PATHOLOGICAL STUDIES OF THE "BUD BLIGHT" OF MULBERRY TREES

IV. PHYSIOLOGICAL CHARACTERS OF THE CAUSAL FUNGUS, GIBBERELLA LATERITIUM (NEES) SNYDER ET HANSEN*

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With 5 Figures in the Text

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INTRODUCTION

The "bud blight" of mulberry trees is mainly caused by *Gibberella lateritium* (NEES) SNYDER et HANSEN, [7] though recently another causal fungus, *Hypomyces solani* (RKE. et BERTH.) S. et H., has been discovered by SAKURAI and the writer. [15] Several papers[8, 9, 10, 11, 12, 13] have been published by the writer concerning the pathological studies of this disease. This paper deals with the physiological characters of *Gibberella lateritium* (NEES) S. et H. It is necessary

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for the future advancement of the pathological studies of this disease that the physiological characters of this causal fungus are surveyed and made clear.

The writer must record here his hearty thanks to Mr. Y. SAKURAI who assited him in the experiments of these studies.

I. EFFECT OF TEMPERATURE AND HYDROGEN-ION CONCENTRATION OF MEDIUM ON GROWTH OF THE CAUSAL FUNGUS

1. Effect of temperature on growth of the causal fungus

This investigation was done, as usual, by means of culturing the causal fungus, *Gibberella lateritium*, on 3 media; potato decoction–1.5% sucrose agar, citrus skin decoction agar, and Dox's synthetic agar, which were poured in Petri dishes. The inoculation was done of conidia or hyphae of the causal fungus. 5 Petri dishes were used for each plot. The experiment was repeated three times. The results are shown in Table 1–a, b. The figures in the table are the average diameters (mm) of the colonies on the 6th day after the inoculation (potato decoction–1.5 % sucrose agar) or on the 7th day after (the other 2 media).

Table 1–a, b shows that the cardinal points for the vegetative growth of *G. lateritium* are minimum, slightly lower than 4°C; optimum, 24°C–26°C; and maximum, about 36°C.

2. Effect of hydrogen-ion concentration of medium on growth of the causal fungus

The basic medium of this experiment is the glucose–asparagine culture solution (glucose 10g, asparagine 2g, KH_2PO_4 1g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeCl_3$·

Table 1–a Effect of temperature on growth of *G. lateritium*, strain No. 1

medium \ Temp. °C		4°	4–10°	7–14°	16°	20°	22°	24°	26°	28°	30°	32°	36°
Potato decoction agar	1	/	/	/	19.4	26.9	36.7	37.3	36.5	28.8	21.5	−	−
	2	+	4.9	6.7	18.5	27.6	30.4	34.5	35.6	33.6	14.4	±	−
	3	+	10.0	15.3	26.3	37.8	33.9	44.3	39.9	34.7	12.3	±	−
	Aver.	+	7.5	11.0	21.4	30.8	33.7	38.7	37.3	32.4	16.0	±	−
Citrus skin decoction agar	1	+	++	8.8	21.7	30.5	36.2	38.1	38.5	36.6	8.1	1.9	±
	2	+	3.0	11.2	20.5	30.8	35.1	38.2	38.1	34.1	19.6	3.0	±
	3	±	0.8	11.0	23.3	31.4	37.4	38.1	36.8	28.4	13.8	+	±
	Aver.	+	1.3	10.3	21.8	30.9	36.2	38.1	37.8	33.0	13.8	1.6	±
Dox's synthetic agar	1	+	+	13.0	24.0	33.0	37.4	37.4	36.8	35.4	23.4	1.0	±
	2	±	+	12.8	26.2	34.2	36.3	39.4	33.4	26.2	23.0	1.0	−
	3	±	+	12.8	24.0	31.0	37.4	38.0	35.4	26.0	23.4	1.5	±
	Aver.	+	+	12.9	24.7	32.7	37.0	38.3	35.2	29.2	23.3	1.2	±

Table 1-b Effect of temperature on growth of *G. lateritium*, strain No. 2

Medium		Temp. °C 4°	4–10°	7–14°	16°	20°	22°	24°	26°	28°	30°	32°	36°
Potato decoction agar	1	/	/	/	28.3	47.8	56.7	61.7	66.3	56.5	45.9	—	—
	2	±	7.8	15.6	30.2	41.7	43.2	51.6	49.2	48.5	25.1	10.0	—
	3	±	3.3	16.9	34.6	43.6	57.5	68.4	69.5	60.9	33.7	3.8	—
	Aver.	±	5.6	16.3	31.0	44.4	52.5	60.6	61.7	55.3	34.9	4.6	—
Citrus skin decoction agar	1	+	+	14.4	33.5	47.0	54.2	65.0	65.4	61.8	18.8	4.4	±
	2	+	8.1	19.7	34.2	47.5	54.1	61.4	64.3	61.2	27.1	6.5	±
	3	+	5.6	18.6	38.0	50.8	58.8	63.9	68.4	67.3	23.4	7.0	±
	Aver.	+	4.6	17.6	35.2	48.4	55.7	63.4	66.0	63.4	23.1	6.0	±
Dox's synthetic agar	1	+	9.4	21.2	39.8	54.5	61.6	64.6	60.6	53.4	36.0	11.8	±
	2	+	9.4	22.6	38.2	49.3	60.4	71.0	70.8	67.8	53.4	5.8	±
	3	+	8.0	23.0	39.0	48.0	60.1	63.2	64.5	61.6	53.4	5.7	±
	Aver.	+	8.9	22.3	39.0	50.6	60.7	66.3	65.3	60.9	47.6	7.8	±

$6H_2O$ 0.001g, $ZnSO_4 \cdot 7H_2O$ 0.001g, $MnSO_4 \cdot 4H_2O$ 0.001g, water 1000ml). 40 ml of the solution was put into each of 200 ml Erlenmeyer flasks with the cotton plug. Then it was regulated to its own definite pH value with HCl or NaOH using the glass electrode pH meter. All the culture solutions were

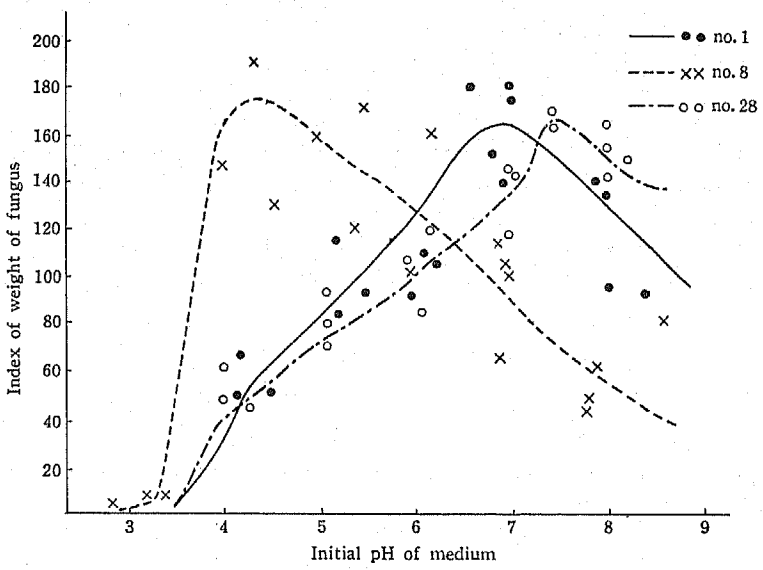

Fig. 1-a Effect of hydrogen-ion concentration of medium on growth of causal fungus

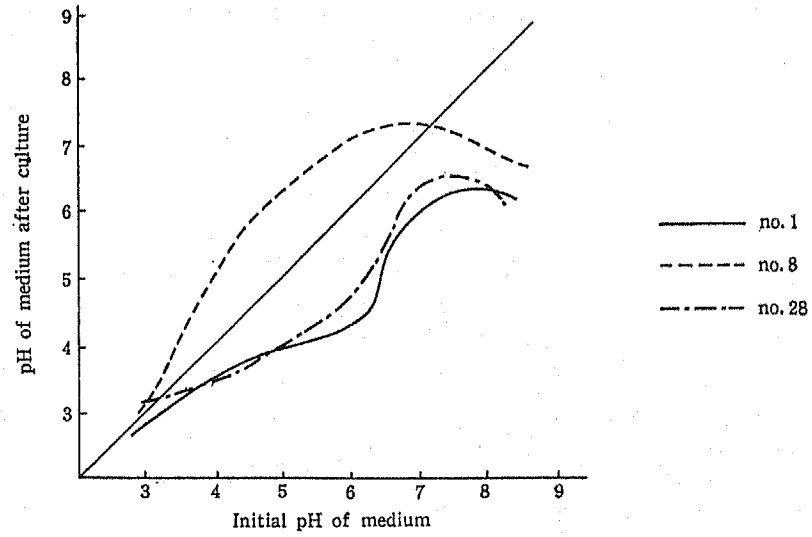

Fig. 1–b Changes of hydrogen-ion concentration of medium by culturing of causal fungus

sterilized in an autoclave at 2kg/cm².

Then the pH values of the solutions were examined again (some of them changed to some extent by the sterilization). The culture was continued for 10 days at 25°C using 5 Erlenmeyer flasks for each pH plot and was repeated 3 times. The effect of the hydrogen-ion concentration of the medium on the growth of nos. 1, 8 and 28 of the causal fungus is shown in Fig. 1–a. The changes of pH values of the culture solution by culturing are shown in Fig. 1–b.

Fig. 1–a shows that the pH range of the growth of the causal fungus on the glucose-asparagine culture solution is from about 3 (no. 8) or 3.5 (nos. 1 and 28) to 9 or a little higher. The optimum pH value is different for the strains of the causal fungus, that is, 6.5~7 for no. 1, 7.5 for no. 28, and about 4.5 for no. 8. Fig. 1–b shows that the pH values of the culture solutions fell towards the acid side after the culturing of nos. 1 and 28, but on the contrary rose after the culturing of no. 8 in the plots of the acid part.

II. EFFECT OF TEMPERATURE AND MOISTURE ON FORMATION OF MACROCONIDIA AND PERITHECIA OF THE CAUSAL FUNGUS

1. Effect of temperature on formation of macroconidia and perithecia of the causal fungus

(1) Effect of temperature on formation of macroconidia

The macroconidium, the most important infectious agent, is mainly produced on the sporodochium which is formed on the mulberry stem in the field. As the typical sporodochium is also formed on the mulberry stem

medium*, the effect of the temperature on the formation of the macroconidia was investigated using the mulberry stem media. Table 2–a shows the results.

Table 2–a Effect of temperasture on formation of sporodchia on mulberry stem medium

No. of exp. \ °C		4°	4–11°	9–14.5°	16°	20°	22°	24°	26°	28°	30°	32°	36°
1	After 2 weeks	−	−	−	±	+	+	+	+	+	−	−	−
	After 4 weeks	−	−	+	+	+	++	+++	+ +++	+++	+++	−	−
2	After 2 weeks	−	−	+	+	+	+	++	++	+	+	±	−
	After 4 weeks	−	−	+	++	+	+	+++ +++	+ +++	+++ +++	+++ +++	±	−
3	After 2 weeks	−	−	−	+	+	+	++	+	+	+	±	−
	After 4 weeks	−	−	+	+	+	+++	++ +++	+++ +++	+++ ++++	+++ ++++	±	−

The mark + in the table shows the formation of 1∼10 sporodochia on a mulberry stem medium, and ++ shows the formation of 11∼20 sporodochia, and so forth. No. 1 of the causal fungus was supplied for this investigation, and 5 mulberry stem media were used for each plot.

The results of the measurement after 4 weeks showed that the sporodochia were formed between 9–14.5°C and about 32°C, most vigorously between 24°C and 30°C. These optimum temperatures (24°C ∼ 30°C) were confirmed again by the observation after more than 5 weeks. At the same time, a few sporodochia were found on the mulberry stem media of 4–11°C plot. After 3 months, they were discovered at 4°C plot.

Secondly, the effect of the temperature on the formation of the conidia on hyphae or pionnotes on the potato decoction–1.5% sucrose agar was investigated. The results are shown in Table 2–b. The number of + in the table shows the degree of the formation of conidia, and (+) means a half degree of + or less. No. 1 of the causal fungus was also used for this investigation.

According to Table 2–b, the cardinal points for the formation of the conidia on hyphae or pionnotes on the potato decoction – 1.5% sucrose agar are as follows:—— minimum, 4°C or a little lower; optimum, 20°C ∼ 28°C; and maximum, 32°C∼36°C. On the mulberry stem medium also these conidia

* The mulberry stem medium is made as follows. The mulberry stem is cut into the pieces of 8 cm length. Each piece was put in a test tube having 2 ml water in it as well as a cotton plug at the mouth, and was sterilized in an autoclave at 2kg/cm².

Table 2-b Effect of temperature on formation of macroconidia on potato decoction—1.5% sucrose agar

No. of exp. \ °C		4°	4–11°	9–14.5°	16°	20°	22°	24°	26°	28°	30°	32°	36°
1	After 1 week	−	+	+	+++	+	+	+	+	+	++	+	−
	After 3 weeks	−	++(+)	+	++	+++	+++	+++	++(+)	++(+)	+	++	−
2	After 1 week	−	+	+	+++	+	+	++	++	+	++(+)	(+)	−
	After 3 weeks	+	+++	++	++(+)	+++	+++	+++	+++	+++	++	++	−
3	After 1 week	−	+	+	+++	+	+	+	++	+	+	(+)	−
	After 3 weeks	+	+++	++	+++	+++	+++	+++	+++	+++	++	+	−

on hyphae or pionnotes were sometimes formed in addition to the typical sporodochia which were explained above. The effect of the temperature on the formation of these conidia on hyphae or pionnotes on the mulberry stem medium showed the same tendency as in the case of on the potato decoction -1.5% sucrose agar.

In the field the macroconidia of the causal fungus are formed mainly on sporodochia. However, it is supposed that the macroconidia on the hyphae or the pionnotes are also formed, when the mulberry stems are piled and moistened.

(2) Effect of temperature on formation of perithecia

The ascospore, one of the important infectious agents, is produced in the perithecia. The perithecia are formed very easily on the mulberry stem medium. So, the relation between the formation of the perithecia and the environmental temperature was investigated using the mulberry stem medium.

Two kinds of investigation were carried out. Firstly, to make clear the effect of the temperature on the formation of perithecia from the hyphae which have spread over the mulberry stem media, the writer divided the mulberry stem media, which had already been kept in 24°C for 15 ~ 20 days after the inoculation, into plots of various degrees of temperature (A-exp.) On the other hand, to make clear the effect of the temperature from the inoculation to the formation of perithecia, the mulberry stem media were divided into various plots of temperature (B-exp.) immediately after having been inoculated. No. 1 of the causal fungus was made use of, and 5 culture tubes of the mulberry stem medium were used for each plot. The results of investigation are shown in Table 3. ± means the obscure formation of the perithecial stroma; (+), 1~5 perithecial stromata formed distinctly on a mulberry stem medium; +, 15–30 formation; and ∞, very numerous

Table 3 Effect of temperature on formation of perithecia on mulberry stem medium

Experiment			°C /	4°	4–13°	7–14.5°	16°	20°	22°	24°	26°	28°	30°	32°
A	1	Formation of perithecial stroma	after 30 days	−		+(+)	++	++	(+)+	(+)	−	±	−	−
			after 60 days	±		+(+)	++(+)	++(+)	++(+)	+(+)	+	+	−	−
		Size of perithecium after 60 days				+	##	###	###	##	##	+		
	2	Formation of perithecial stroma	after 30 days	−		+(+)	++	++	+(+)	+	−	−	−	−
			after 90 days	+		∞	∞	∞	∞	∞	+ +++	+	±	−
		Size of perithecium after 90 dags		##		##	###	##	###	##	##	##		
B	1	Formation of perithecial stroma	after 30 days	−	−	−	+	(+)	+(+)	)+)	−	−	−	−
			after 75 days	−	+	+	+(+)	(+)	++	(+)	−	−	−	−
	2	Formation of perithecial stroma	after 30 days	−	−	(+)	+	+(+)	++	−	−	−	−	−
			after 75 days	−	+++	+++	+++	++	∞	+++	++ +++	+ +++	−	−
	3	Formation of perithecial stroma	after 30 days	−	(+)	(+)	(+)	(+)	++	+	−	−	−	−
			after 75 days	−	(+) +++	++	+++	+++	∞	(+) +++	+++	+(+)	−	−

formation. The marks †, †† and ††† show the sizes of perithecia. The size of the perithecium was represented by the largest diameter of the perithecia among the materials sectioned several times by hand; †, less than 100μ; ††, 100μ~150μ; †††, more than 150μ.

According to the A-exp. of Table 3, the formation of the perithecium occurs from 4°C to about 30°C and its optimum temperature ranges from 7-14.5°C to 24°C. And the results of the B-exp. of Table 3 show that the perithecia are formed in the temperature from 4-13°C to 28°C, and the optimum temperature is in the range of 16°C ~ 24°C, and the fittest is especially 22°C. In these experiments the ripened perithecia or ascospores could not be found. When the pieces of the diseased mulberry stems, which are collected in the field, are put in the test tubes with the cotton plugs and kept in 20°C~25°C, the ripened perithecia or ascospores are easily formed on the surface. This fact seems to suggest that the mulberry stem medium loses the component which promotes the ripening of the perithecia when the sterilization of it is carried out in the autoclave at 2kg/cm^2.

2. **Effect of moisture on formation of macroconidia and perithecia**

(1) Effect of moisture on formation of macroconidia

If the diseased mulberry stems, which are collected in the field, are cut into pieces of about 10 cm length, put in the sterilized test tubes having cotton plugs and kept at 20 ~ 28°C in a thermostat, many sporodochia are produced on the surface. Accordingly, we can easily confirm that the formation of the sporodochia on the moistened mulberry stem is easier than that on the dry mulberry stem. But, the influence of the relative humidity of the atmosphere on the new formation of sporodochia could not be investigated, because the sporodochia were never newly formed on the mulberry stems which were closed completely in the glasswares in order to keep definite relative humidities. However, the influence of the relative humidity of the atmosphere upon the formation of macroconidia on the already formed sporodochia could be easily investigated. The method of the investigation is as follows. The sporodochia on the surface of the mulberry stem were washed with a brush for the purpose of removing the old macroconidia on them, and were dried in the room temperature. The pieces of mulberry stem having the sporodochia were put in the glasswares, the relative humidities of which were made definite using H_2SO_4 of various concentrations[18] The glasswares were closed completely and were kept at 25°C in the thermostat. 1~5 days after the treatment, the formation of the macroconidia on the sporodochia was investigated using the microscope. This experiment was repeated 5 times using the mulberry stem media (No. 1 of the causal fungus was cultured for about a month) as well as the diseased mulberry stems collected in the field. The average value of the results is shown in Table 4.

Table 4 shows that the new macroconidia were observed on the sporodochia in 94.8% and higher plots after a day, and on those in 85% plot after 3 days, but could not be observed in 80% plot even after 5 days.

(2) Effect of moisture on formation of perithecia

The effect of the moisture on the formation of the perithecia on the

Table 4 Effect of relative humidity of atmosphere on formation of macroconidia on sporodochia

	Relative humidity %	Days elapsed			
		1	2	3	5
On diseased mulberry stem	100	++	+++	+++	+++++
	97.5	++	++	+++	+++
	94.8	+	+	+++	+++
	89.0	−	+	+++	+++
	85.0	−	−	+	++
	80.0	−	−	−	−
On mulberry stem medium	100	++	++	+++	+++++
	97.5	++	++	+++	+++
	94.8	+	+	+++	+++
	89.0	−	+	++	++
	85.0	−	−	+	+
	80.0	−	−	−	−

Note : The mark + shows the formation degree of macroconidia.

Table 5 Effect of moisture of mulberry stem on formation of perithecia

No. of exp. / Plot	1	2	3	4	5	Average
Dry mulberry stem	26.2	51.1	3.1	13.3	29.8	24.7
moist mulberry stem	391.3	430.2	251.2	95.4	318.5	297.3

Note : The average number of perithecial stromata on a mulberry stem (10 cm length) is shown.

diseased mulberry stems, which were collected in the field, is shown in Table 5. This experiment was carried out as usual using the test tubes with the cotton plugs. The diseased mulberry stems collected in April are convenient for this experiment, because no perithecium is yet formed on them. According to Table 5, the moistened mulberry stem is more suitable for the formation of perithecia than the dry mulberry stem.

Though the perithecia were easily formed on the mulberry stem in the culture tube having the cotton plug as shown in Table 5, they were scarcely formed in the glassware which was closed completely. So the effect of the relative humidity of the atmosphere on the formation of perithecia could not be investigated. This fact seems to suggest that some ventilations (or some amount of oxygen in the air) are necessary for the formation of the perithecia.

III. EFFECT OF LIGHT ON FORMATION OF SPORODOCHIA AND PERITHECIA OF THE CAUSAL FUNGUS

Many investigations have been published concerning the effect of light upon the formation of conidia and perithecia of various fungi. According to them, the effect of light is different according not only to the kinds of light and its strength but also to the kinds of fungi and their condition.[3,6] In this chapter the experimental results on the effect of light and darkness, and the ultraviolet radiation upon the formation of the sporodochia and perithecia of this causal fungus will be described.

1. Effect of light and darkness upon formation of sporodochia and perithecia of the causal fungus

Material and method The mulberry stem media, on which the fungus had been cultured at 25°C for 7 days, were divided into 3 plots (light I, light II and dark). In the light I plot the mulberry stem media (the quality of glass of the test tubes is common) were arranged on the test tube racks which were situated near the southern window of the laboratory. In the light II plot the mulberry stem media, which were arranged as in the light I plot, were covered with a sheet of thin art paper. In the dark plot the mulberry stem media were put in a dark box which was covered with black cloth. A little sterilized water was often poured into each medium to prevent drying up.

Results and discussion 5 mulberry stem media were used for each plot, and the experiment was repeated twice concerning Nos. 1, 10, 22 and 28 of the causal fungus. At the first experiment, the fungi were inoculated on the media on May 29, and the media were divided and placed in each plot on Jun. 5. At the second experiment, the inoculation was done on Jun. 18 and the division was done on Jun. 25. The measurements of the sporodochia and the perithecia were respectively carried out 20 days and 2 months after the division into each plot (Table 6-a, b).

According to Table 6-a, the difference between the formation of sporodochia of the light I plot or the light II plot and that of the dark plot is

Table 6-a Effect of light and darkness on formation of sporodochia

Plot	1st exp.					2nd exp.					Average
	*1	10	22	28	Aver.	1	10	22	28	Aver.	
Light I	9.2	13.2	28.6	44.4	23.9	43.0	13.6	36.8	45.8	34.8	29.3
Light II	6.0	9.4	12.6	39.4	16.9	41.2	12.6	27.4	41.8	30.8	23.8
Dark	5.6	8.2	9.2	21.0	11.0	26.4	10.6	25.6	16.8	19.9	15.5

Notes: 1) * showing no. of fungus. 2) L.S.D. (5%) $\geqq$ 6.4 3) Average values of maximum temperatures of 3 plots are as follows. 1st exp.— light I 25.2°C, light II 23.8°C dark 22.7°C. 2nd exp.—light I 26.3°C, light II 25.2°C, dark 24.6°C.

Table 6-b Effect of light and darkness on formation of perithecial stromata

Polt	1st exp.					2nd exp.					Average
	*1	10	22	28	Aver.	1	10	22	28	Aver	
Light I	74.3	202.0	168.6	190.0	158.7	92.4	74.0	92.8	128.8	97.0	127.9
Light II	70.2	168.0	128.2	178.0	136.1	88.5	56.3	92.1	130.0	91.7	113.9
Dark	58.4	148.0	118.0	152.0	119.1	72.3	22.7	74.1	86.2	63.8	91.5

Notes : 1) * showing no. of fungus. 2) L.S.D. (5%) ≧ 11.0 3) Average values of maximum temperatures of 3 plots are as follows. 1st exp. —light I 27.2° C, light II 26.2°C, dark 25.5°C. 2nd exp—light I 28.6°C, light II 27.0°C, dark 26.3°C.

significant as L.S.D. (5%) is 6.4. The differences of light condition are usually accompanied with the differences of temperature. However, in these experiments, the remarkable differences of temperature among the plots occurred only in several hours of the daytime, and in the other times the temperatures of all plots were almost the same. According to the notes of Table 6-a, the difference between the mean of maximum temperatures of the light II plot and that of the dark plot is only about 1°C. This fact seems to suggest that the difference of the formation of sporodochia between the light plots and the dark plot depends not on the difference of temperature, but on the difference of light condition.

According to Table 6-b, the differences of the formation of perithecia among the 3 plots were significant, and the more the light was, the more the perithecium was formed. As to the temperature of the 3 plots, it is a matter of course that the more the light is, the higher the temperature is, as shown at the notes of Table 6-b. As the optimum temperature for the formation of perithecia is 7-14.5°C~24°C (Chapt II Sect. 2), the comparatively low temperature in summer is considered to be suitable for the formation of the perithecia. From these facts, it is concluded that the light promotes the formation of the perithecia of *G. lateritium*.

HAWKER[3] classified the fungi into 4 groups. The first group is the fungi which form spores in complete darkness and do not show increased spore production in the light. The second group is the fungi which produce spores more freely when illuminated than in darkness. The third group is the fungi which need light to initiate spore production at some stage of thier development. The fourth group is the fungi, the spore formation of which is inhibited by the light. It is clear that *Gibberella lateritium* (NEES) S. et H. belongs to the second group.

2. Effect of ultraviolet radiation on formation of sporodochia and perithecia of the causal fungus

Material and method The fungi, which had been cultured on the mulberry stem media at 25° C for 7 days, were exposed to the ultraviolet radiation from the heliolamp (Shimazu Co. 100V, 60~, 0.25A, λ 2600Å~6000Å, and the peaks lie at 3100Å, 3600Å, 4000Å, 4300Å, and 5400Å). The method

of the exposure is as follows. The mouths of the culture tubes were faced to the lamp tube of the heliolamp at the distance of 15 cm, and the cotton plugs were taken away from the culture tubes. The light was irradiated for 10 seconds and the cotton plugs were restored. This treatment was carried out once a day from one day to 7 days. After that, the mulberry stem media were kept in the room, and the formation degrees of the sporodochia and the perithecia were investigated.

Results 5 mulberry stem media were used for each plot, and the experiment was repeated twice concerning Nos. 1, 10, 22 and 28 of the causal fungus. At the first experiment, the inoculation of the fungi on the media was done on May 12, and the first irradiation was done on May 19. At the second experiment, the inoculation of the fungi was done on May 20, and the first irradiation was carried out on May 27. The sporodochia and the perithecia were measured 20 days or 60 days after the first irradiation respectively (Table 7-a, b and Fig. 2).

Table 7-a, b and Fig. 2 show that the sporodochia and the perithecia were formed most vigorously at the one time irradiated plot, and the more the times of irradiation were, the less the sporodochia and the perithecia were formed. Their formation degree of the not-irradiated plot was between that of the one time irradiated plot and that of the two times irradiated plot.

Table 7-a Effect of ultraviolet radiation on formation of sporodochia

Time of irradiation	1st exp.					2nd exp.					Average
	*1	10	22	28	Aver.	1	10	22	28	Aver.	
0	28.8	19.6	53.2	54.0	38.9	67.0	19.2	39.8	45.3	42.8	40.9
1	30.2	26.0	70.6	82.6	52.4	94.0	32.8	63.6	54.0	61.1	56.8
3	22.0	19.0	46.6	48.2	34.0	48.8	18.6	41.2	34.4	35.8	34.9
5	19.8	14.8	26.4	25.8	21.7	39.6	17.0	39.0	20.0	28.9	25.3
7	19.4	8.6	23.2	18.4	17.4	21.6	8.8	21.2	19.0	17.7	17.6

Notes : 1) * showing no. of fungus. 2) L.S.D. (5%) $\geqq$ 10.4

Table 7-b Effect of ultraviolet radiation on formation of perithecial stromata

Time of irradiation	1st exp.					2nd exp.					Average
	*1	10	22	28	Aver.	1	10	22	28	Aver.	
0	96.3	129.0	102.0	102.4	107.4	80.2	90.0	76.6	82.2	82.3	94.9
1	108.0	133.0	98.4	120.0	114.9	96.0	86.3	94.0	92.1	92.1	103.5
3	86.1	132.2	98.4	97.6	103.6	72.5	74.5	72.7	58.3	69.5	86.6
5	73.2	113.3	82.6	84.3	88.4	54.1	66.2	48.3	48.0	54.2	71.3
7	66.3	88.4	72.3	68.2	73.8	18.3	56.1	20.4	36.2	32.8	53.3

Notes : 1) * showing no. of fungus. 2) L.S.D. (5%) $\geqq$ 9.0

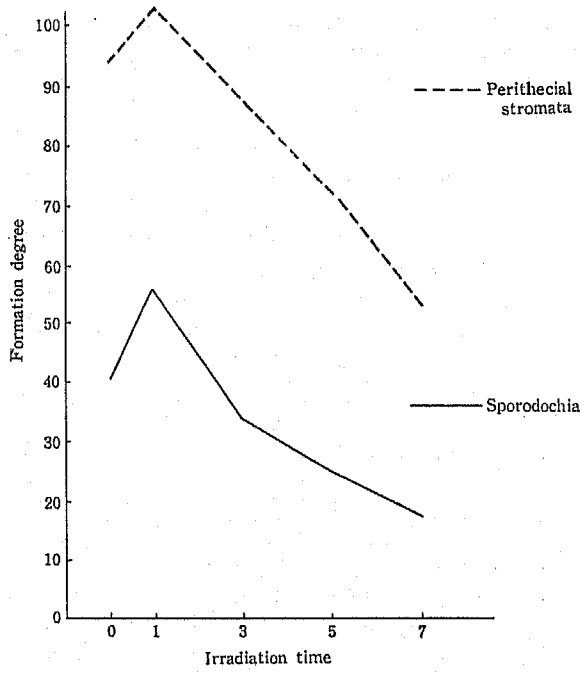

Fig. 2 Effect of ultraviolet radiation on formation of sporodochia and perithecial stromata on mulberry stem medium

According to RAMSEY and BAILEY,[13] the formation of conidia of *Fusarium cepae* and *Macrosporium tomato* was promoted by the adequate irradiation of the ultraviolet ray. SAKAI[14] also reported that the formation of conidia of *Phytopthora infestans* was promoted by its adequate irradiation. The results of the experiment in this section seem to suggest also that the adequate irradiation of the ultraviolet ray promotes the formation of sporodochia and perithecia of *Gibberella lateritium* (NEES) S. et H. STEVENS[16,17] investigated the response of various races of *Glomerella cingulata* to the ultraviolet irradiation. According to him, some races of *Glomerella cingulata* are not influenced by the irradiation. But the other races were influenced, and the formation of their perithecia was promoted or inhibited by the same irradiation. 4 strains of *G. lateritium*, which were investigated above, showed the same response to the ultraviolet radiation.

IV. EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY OF ATMOSPHERE ON GERMINATION OF MACROCONIDIA OF THE CAUSAL FUNGUS

1. Effect of temperature on germination of macroconidia

The macroconidia, used for this experiment, were obtained from the

fungi which had been cultured at 25°C on potato decoction-1% sucrose agar for 10~14 days. The spore suspension was made with 1 % sucrose solution, and several drops of the spore suspension were placed on sterilized slide glasses. Each of these slide glasses was placed in a glassware of 100 % relative humidity and was kept in a thermostat which had a certain temperature. After definite hours the germinability of the macroconidia of each temperature plot was counted. The experiment was repeated 5 times concerning 2 strains (Nos. 1 and 36). Table 8 shows the average values of the resullts of the investigation.

Table 8 Effect of temperature on germinability (%) of macroconidia

	Hours elapsed \ °C	2°	5—6°	10°	16°	20°	22°	24°	26°	28°	30°	32°	36°
No. 1	4	—	—	—	0	0	0.5	1.2	1.3	1.2	0.3	0	0
	6	—	0	0	3.5	34.3	52.3	79.0	80.1	54.0	5.6	5.1	0
	16	0	0	1.5	91.0	95.5	99.1	99.5	99.5	99.4	95.4	65.9	0
No. 36	4	—	—	—	0	0	0.9	1.5	1.8	1.4	0.1	0	0
	6	—	0	0	4.2	33.9	57.3	80.1	82.4	59.4	10.4	4.9	0
	16	0	0	1.3	90.3	97.2	98.5	99.4	99.7	99.1	93.2	54.3	0

Notes : At 5—6°C, 1.2% (No. 1) or 1.3% (No. 36) spores germinated after 24 hours. At 2°C, no spore germinated after 24 hours, but 0.5% spores (Nos. 1 and 36) germinated after 48 hours.

According to Table 8, the optimum temperature for the germination of macroconidia is 24°C~26°C especially 26°C. As to the relation between the lower temperature and the germinability, at 10°C 1.3~1.5% spores germinated after 16 hours, at 5-6°C 1.2~1.3% spores germinated after 24 hours, and at 2°C 0.5% spores germinated after 48 hours.

The relation between the germinability of spores and the temperature is considered to be deeply connected with the relation between the infection of the causal fungus to the mulberry stems and the environmental temperature.

2. Effect of relative humidity of atmosphere on germination of macroconidia

The source of the macroconidia, used for this experiment, is the same as in the experiment of the previous section. The spore suspension was made with the water, in which the bark of mulberry stem was boiled (bark 200g, distilled water 1l). Several drops of the spore suspension were placed on sterilized slide glasses and were dried at the room temperature. These slide glasses were kept at 25°C for 15—16 hours in the atmospheres with definite relative humidities which were made by using sulphuric acid of various concentrations[18], and the germinabilities of the spores were counted. This experiment was repeated 5 times concerning 4 strains (Nos. 1, 4, 18, and 28). The average values of the results are shown in Table 9.

According to Table 9, the macroconidia germinate most vigorously in 100%

Table 9 Effect of relative humidity of atmosphere on germinability of macroconidia

No. of strain	Relative humidity % / Item of investigation	100	97.5	94.8	89.0	85.0	80.0
1	Spores counted	1155	1226	1415	1226	1342	1418
	Germinability (%)	82.4	12.4	0.8	0.3	0.1	0
	Average of germination tubes (μ)	197.1	18.9	9.1	6.6	5.5	0
	Average of longest germination tubes (μ)	449.4	37.7	9.9	7.8	5.7	0
4	Spores counted	1187	1251	1275	1113	1521	1781
	Germinability (%)	81.5	16.3	0.6	0.3	0.2	0
	Average of germination tubes (μ)	281.9	33.2	30.8	13.4	3.6	0
	Average of longest germination tubes (μ)	658.3	88.8	40.2	14.0	5.0	0
18	Spores counted	1421	1318	1278	1621	1331	1951
	Germinability (%)	79.3	13.3	1.4	0.7	0.3	0
	Average of germination tubes (μ)	191.3	45.3	13.4	3.6	9.2	0
	Average of longest germination tubes (μ)	542.4	89.3	19.3	5.0	10.4	0
29	Spores counted	1181	1225	1326	1511	1354	1784
	Germimabibity (%)	61.9	11.6	1.3	3.2	0.5	0
	Average of germination tubes (μ)	380.4	49.2	11.5	3.4	3.9	0
	Average of longest germination tubes (μ)	731.3	91.3	19.3	5.8	4.8	0

relative humidity. In the lower relative humidities (97.5% ~ 85.0%), the germinability falls remarkably and in 80% relative humidity the macroconidia do not germinate.

V. INFLENCE OF LOWER TEMPERATURE AND RELATIVE HUMIDITY OF ATMOSPHERE ON LONGEVITY OF MACROCONIDIA OF THE CAUSAL FUNGUS

Generally it is supposed that the lapse of days causes the decline of the germinability (or the longevity) of spores, and consequently the decline of the pathogenicity of the spores. In this chapter the experimental results of the influence of lower temperature and various relative humidities of atmosphere upon the longevity of the macroconidia will be described.

1. Influence of lower temperature upon longevity of macroconidia

Material and method . The investigation was carried out by the method

of ABE,[1] HEMMI and AKAI[5] and AKAI[2] who investigated the influence of lower temperature upon the longevity of the spores of various fungi. The macroconidia used for this investigation were collected in the field or obtained from the culture. In the 'dry spore' experiment, the diseased mulberry stem having many sporodochia on the surface was cut into 10 cm length, put in the sterilized test tube with the cotton plug, and was kept at $-5° \pm 1°C$ in the thermostat. On the other hand, in the 'frozen spore' experiment, the spore suspension, made with 1 % sucrose solution, was poured in the test tube with the cotton plug (1 ml a test tube) and was kept at $-5° \pm 1°C$ in the thermostat. After the elapse of the definite days the germinabilities of the spores were investigated by the slideglass-drop method (see chapt. IV, Sect. 1).

In addition, the word 'dry spore' was used as the antonym of 'frozen spore' and did not mean the real dry spore, because the relative humidity of atmosphere in the thermostat of lower temperature was almost 100%.

Results and discussion The experiment was repeated twice. The first experiment was begun on May 10, and the second one on May 15. The results are shown in Table 10 and Fig. 3. According to Table 10 and Fig. 3, the frozen spores are plainly weaker than the dry spores, and after 160 days the

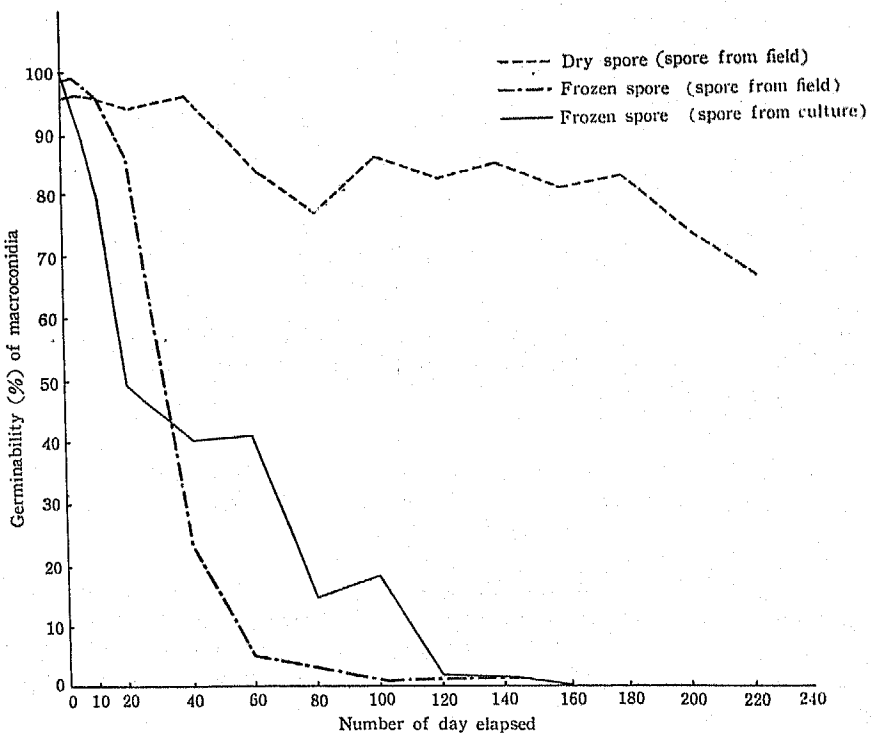

Fig. 3 Influence of lower temperature (−5°C) on longevity of macroconidium

Table 10 Influence of lower temperature (−5°C) on logevity of macroconidia

Days elapsed	Dry spore exp.					Frozen spore exp.									
	Spore from field					Spore from field					Spore from culture				
	1st exp.		2nd exp.		Average Germinability (%)	1st exp.		2nd exp.		Average Germinability (%)	1st exp.		2nd exp.		Average Germinability (%)
	Spores counted	Germinability (%)	Spores counted	Germinability (%)		Spores counted	Germinability (%)	Spores counted	Germinability (%)		Spores counted	Germinability (%)	Spores counted	Germinability (%)	
0	1,481	97.2	1,492	94.9	96.1	1,481	97.2	1,532	98.6	97.9	1,446	98.3	1,521	97.3	97.8
5	1,641	97.3	1,651	96.5	96.9	1,677	98.6	1,672	98.9	98.8	1,686	88.6	1,674	92.4	90.5
10	1,757	96.8	1,759	96.8	96.8	1,641	95.6	1,673	96.6	96.1	1,776	74.2	1,733	81.3	77.8
20	1,732	95.4	1,593	94.4	94.9	1,851	83.2	1,754	89.5	86.4	1,716	45.2	1,719	53.2	49.2
40	1,592	96.6	1,734	95.9	96.3	1,744	22.8	1,749	23.2	23.0	1,716	40.5	1,719	41.3	40.9
60	1,874	82.3	1,875	87.3	84.8	1,516	4.6	1,741	5.6	5.1	1,662	39.6	1,691	42.3	41.0
80	1,744	65.5	1,749	87.3	76.4	1,921	1.5	1,981	5.1	3.3	1,762	13.8	1,633	14.5	14.2
100	1,691	91.3	1,692	80.2	85.8	1,934	0.5	1,983	0.3	0.4	1,941	24.8	1,921	11.2	18.0
120	1,741	95.3	1,829	69.3	82.5	1,325	0	1,211	1.0	0.5	1,951	1.5	1,762	0.7	1.1
140	1,593	89.4	1,845	80.2	84.8	1,411	0	1,421	2.0	1.0	1,924	0	1,725	0.2	0.1
160	1,824	86.4	1,921	74.1	80.3	1,425	0	1,424	0	0	1,231	0	1,531	0	0
180	1,914	80.3	2,110	84.1	82.2	1,935	0	1,681	0	0	1,921	0	1,432	0	0
200	1,843	64.5	1,921	82.1	73.3										
220	1,921	67.1	1,835	66.3	66.7										

Note : The thermostat was out of order on the 77th day of 1st exp. (or the 72th day of 2nd exp.) and the temperature rose to 5°C. But it was restored after 20 hours.

former's germinability was completely lost though the latter reserved the germinability of more than 80%. In comparison the spores collected from the field were proved in this experiment to be stronger at the beginning but weaker than the spores from the culture after 40 days. However, both spores lost all the germinability after 140~160 days. The duration of the spores is considered to be much influenced by the environmental factors, under which they are formed, as HEMMI and AKAI,[5] and AKAI[2] have pointed out.

From the fact that the frozen macroconidia as well as the dry macroconidia which were kept at-5°C survive more than 4 months, it is clear that the macroconidia of *Gibberella lateritium* can pass the winter even in the provinces of severe cold.

On the other hand, when the pieces of the diseased mulberry stem which were put in the test tubes with the cotton plug, were kept for long days in the room, the spores on them showed the germinabilities of 80 %, 47.5 %, and 39.2% respectively after 60 days, 80 days and 100 days. In the natural condition the influences of environmental factors, especially drying as well as the temperature, are not so simple as in this experiment. The influence of drying or the relative humidity of the atmosphere on the longevity of the macroconidia will be dealt with in the next section.

2. Influence of relative humidity of atmosphere on longevity of macroconidia

Material and method The bark of the diseased mulberry stems or the mulberry stem media was separated from the wood and dried in the room for a week. The pieces of the bark were put in the glasswares having the atmospheres of definite relative humidities which were made by using sulphuric acid of various concentrations,[18] and were kept at 25°C. After the elapse of the definite days, the germinabilities of the macroconidia on the sporodochia of the bark were investigated. Once in ten days the air in the glasswares was ventilated and the concentrations of the sulphuric acid were confirmed.

Table 11 Influence of relative humidity of atmosphere on longevity of macroconidia (at 25°C)

Relative humidity % / Days elapsed	Spore from field					Spore from culture				
	100	94.8	85.0	74.6	49.5	100	94.8	85.0	74.6	49.0
0	96.2	96.2	96.2	96.2	96.2	98.0	98.0	98.0	98.0	98.0
5	95.4	92.0	88.1	86.5	86.6	97.5	97.3	94.3	92.5	91.0
10	91.2	86.9	87.1	79.5	76.0	94.1	93.6	91.3	89.8	71.1
20	84.3	82.7	72.2	70.8	51.2	85.2	80.7	69.0	61.2	35.5
40	76.0	75.6	65.3	55.0	33.5	82.3	73.8	65.2	56.0	34.9
60	74.5	63.3	45.2	33.1	18.5	75.3	64.4	44.9	32.2	20.3
80	71.3	53.4	36.4	20.3	12.1	72.4	54.4	36.2	21.3	11.3
100	68.3	49.5	21.8	5.1	3.1	59.4	47.3	19.3	4.0	4.2

Note : The germinability (%) of more than 2000 spores is shown.

Results and discussion The experiment was repeated twice respectively concerning the sporodochia on the diseased mulberry stem from the field and those from the mulberry stem media. The average values of the experimental results are shown in Table 11.

According to Table 11, the spores from the field and those obtained from the culture are not different concerning the response to the relative humidity of atmosphere. The response appears already 5 days after the treatment, and 100 days after the treatment the germinability of the macroconidia falls to 3~4% in 49% relative humidity though in 100 % relative humidity it shows more than 59 %. The average values of the experimental results of both spores are shown in Fig. 4.

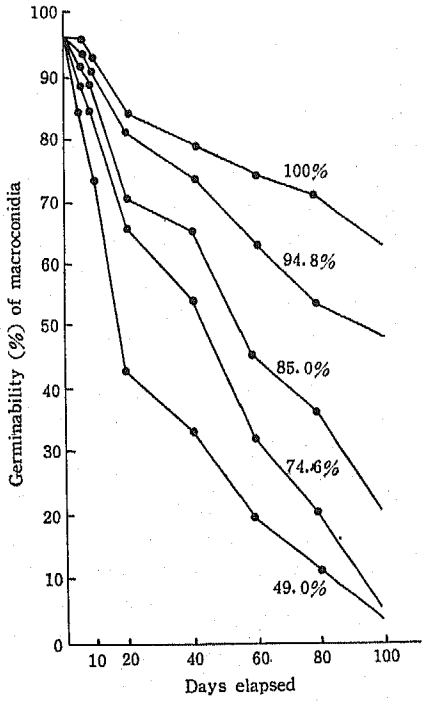

Fig. 4 Influence of relative humidity of atmosphere on longevity of macroconidia (at 25°C)

From the above results, it is concluded that the longevity of the macroconidia is influenced remarkably by the relative humidity of atmosphere and is shortened by the lower relative humidity. Looking over the data from the various provinces in Japan, we can sometimes find the cases that the average value of relative humidity of atmosphere during a day is less than 40%. But the average value of the relative humidity for a month falls hardly less than 60%. At any rate, the influence of the ever-changing relative humidity of atmosphere in the natural condition upon the longevity of the macroconidia must be investigated newly in future.

VI. GROWTH FACTORS, CARBON SOURCES AND NITROGEN SOURCES OF THE CAUSAL FUNGUS

1. Growth factors of the causal fungus

The basic culture solution used for this experiment is the glucose-asparagine solution (glucose 10g, asparagine 2g, KH_2PO_4 1g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeCl_3 \cdot 6H_2O$ 0.001g, $ZnSO_4 \cdot 7H_2O$ 0.001g $MnSO_4 \cdot 4H_4O$ 0.001g, and distilled water 1000 ml). The pH value of the solution was regulated to 5.5. Active carbon was added to the solution (5g per 1000ml), and boiled for 30 minutes to remove the surplus growth factors which are contained in the solution. And the solution was filtrated.

For the purpose of determining the growth factors necessary for the causal fungus, 5 plots (in the first plot no growth factor was added, in the second plot Thiamine was added, in the third Biotin was added, in the fourth Thiamine and Biotin were added, and in the fifth Thiamine, Biotin, Inositol and pyridoxine were added) were set up following LILLY and BARNETT.[6] The amounts of growth factors added to each 1000 ml of the glucose-asparagine solution are as follows:——Thiamine (hydrochloride, Wakojunyaku Co.) 5mg, Biotin (Mann Research Lab. Inc., N.Y.) 5μg, Inositol (Wakojunyaku Co.) 5mg, Pyridoxine (hydrochloride, Merk Co.) 100μg. Nos. 1 and 8 of *Gibberella lateritium* were used for this investigation.

10 ml of each of these solutions was poured in a culture tube (diameter 2.5cm) with the cotton plug and was sterilized in an autoclave at 2kg/cm^2. 5 culture tubes were used for each plot each time. The fungus was inoculated with the spore suspension 1 öse to 1 culture tube) or a bit of the hyphae. The culturing was carried out at 25°C for 10~15 days, and the dry matter of the fungus was measured. The results are shown in Table 12-a.

Table 12-a Effect of growth factors upon growth (mg) of causal fungus

No. of strain	Plot	No. of exp.					
		1	2	3	4	5	Average
1	No growth factor	116	88	51	29	79	72.6
	+ T	125	55	33	29	22	52.8
	+ B	157	87	66	38	65	82.6
	+ TB	114	78	33	34	60	63.8
	+ TBIP	122	67	36	41	63	65.8
8	No growth factor	198	120	43	121	103	117.0
	+ T	140	87	31	66	63	77.4
	+ B	176	102	55	154	106	118.6
	+ TB	139	94	40	85	60	83.6
	+ TBIP	122	96	26	84	64	78.4

Notes : T—Thiamine, B—Biotin, I—Inositol, P—Pyridoxine. L. S. D. (5%) of No. 1 $\geqq$ 16.2, L.S.D. (5%) of No. 8 $\geqq$ 26.6

Table 12-b Effect of growth factors on growth of No. 64—1

Plot	1	2	3	Average
No growth factor	0	(3)	0	(1)
+ T	47	48	51	48.7
+ B	(2)	(2)	(1)	(1.7)
+ TB	56	43	48	49.0
+ TBIP	75	72	55	67.3

Note : small amount of growth in ( ) is regarded to have been caused by trace of thiamine which was contained in inoculum hyphae.

According to Table 12–a, nos. 1 and 8 of *G. lateritium* do not require any growth factors, that is, they are self–sufficient. Comparing the plots having thiamine (T, TB, or TBIP) with those having no thiamine (no growth factor, or B), the growth in the former is inferior to that in the latter. This result seems to show that the addition of thiamine controls the growth of nos. 1 and 8 of *G. lateitium* a little.

The relation between the concentration of thiamine and the growth of No. 1 of *G. lateritium* is shown in Fig. 5. This is the average value of the experimental results which were repeated three times.

As shown in Fig. 5, the function of thiamine controlling the growth of *G. lateritium* appears at the concentrations from 5 μg/1000 ml to 200 μg /1000 ml almost equally.

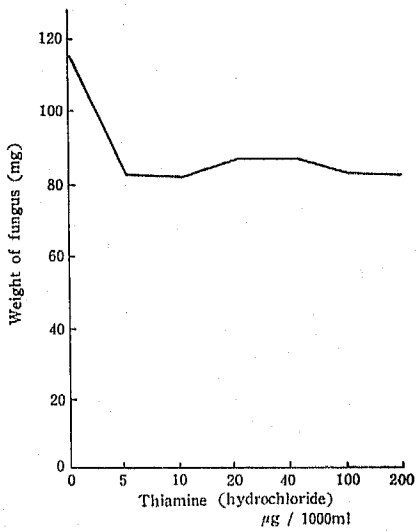

Fig. 5 Effect of concentration of thiamine on growth of causal fungus

As mentioned above, nos. 1 and 8 of *G. lateritium* are self–sufficient and need no addition of any growth factor for their growth. In addition the other 38 strains of *G. lateritium* were tested. The results of the test proved that all strains except 1 saltant (no. 64–1) are self–sufficient. The experimental results of no. 64–1 are shown in Table 12–b. No. 64–1 needs the addition of thiamine for its growth, that is, No. 64–1 is the thiamine–defficient.

2. **Carbon sources and nitrogen sources of the causal fungus**

(1) Carbon sources of the causal fungus

Material and method The component of the basic culture solution used for this experiment is the same as the glucose–asparagine solution of the previous section excepting that KNO_3 (3g) was used instead of asparagine (2g). But various carbon compounds equivalent to the glucose 10g were respectively added to the solutions in place of glucose. Growth factors were not added. 30 ml of the solutions was poured in each 200 ml Erlenmeyer flask with the cotton plug and was sterilized in an autoclave at 2kg/cm^2. 4 Erlenmyer flasks were used for each plot each time. All the pH values of the solutions were regulated to 6.3~6.8 with NaOH or HCl before the sterilization.

The inoculation of the fungus (no. 1 of *G. lateritium*) was done with the spore suspension (1 öse to 1 flask). The culturing was carried out at 25°C for 10 days, and the dry matter of the fungus, the formation degree of the spore and the pH value of the solution were measured.

Results 19 kinds of carbon compounds, that is, xylose, arabinose (pentoses), glucose, fructose galactose, sorbose (hexoses), sucrose, maltose,

Table 13 Effect of various carbon sources on growth of causal fungus

Carbon source	Weight of fungus (mg)	Formation of spore	pH	
			Before culturing	After culturing
Xylose	189	++	6.8	6.9
Arabinose	230	+++	6.6	7.0
Glucose	229	++	6.7	6.9
Fructose	235	+++	6.6	7.5
Galactose	239	++	6.5	7.0
Sorbose	188	+	6.6	6.9
Sucrose	292	+++	6.8	7.5
Maltose	385	+++	6.7	8.4
Lactose	199	++	6.8	8.0
Soluble Starch	276	+++	6.6	8.4
Inulin	slight	−	6.5	6.8
Glycerine	468	+++	6.8	8.5
Mannit	435	+++	6.7	8.6
Formic acid	14	−	6.4	8.6
Acetic acid	74	−	6.5	7.7
Oxalic acid	slight	−	6.6	6.8
Succinic acid	173	+	6.5	9.3
Malic acid	188	−	6.3	9.3
Citric acid	50	−	6.3	8.5

Note : The average values of experimental results of 3 times are shown.

lactose (disaccharides), soluble starch, inulin (polysaccharides), glycerine, mannit (higher alcohols), formic acid, acetic acid, oxalic acid, succinic acid, malic acid, citric acid (organic acids) were tested. The results are shown in Table 13.

According to Table 13, glycerine and mannit (higher alcohols) were the best carbon sources. And maltose and sucrose (disaccharides) and soluble starch (polysaccharide) were good sources. But inulin, though it is another polysaccharide, was very bad carbon source. Galactose, fructose, arabinose, glucose, xylose and sorbose (monosaccharides) were fairly good sources. And organic acids were bad for the growth.

The formation degree of the spores was generally in proportion to the growth degree except a few substances. Generally speaking, the pH values of the solution rose after the culturing of the causal fungus. This phenomena is considered to be caused by the unequivalent loss of NO_3-ion of KNO_3 in the culture solutions.

In conclusion, the order of good carbon sources is as follows:——glycerine, mannit, maltose, sucrose, soluble starch, galactose, fructose, arabinose, glucose, lactose, xylose, sorbose, malic acid, succinic acid, acetic acid, citric

acid, formic acid, inulin, oxalic acid.

(2) Nitrogen sources of the causal fungus

Material and method The basic culture solution of this experiment is the glucose-asparagine solution. Various nitrogen sources equivalent to asparagine 2 g were added respectively in place of the asparagine. Growth factors were not added. 7 ml of each of these solutions was poured in a culture tube (the diameter of the mouth is 2.5 cm) with the cotton plug and was sterilized in an autoclave at 2kg/cm^2. 5 culture tubes were used for each plot each time. All pH values of the solutions were regulated to 6.3—6.8 with NaOH or HCl before the sterilization. The inoculation of the fungus (no. 1 of *G. lateritium*) was done with the spore suspension (1 öse to 1 flask). The culturing

Table 14 Effect of various nitrogen sources on growth of causal fungus

Nitrogen source	Weight of fungus(mg)	Formation of spore	pH	
			Before culturing	After culturing
KNO_3	78	++	6.4	6.3
$NaNO_3$	73	++	6.7	6.4
$Ca(NO_3)_2 \cdot 4H_2O$	97	++	6.3	6.1
$(NH_4)_2SO_4$	8	−	6.6	5.4
$(NH_4)H_2PO_4$	10	−	6.6	5.4
$(NH_4)_2C_2O_4 \cdot H_2O$	53	+	6.8	6.1
$(COONH_4 \cdot CHOH)_2$	22	−	6.5	5.1
NH_4NO_3	11	−	6.7	5.5
$NaNO_2$	0	−	6.3	6.3
Glycocoll	82	++	6.7	6.3
l-Alanine	47	+	6.7	5.6
dl-Serine	30	(+)	6.4	5.0
dl-Valine	33	(+)	6.8	6.0
l-Leucine	51	(+)	6.8	5.9
dl-Aspartic acid	101	+++	6.7	7.0
l-Glutamic acid	102	+++	6.5	6.4
dl-Phenylalanin	17	(+)	6.5	5.9
l-Tyrosine	25	(+)	6.5	5.7
l-Histidine monohydrochloride	72	++	6.4	6.2
l-Proline	46	+	6.6	5.5
l-Tryptophane	50	(+)	6.6	5.7
dl-Lysine monohydrochloride	13	(+)	6.6	5.7
Creatine	slight	−	6.6	6.5
Taurine	slight	−	6.7	6.5
Urea	55	(+)	6.5	6.4
Asparagine	66	++	6.6	6.1

Note : The average values of experimental results of 3 times are shown.

was carried out at 25°C for 15 days, and the dry matter of the fungus, the formation degree of the spore and the pH value of the solution were measured.

Results 26 kinds of nitrogen compounds, that is, KNO_3, $NaNO_3$ and $Ca(NO_3)_2 \cdot 4H_2O$ as nitrates, $(NH_4)_2SO_4$, $(NH_4)H_2PO_4$, $(NH_4)_2C_2H_4 \cdot H_2O$ and $(COONH_4 \cdot CHOH)_2$ as ammonium compounds, $NaNO_2$ as a nitrite, and glycocoll, l-alanine, dl-serine, dl-valine, l-leucine, dl-aspartic acid, l-glutamic acid, dl-phenylalanine, l-tyrosine, l-histidine monohyarochloride, l-proline, l-tryptophane, dl-lysine monohydrochloride, creatine, taurine, urea, asparagine as organic compounds were tested. Among these, 11 amino acids from glycocoll to l-proline have been found in the bark of the mulberry stem by the writer[9] The results are shown in Table 14.

Table 14 shows that the nitrates are good nitrogen sources despite the kind of cation. The pH values of the solutions containing ammonium compounds or NH_4NO_3 dropped by culturing the fungus, and the growth of the fungus in the solutions was inferior to that in nitrates. $NaNO_2$ was the bad nitrogen source and the fungus could not grow in the solutions containing $NaNO_2$. Among organic nitrogen sources, l-glutamic acid, l-aspartic acid and glycocoll were the best and nearly of the same value as the nitrates or superior to them. L-histidine monohydrochloride, asparagine and urea were the good nitrogen sources, but inferior to nitrates. The order of good nitrogen sources is as follows:—— l-glutamic acid, l-aspartic acid, $Ca(No_3)_2 \cdot 4H_2O$, glycocoll, KNO_3, $NaNO_3$, l-histidine monohydrochloride, asparagine, urea, $(NH_4)_2C_2O_4 \cdot H_2O$, l-leucine, l-tryptophane, l-alanine, l-proline, dl-valine, dl-serine, l-tyrosine, $(COONH_4 \cdot CHOH)_2$, dl-phenylalanine, dl-lysine monohydrochloride, NH_4NO_3, $(NH_4)H_2PO_4$, $(NH_4)_2SO_4$, creatine, taurine. The formation degree of the spores was generally in proportion to the growth degree, but a few exception was found as shown in Table 14.

SUMMARY

This paper deals with the physiological characters of *Gibberella lateritium* (NEES) SNYDER et HANSEN which is the main causal fungus of the "bud blight" of mulberry trees.

(1) The cardinal points of temperature for the vegetative growth of this fungus are —— minimum, slight lower than 4°C; optimum, 24°C~26°C; and maximum, about 36°C. The pH range of the growth of the fungus on the glucose-asparagine culture solution is from about 3 (No. 8) or 3.5 (Nos. 1 and 28) to 9 or a little higher. The optimum pH value is different for the strains of the causal fungus, that is, 6.5—7 for No. 1, 7.5 for No. 28, and about 4.5 for No. 8.

(2) The sporodochia are formed on the mulberry stem media between 4° C and about 32°C, most vigorously between 24° C and 30°C. But, the conidia on hyphae or pionnotes are formed on the potato decoction—1.5% sucrose agar between 4° C or a little lower and 32°C~36°C, most vigorously 20°C~28°C. The formation of the perithecium occurs on the mulberry stem media from 4°C to about 30°C and its optimum temperature ranges from 7—14.5°C

to 24°C.

(3) The formation of sporodochia and perithecia on the moistened mulberry stem is easier than that on the dry mulberry stem. The new macroconidia are observed on the sporodochia in 94.8% or higher relative humidities after a day, and on those in 85% after 3 days.

(4) The light promotes the formation of sporodochia and perithecia of this fungus on the mulberry stem medium, though they are also formed in the darkness. The adequate irradiation of the ultraviolet ray from the heliolamp promotes the formation of sporodochia and perithecia of this fungus.

(5) The optimum temperature for the germination of macroconidia is 24°C~26°C. As to the relation between the lower temperature and the germinability, 1.3~1.5% spores germinated at 10°C after 16 hours, 1.2~1.3% spores germinated at 5—6°C after 24 hours, and 0.5% spores germinated at 2°C after 48 hours. The macroconidia germinate most vigorously in 100% relative humidity. In the lower relative humidities (97.5%~85.0%), the germinability falls remarkably and in 80% relative humidity the macroconidia do not germinate.

(6) The frozen spores which were kept at—5°C in the thermostat decreased their germinability day after day and lost all the germinability after 160 days. But the dry spores which were kept in the same thermostat showed the germinability of 80% after 160 days. The longevity of the macroconidia is influenced remarkably by the relative humidity of atmosphere and is shortened by the lower relative humidity, that is, 100 days after the treatment the germinability of the macroconidia falls to 3~4% in 49% relative humidity, though in 100% relative humidity it shows more than 59%.

(7) Generally, this fungus needs no addition of any growth factor for its growth, and the addition of thiamine controls its growth a little. But one saltant which is thiamine defficient is discovered.

The order of good carbon sources for the growth of this fungus is as follows:—— glycerine, mannit, maltose, sucrose, soluble starch, galactose, fructose, arabinose, glucose, lactose, xylose, sorbose, malic acid, succinic acid, acetic acid, citric acid, formic acid, inulin, oxalic acid. And the order of good nitrogen sources for it is as follows :—— l-glutamic acid, l-aspartic acid, $Ca(NO_3)_2 \cdot 4H_2O$, glycocoll, KNO_3, $NaNO_3$, l-histidine monohydrochloride, asparagine, urea, $(NH_4)_2C_2O_4 \cdot H_2O$, l-leucine, l-tryptophane, l-alanine, l-proline, dl-valine, dl-serine, l-tyrosine, $(COONH_4\ CHOH)_2$, dl-phenylalanine, dl-lysine monohydrochloride, NH_4NO_3, $(NH_4)H_2PO_4$, $(NH_4)_2SO_4$. Creatine and taurine are very bad nitrogen sources, and $NaNO_2$ was not utilized.

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