel," the cell of the conidium itself changes to a chlamydospore, or the conidia by germination immediately produce chlamydospores or "Dauelmycel."

For such strong adaptability and resistance to several food conditions and unfavorable influences, the lack of higher fruiting bodies is no disadvantage to the life of the fungus in nature, and it causes an obstinate soil disease.

II. Tannin Culture

The effects of tannic acid on fnngi have attracted the attention of many authors and considerable work on this problem has been done. Tannin has been supposed to be a byproduct of the metabolism of plants, and it serves more or less as a protective agent against the attacks of organisms.

Pfeffer (1897) says that tannins are undoubtedly produced for definite purposes, and are not a mere byproduct produced under all circumstances. Cook (1911) also says that tannin serves no doubt as a protective agent. Clinton (1913), who studied *Endothia parasitica* and *Endothia gyrosa*, noticed that these two fungi can use tannic acid, at least in dilute solutions, as food. T. Hemmi (1915), who made studies on *Valsa japonica*, says in his summary that the fungus grows more luxuriantly on cultures containing a low percentages of tannic acid than without it, but high percentages of tannic acid inhibit its growth entirely.

It seems to me somewhat unlikely that the tannin production of plants has any ecological meaning. It would seem much more natural and rational to think that tannin is produced in the bodies of plants merely as a bypro-

duct of metabolism or is produced by the irritation of parasitic organisms without any ecological meaning. We must consider about the physiology of tannin formation instead of trying to imagine its ecological meaning.

EXPERIMENT 1.

The effects of tannic acid on the hyphal growth of the fungus.

For standard medium I have used a solution having the following formula.

Ammonium nitrate NH_4NO_3	10.0 g.
Potassium biphosphate KH_2PO_4	5.0 g.
Magnesium sulphate (cryst.) $MgSO_4$	2.5 g.
Cane sugar $C_{12}H_{22}O_{11}$	50.0 g.
Distilled water H_2O	1000.0 cc.

Portions of the standard nutrient solution, 50 cc. each, were placed in a number of Erlenmeyer's flasks. To these tannic acid was added in varying percentages, three flasks of each different percentage being prepared. They were sterilized in Koch's steam sterilizer for half an hour twice with a day interval, and inoculated with a bit of mycelium or conidia. If the spores could easily be separated from the mycelium, the result would be more accurate, but as it was very difficult to do, I have followed a simple method of inoculating it with a mixture of the mycelium and conidia. Then incubated them at 25°C., and after ten days compared their growth.

(a) At first the cultures which contained tannin lower than 0.4% were observed. To compare the degree of their growth I have adopted a method of weighing their mycelium after ten days from inoculation. For this purpose I put the filter papers in a desiccator and dryed them absolutely, and then weighed every one of them. Through these filter paper the cultures were filtered, and after three or four times washing with distilled water the mycelium retained in the filter paper as a packet. Evaporate the moisture of the packets in a boiling hot water desiccator, and then kept them in a calcium chloride desiccator for some days, and weighed them. By subtracting the weights of the filter papers from the obtained weights of the packages, the weight of the mycelium developed in each culture was found. But in this

method, there may be so many sources of inaccuracy as to make one accept the results of the experiment simply as relative and not as absolute.

Serial number	% of tannin	Average weight of mycelium	Appearance
1.	0.00	0.787	very good
2.	0.01	0.521	very good
3.	0.05	0.512	very good
4.	0.10	0.517	good
5.	0.15	0.519	good
6.	0.20	0.504	good
7.	0.25	0.325	moderate
8.	0.30	0.300	moderate
9.	0.35	0.240	fair
10.	0.40	0.222	fair

There occured occasionally abnormally good or poor development of the fungus in some cultures. I have omitted such cultures.

(b) In the second series, the cultures which contained tannin higher than 0.45% were observed. The greater part of the cultures did not develop so well as to enable me to weigh the mycelium. I have, therefore, classified them by the appearance of the cultures.

Serial No.	Percentage of tannin	Development of mycelium
11	0.45	fair
12	0.50	a little
13	0.55	slight
14	0.60	,,
15	0.65	trace
16	0.70	,,
17	0.80	,,
18	0.90	,,
19	1.00	,,
20	1.10	,,
21	1.20	,,
22	1.30	,,

In the cultures No. 11, and No. 12, the growth of thin aerial mycelium took place on the surface of the cultural medium. In No. 13 and No. 14 only a little aerial mycelium was seen. In No. 19 there was no aerial mycelium, and the fungus appeared as a small brown mass in the bottom of the solution. This brown colour was caused by the chlamydospores and "Dauermycel" which have brown thick wall and granulated cell contents.

In No. 20 to No. 22, there was hardly a trace of mycelial development. The mycelium of the fungus appeared as only a very small brown mass, which may probably be a little larger than when it was inoculated. After three weeks, such a brown mass was taken out of the tannin solution, and having been washed with sterilized distilled water several times, put in a nutrient solution and incubated, then immediately it began to grow and produced aerial mycelium vigorously, covering all over the surface of the nutritive medium.

EXPERIMENT 2.

The effects of tannic acid on the germination of the conidia.

In this experiment I have used the same cultural medium as in the preceding experiment, and adopted the drop culture with the Van Tieghem's cells.

Cover glasses were washed very carefully with acetic acid and caustic soda and at last with redistilled water.

The spores were obtained from a bean agar medium which had grown for two weeks in an incubator at 25°C.

The number of cultures was seven which contained different percentages of tannin. Every number was duplicated. They were inoculated with the conidia and incubated at 25°C.

The results were as in the following table.

Serial number	% of tannin	Germination of conidia		
		24h.	48h.	72h.
1	0.3%	++	++++	+++++
2	0.6	+	+++	+++
3	0.7	+	++	++

4	0.8	+	++	++
5	0.9	+	+	++
6	1.0	−	+	+
7	1.3	−	+	+

In the culture No. 1, after 24 hours many of the inoculated spores swelled and enlarged. The contents of these spores became homogeneous and nearly transparent. But only a small number of the spores germinated. Some of them shrank and their contents became granular, and died. There were spores in which some cells shrank while the other cells swelled or germinated.

After 48 hours many of them germinated. Germ-tubes had grown to the length of 10 to 150 microns, and some of them branched.

After 72 hours, the mycelium had developed to such an extent that it could be seen macroscopically. At this time the vigorously branched mycelia produced the microtype conidia at the end of the side branches. Chlamydospores were produced in large numbers. The shrunken spores remained as before without swelling.

In cultures No. 2 to No. 5, after 24 hours I found very few germinated spores. The swelled spores were less in number and the shrunken spores were more, the proportion between them varying in accordance with the increase in the percentage of tannin.

After 48 hours, a small number of the conidia germinated, and in the cultures No. 5 germinated spores were still more rare.

After 72 hours, some of the well developed mycelium had branched and produced a very few microconidia which are transformed directly into chlamydospores, the cell-wall of the conidia becoming thick and brown in colour.

In cultures Nos. 6 and 7, after 24 hours none of the spores had germinated. Only 1/3 of the inoculated spores swelled more or less, there being almost no spores in which all of the cells composing them swelled.

After 48 hours a very small number of the spores germinated.

After 72 hours there was observed neither branched mycelium nor conidial formation. Chlamydospores were fairly produced. Almost all of the germi-

nated spores immediately formed chlamydospores.

If such a shrunken spore was sown in a nutrient solution, it did not germinate at all.

From the results of these tannic acid cultures, the following conclusions may be drawn:

1. This fungus can not assimilate tannin even to a slight degree as food material, and its hyphal growth is retarded proportionately according to the degree of the concentration of tannin.

2. When the concentration of tannin becomes more than 1%, the hyphal growth of the fungus is almost prevented.

3. In tannin solutions of over 1% concentration the germination of the conidia is retarded heavily and many of the spores are killed.

4. But even 3% concentration of tannin is not fatal to this fungus. The conidia can still germinate and produce chlamydospores. Moreover the fungus which was kept in this solution for three weeks, has maintained its vitality.

5. Tannin retards the growth of the fungus and thereby causes the formation of the resting spores.

III. Citric acid culture

After the experiments with tannic acid cultures, I undertook to investigate, whether or not tannic acid has special effect in preventing the attack of the fungus, compared with other acids which are generally found in the plant-body. I have used citric acid for the comparison, as it is ordinarily used for such experiments. For the standard nutrient solution, both the synthetic solution as in the preceding formula, and potato decoction hard agar were used, in order to show what influences of standard nutritive media may have on the relation between the growth of fungus and toxicity of chemicals.

Experiment 1.

Cultural media were prepared as in the case of tannic acid cultures. They were inoculated and incubated at 25°C. Four cultures were used for each percentage. The percentages and results are shown in following table.

Serial number	Percentage of citric acid	Growth of the fungus after 10 days
1	0	very good
2	0.10	moderate
3	0.50	a little
4	1.00	slight
5	1.50	trace
6	2.00	none
7	3.00	,,
8	4.00	,,
9	5.00	,,

In the control culture, No. 1, aerial mycelium grew vigorously, covering thick all over the surface of the nutritive solution.

In No. 2 aerial mycelium grew far more poorly than No. 1, covering thinly and deficiently over the surface of the nutritive solution.

In No. 3 the fungus appeared as brown masses submerged in the solution, and aerial mycelium scarcely grew from these masses on the surface of the nutritive solution.

In No. 4 and No. 5 the fungus was recognized only as small brown masses sinking to the bottom of the solution, and there appeared on aerial mycelium over the surface of the solution.

In the cultures containing higher percentages of citric acid, above 2%, I could not find the hyphal growth of the fungus macroscopically.

The conidia were produced very sparsely in all of three cultures. Chlamydospores were produced richly in almost all cases except No. 1.

In the cultures of No. 3 the greater part of the inoculated conidia shrank and did not germinate. When a conidium germinated, the hypha did not grow long, but its cell contents became granular, and the wall of the cells thickened, coloured brown, thus changed into "Dauermycel" or chlamydospore. The brown colour of the mass is due to the chlamydospores and "Dauermycel." Such a chlamydospore-formation was demonstrated even in the cultures of No. 9 microscopically.

If such a brown mass of the fungus-hyphae be taken out and washed well, and then brought into a new culture medium, it begins to grow and

covers all over the surface of the medium with the aerial mycelium.

The fact shows that a comparatively higher concentration of citric acid does not injure the fungus so severly as to casue its death, at least of its chlamydospores.

As the synthetic nutritive solution itself has somewhat weak acidity by the potassium biphosphate (KH_2PO_4), the addition of citric acid intensifies it greatly.

In this experiment I have observed, that citric acid retards the growth of the fungus in nearly the same manner as tannic acid, especially in higher concentrations. Comparing the tannic acid cultures with the citric acid cultures, it does not seem that tannin is more toxic than citric acid for the fungus in this synthetic solution.

Flax is a non-tannin-bearing plant, therefore *Fusarium lini* may be considered to have no adaptation to tannin in its natural condition. Then it must be said that for such a fungus tannin is no more toxic than citric acid. I do not think it reasonable to give an ecological meaning to the tannin production of plants and to regard tannin especially as a toxic or preventive substance for parasites generally. But tannin production is merely a characteristic of a species or genus of plant, and tannin is no more than a physiological product by assimilation or metabolism of a plant.

EXPERIMENT 2.

In the present experiment I have used potato decoction hard agar for the standard cultural medium in order to investigate the effect of the change of a cultural medium containing different percentages of citric acid upon the growth of the fungus.

The standard medium was prepared in the ordinally method. The formula of the medium is as follows.

Potato decoction	1000 cc.
Cane sugar	100 grams
Agar	20 „

The medium was sterilized in a Koch's steam sterilizer for half an hour twice with one day's interval.

The cultural media which contained above 0.5% of citric acid did not solidify. The cultures No. 1 to No. 3 were used as slants in test tubes and those above No. 4 (0.5% citric acid) were used in liquid form in Erlenmyer's flasks.

They were inoculated with a bit of the mycelium or conidia which were produced in a culture grown two weeks in an incubator at 25°C on apricot agar medium.

The results of the cultures after 10 days were as in the following table.

Serial number	Percentage of citric acid.	Development of mycelium.	Formation of conidia.	Formation of chlamydospores.
1	0.00	good	very good	slight
2	0.06	very good	very good	slight
3	0.10	very good	good	slight
4	0.50	very good	fair	slight
5	1.00	moderate	fair	a little
6	1.50	fair	a little	a little
7	2.00	a little	slight	a little
8	3.00	slight	slight	fair
9	5.00	none	none	slight
10	apricot agar for control	good	good	moderate

Twenty-four hours after inoculation, the cultures No. 1 to No. 3. showed the visible growth of the mycelium.

Two days after inoculation, in No. 4, No. 5 and No. 10 (apricot agar) a visible growth of the mycelium had begun. No. 1 to No. 3 produced conidia already a little.

After 4 days, No. 6 and 7 began to show a visible mycelial growth. At this time, in No. 1 to No. 4 vigorous growth had taken place, the aerial mycelium covering all over the surface of the medium. No. 10 was not yet so good as in No. 1.

After 5 days, No. 8 began to grow, but in No. 9 the mycelial growth could not be seen with the naked eyes. No. 1 to No. 5 produced the conidia richly. In No. 3 and No. 4 the mycelium presented a light pink colour. This colour was also assumed by many other cultures afterward.

Production of the chlamydospores is generally poor. In the No. 9 cultures I could observe the chlamydospore-formation microscopically, notwithstanding the hyphal growth is scarcely detected macroscopically.

Production of conidia is vigorous in the cultures of low percentages of citric acid.

From the results of these experiments I have drawn the following conclusions :—

1. Small quantities of citric acid existing in the culture media are favorable for the fungus. Citric acid seems, however, not to serve much for nutrition, it may only do so for a carbon source, but as there is cane sugar present in the culture medium, there may not be any necessity for the fungus to utilize the carbon of another carbon-source so difficultly assimilable as citric acid.

2. Citric acid retards the formation of the conidia. In the acid-free medium the fungus produces conidia abundantly. With the increase of the acid percentage conidial formation become gradually less.

3. In the preceding citric acid cultures with the synthetic solution, the growth of the mycelium was checked even by 1.5% concentration of citric acid, but in the case of the potato agar medium even by the 3% concentration of acid the growth of the fungus was not checked. Acidity of the standard medium may have caused such a difference.

4. Chlamydospore-formation was induced by citric acid. In the acid-free cultures the chlamydospore-formation was observed rarely after 10 days from inoculation. In the cultures of higher acid percentage it was somewhat vigorous considering the poor growth of the mycelium. When the cultures became old, chlamydospores were produced more and more even in the cultures which contain citric acid in low percentage.

IV. Effects of Temperature on the Fungus

Investigation of the relation between temperature and growth of the fungus or infection is very interesting biologically, and at the same time very important economically.

There are many reports of temperature studies on different kinds of fungi.

On the genus *Fusarium* a considerable amount of such work has been done.

Fusaria, as a rule, seem to require a high temperature for their most virulent attack.

Jones (1908) says that the damping off of coniferous seedings was facilitated by a high temperature.

Wollenweber (1913) says that the wilt diseases, caused by *Fusarium*, occur most severely in warmer climates.

Wolf (1910) says that the wilt diseases of pansy, caused by *Fusarium Violae*, are found only in July, and then only when the beds in which the plants were growing has been heavily covered with fresh horse manure, both of which facts suggest a dependence of the fungus on high temperature.

Humphrey (1914) says that *Fusarium orthoceras* and *Fusarium oxysporum* cause the tomato blight when the temperature is high. The optimum temperature of the fungi is about 30°C.

Gilman (1916), who studied the cabbage yellow and the relation of temperature to its occurrence, observed that a high temperature (as 25°C) was favorable to the mycelial growth of *Fusarium conglutinans*, and the germination of the conidia occurred within only three hours at 33°C, and cabbage seedlings were attacked most severly at 28°C. to 30°C., but in low temperature (as 10° to 12°C.) the conidia could not germinate.

Tisdale (1917), who studied the relation of temperature to the growth and infecting power of *Fusarium lini*, reported that for the growth of this fungus the minimum temperature is 10° to 11°C., the optimum is 26° to 28°C., and the maximum is 35° to 36°C., on the potato agar medium; and the critical temperature for the infection of this fungus is 14° to 16°C.

Experiment 1.

Effect of temperature on the mycelial growth of the fungus.

I used the synthetic solution of following formula for the cultural medium:

Ammonium nitrate (NH_4NO_3)	1.00 gram
Potassium biphosphate (KH_2PO_4)	0.50 ,,
Magnesium sulphate crystal ($MgSO_4$)	0.25 ,,
Cane Sugar ($C_{12}H_{22}O_{11}$)	20.00 ,,

Redistilled Water (H_2O) 1000.00 cc.

Took 50 cc. of the solution in an Erlenmyer's flask of the capacity of 200 cc. Sterilized, and inoculated with a bit of the mycelium and conidia. Incubated them at the various temperatures. After ten days, strained the mycelium through pieces of filter paper of known weights, in order to compare the growth of the fungus for every temperature in the dry weight. Cultures for each temperature were in triplicate.

The obtained results are following.

1. At 3° to 5° C. (in a cold chamber.)

   No growth.

   But the fungus maintained its vitality, and by incubating them at 25°C., the fungus grew vigorously.

2. At 10° to 12°C. (in an incubator.)

   a trace.

3. At 13° to 15°C. (in an incubator.)

   A little.

   Dry weight of mycelium....0.007 g.

4. At 20°C. (in an incubator.)

   Moderate.

5. At 25°C. (in an incubator.)

   Good. Aerial mycelium grew vigorously, covering all over the surface of the medium.

   Dry weight of the mycelium....0.462 g.

6. At 28°C. (in an incubator.)

   Very good. Aerial mycelium grew vigorously, covering all over the surface of the medium.

   Dry weight of the mycelium....0.550 g.

7. At 30°C. (in an incubator.)

   Very good. Aerial mycelium grew most vigorously, covering thick all over the surface of the medium.

   Dry weight of the mycelium....0.610 g.

8. At 34° to 35°C. (in an incubator.)

   Fair. Aerial mycelium grew poorly, scarcely covering over the

surface of the medium.

Dry weight of the mycelium....0.094 g.

9. At 36° to 37°C. (in an incubator.)

A trace. Mycelium grew very little and did not produce the aerial mycelium, sinking to the bottom of the cultural solution.

10. At 40°C. (in an incubator.)

No growth.

The conidia shrank and did not germinate.

From these results it may be said that in this nutrient solution the minimum temperature for the growth of the fungus is 10° to 12°C., the optimum temperature about 30°C. and the maximum temperature is about 36°C to 37°C.

Experiment 2.

The effects of a high temperature on the germination of the conidia.

I have adopted 50°C. as the high temperature. The test tubes of bean agar cultures of the fungus were placed in an incubator at 50°C. The conidia were taken out from these test tubes with an hour's interval, and were sowed in drops of the nutrient solution, and incubated at 25°C.

The conidia which were incubated at 50°C. for an hour about half germinated, and the other half shrank and died.

The conidia which were incubated at 50°C. for two hours did not germinate at all, they all shrank and were killed.

Above three hours no conidium survived.

Experiment 3.

Resistance of the fungus to low temperature.

I have used ice and NaCl for the cooling agent. The test tubes of the vigorously growing bean agar cultures of the fungus in an incubator at 25°C. were placed in the thermos bottles which contained the cooling agent, and after a certain interval they were removed and the conidia were sown in the nutrient solution and incubated at 25°C. The results were as following table.

Temperature	Duration of cooling	Growth of fungus
−12° to −15°C.	1 day	+
−17° to −20°C.	1 day	+
−21°C.	1 day	+
−12° to −21°C.	3 days	+

They germinated vigorously and produced aerial mycelium.

In these results it may be said that the low temperature, as −21°C., does not harm the vitality of this fungus at least within 24 hours.

EXPERIMENT 4.

Resistance of the fungus to high temperature.

I have investigated the resistance with wet heat. The test tubes, which contained 10 cc. of nutritive solution, were inoculated with a bit of mycelium and the chlamydospores of the fungus. The test tubes were incubated at the high temperature in a self-regulating water bath, and with some interval they were removed to an incubator at 25°C. I have adopted 50°C. and 60°C. for the high temperature, and 1 to 6 hours for the intervals. The results obtained were as follows:

Temperature	Intervals					
	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
50°C.	+	+	+	−	−	−
60°C.	+	+	±	−	−	−

+ shows living. − shows died.

± shows some tubes living, some died.

Every sample was triplicated.

The fungus maintained its vitality in this nutritive solution exposed for three hours to the temperature 50°C. By heating at 60°C. for three hours, the fungus in one tube maintained its vitality, while that in the other two tubes was killed.

Above three hours at both temperatures none retained their vitality.

V. Summary

1. On several kinds of the artificial cultural medium, this fungus develops well producing conidia, and when the culture become old chlamydospores are formed.

2. In the synthetic solution which I have used, both tannic acid and citric acid retard the growth of the fungus.

3. In the potato agar medium, citric acid stimulates the growth of the fungus in a low concentration, while in a high percentage of citric acid the growth of the fungus is retarded.

4. In the synthetic solution which I have used, the minimum, optimum and maximum temperatures for the growth of the fungus are 10°C. to 12°C., 30°C. and 36°C. to 37°C. respectively.

5. Wet heat of 50°C. annihilates the germinating power of the conidia within two hours.

6. Wet heat of 60°C. does not kill the chlamydospores or "Dauelmycel" of the fungus within three hours.

7. At the low temperature of −21°C. the vitality of the fungus is not injured.

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摘　　要

亞麻立枯病菌 (Fusarium lini) に關する生理學的研究の一部として、一般培養試驗、單寧培養試驗及び發芽試驗、枸橼酸培養試驗及び温度の影響試驗を行ひ、次の結果を得たり。

1. 一般培養試驗、

本菌は一般培養基の多くのものに良く發育して胞子を作り、天然の狀態に於て亞麻に寄生せる場合とは多少異りたる性質を現す、就中杏、馬鈴薯、菜豆等の寒天培養基は最も適當なるものにして、菌糸及び胞子の發育生成極めて良好なり。合成培養液は菌糸の發育に適し、各種の試驗を行ふ際に使用して便なり。

2. 單寧培養試驗、

余の使用せる合成培養液中に於ては、單寧の存在は本菌の發育に對して有害なる影響を及ぼす事著しく 0.01% の微量も菌體の發育を阻碍する事明かにして、其の含量 0.6% に及べば菌糸の伸長は既に全く之を見ざるに至れり。然れども單寧培養液中にありては、菌の厚膜胞子形成は甚だ活潑に行はれ、爲に菌は單寧の有害なる影響より免かるるものにして、例へば 1.3% の濃度の單寧溶液中に在る事已に二週間に及べる菌體も、尙ほ能く其の生活力を維持し、之を無單寧培養液中に移せば旺盛なる發育をなしたり。

3. 單寧培養液中に於ける分生胞子の發芽試驗、

合成培養液中に於ける單寧の存在は本菌分生胞子の發芽に有害なる影響を及ぼす。攝氏二五度の温度を以て試驗せる所に依れば、0.3% の濃度に於ては接種せる分生胞子の大部分は發芽力を害されず、少數のものは 24 時間以內に發芽し、48 時間の後には多くのもの發芽したり、而して爾後菌糸伸長して 72 時間以內に 150 ミクロンに及ぶもの多く、分岐して小分生胞子を生ずるものあり、枯縮せる胞子は比較的少數に過ぎざりき。然るに 0.6% 乃至 0.9% の濃度に於ては播下せる分生胞子の約半數は枯縮し、24 時間以內に發芽せるものは極めて稀にして、48 時間にして尙ほ僅少の數の發芽を見るに過ぎず、發芽せるものも直ちに厚膜胞子を作り、充分なる菌糸の伸長を見るに至らざりき。1.0% 以上の濃度に於ては 24 時間以內に發芽せるものは皆無にして、48 時間を經て始めて稀に發芽せるものを認め、接種せる胞子の三分の一以上は全々枯縮せり。

4. 枸橼酸培養試驗、

本試驗に於ては、培養基として合成培養液と馬鈴薯寒天培養基を用ゐたるが、前者に於ける成績は單寧の場合と大差なく、後者の場合には著しき差異を現したり、即ち枸橼酸の含量 0.5% 以下のものに在りては菌糸の發育頗る良好にして、寧ろ全々酸を加へざるものに優るもの

あり、1% 以上にして初めて顯著なる惡影響現はれ、5% に至りて全く發育を止めたり。然れども分生胞子の生成は 0.1% に於て既に多少阻碍せらるる傾向を認めたり、之に反して厚膜胞子の生成は比較上略酸の含量に比例して增加する傾向明かなりき。

5. 菌糸の發育に及ぼす溫度の影響試驗、

本菌は Fusarium 屬の通性たる好熱菌の性質を現はし、菌糸發育に對する溫度としては最低 10°C. 乃至 12°C. 最適 30°C. 最高 36°C. 乃至 37°C. 致死溫度約 40°C. を得たり。

6. 胞子の高溫に對する抵抗力試驗、

本菌分生胞子は高溫に對する抵抗力比較的弱くして、50°C. の濕熱に一時間遭遇すれば、供試分生胞子の約半數は枯縮し、半數のみ發芽力を保ちたり、同溫度中に二時間を經過すれば全部枯縮して發芽するもの皆無なりき。然れども厚膜胞子及び厚膜菌糸の高溫に對する抵抗力は更に强きものにして、濕熱 50°C. 中に 3 時間を經過したるもの尙生活力を保ち、4 時間にして始めて全部死滅せり、同じく 60°C. に於ては 3 時間にして大部分は死滅すれども尙生き殘るものあり、4 時間にして始めて全部の死滅を見たり。

7. 菌體の低溫に對する抵抗力試驗、

本菌の低溫に對する抵抗力は比較的强く、-21°C の低溫中に經過する事 24 時間に及びても、毫もその生活力を害さるゝ事なかりき。