PATHOLOGICAL STUDIES OF THE "BUD BLIGHT" OF MULBERRY TREES

II. ON THE INFLUENCE OF MANURE UPON THE DEVELOPMENT OF THE DISEASE AND THE FORMATION OF SPORODOCHIA AND PERITHECIA OF THE CAUSAL FUNGUS*

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With 1 Plate

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Introduction

Many investigations have been published concerning the influence of manure upon the occurrence and development of plant diseases. But it is to be regretted that those investigations have dealt mainly with the diseases of harbacious plants and not with the stem diseases of woody plants. It was known by the writer(30) that the mode of the occurrence and development of the "bud blight" of mulberry trees, one of the stem diseases, is very different from that of the diseases of harbacious plants.

In this paper the results of the writer's experiments and some pathological discussions concerning the influence of manure upon the development of the "bud blight" of mulberry trees and the formation of sporodochia and perithecia of the causal fungus are reported. It is a matter of course that these experiments were planned on the basis of the mode of the occurrence and development of this disease. Inoculations were carried out to make clear the influence of manure upon the development of the disease in the not-growing stage as well as in the growing stage of mulberry trees. In the growing stage the wound cork layers are formed in mulberry stems, and the invasion of the causal fungus is generally repressed by the wound cork layers. On the other hand, in the not-growing stage (autumn, winter and early spring) the wound cork layers are not formed in mulberry stems and the invasion of the causal fungus is easy to occur. When the experiments to make clear the influence of manure upon the formation of sporodochia and perithecia on mulberry stems were carried out, the writer also paid particular attention to the growth stage at which the supplied mulberry stems were cut.

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I. THE INFLUENCE OF MANURE UPON THE DEVELOPMENT OF THE DISEASE

1. Materials and Methods

(1) The Culture of Mulberry Trees

N-experiment (A)-experiment. Preparing; Round pots (75 cm diamater, 120 cm depth and bottomless) were arranged regularly* in a farm of the writer's Faculty and lower parts (about 110 cm) of them were buried in the soil.** Uniform young mulberry trees were planted in the pots, one tree in one pot, in the spring of 1935. These mulberry trees were cultured uniformly as far as possible in the pots by the method of the "Negari" training and summer harvest (all stems of the mulberry trees were cut at the same time near the level of the soil during the last 10 days of June or the first 10 days of July every year). The variety-name of the mulberry trees is Jūmonji. Setting up of the experimental plots; Since the spring of 1945 the following experimental plots were set up. Each plot was composed of 4-5 pots situated at random.

Quantity of manure given per 1 pot

Kind of plot	Ammonium sulphate (g)	Calcium super-phosphate (g)	Potassium sulphate (g)	Calcium carbonate (g)
N-little	50	100	50	100
N-much	200	100	50	100
N-very much	400	100	50	100

These kinds of manure were given in April—May every year. The method of culture except manuring was the same, and the stems which grew every year from the buds near the roots after the harvest (which was done during the last 10 days of June or the first 10 days of July) were used for the inoculation experiments.

(B)-experiment. Preparing; Round pots (95 cm diameter, 120 cm depth and bottomless) were set near the pots of (A)-experiment after the same manner with that of (A)-experiment. Also in this (B)-experiment uniform young mulberry trees were used and each of them was planted in the pot in the spring of 1950. These mulberry trees were also cultured uniformly as far as possible by the method of the "Negari" training and summer harvest. The variety-name of the mulberry trees is Kairyonezumigaeshi. Setting up

* The distance between the centers of the neighbouring pots was kept 150 cm (see Plate Fig. 3).

** The soil of the farm is clayey loam of Younger Terrace.

of the experimental plots; In the spring of 1951 the following experimental plots were set up. Each plot was composed of 4 pots situated at random.

Quantity of manure given per 1 pot

Kind of plot	Ammonium sulphate		Calcium superphosphate (g)	Potassium sulphate (g)
	Quantity (g)	Date given		
N-Apr. 19, little	25	19/IV	50	25
N-Apr. 19, much	100	19/IV	50	25
N-Jul. 10, little	25	10/VII	50	25
N-Jul. 10, much	100	10/VII	50	25
N-Aug. 7, little	25	7/VIII	50	25
N-Aug. 7, much	100	7/VIII	50	25

Note; Calcium superphosphate and potassium sulphate were given on April 19.

The supplied mulberry trees were cut twice in 1951. The first cutting was done on Mar. 30. Every mulberry tree was cut at the part near the root. The second cutting was done on June 20. All the mulberry stems which grew up after the 1st cutting were cut at the part of 20 cm height. The side buds of remaining parts of the stems grew up again some days after the 2nd cutting. These newly grown mulberry stems were used for the experiments.

K_2O-experiment Preparing; Round pots were set after the same manner with that of N-experiment. In this K_2O-experiment also uniform young mulberry trees were used and each of them was planted in the pot in the spring of 1934 (A-exp.) and 1944 (B-exp.). The mulberry trees were also cultured uniformly as far as possible in the pots by the method of the "Negari" training and summer harvest. The variety-name of the mulberry trees is Jūmonji. Setting up of the experimental plots; Since the spring of 1945 the following experimental plots were set up. Each plot was composed of 5-6 pots situated at random.

Quantity of manure given per 1 pot

Kind of plot		Ammonium sulphate (g)	Calcium superphosphate (g)	Potassium sulphate (g)	Calcium carbonate (g)
A-exp.	K_2O-given	200	100	50	100
	K_2O-not given	200	100	0	100
B-exp.	K_2O-given	100	0	50	100
	K_2O-not given	100	0	0	100

These kinds of manure were given in April—May every year. The method of training and harvest was the same. The stems which grew every year from the buds near the roots after the harvest were used for the inoculation experiments.

P_2O_5-experiment Preparing ; Round pots were set after the same manner with N-experiment. In this P_2O_5-experiment also uniform young mulberry trees were planted in the pots, one tree in one pot, in the spring of 1934 (A-exp.) and 1944 (B-exp.). These mulberry trees were also cultured uniformly as far as possible by the method of the "Negari" training and summer harvest. The variety name of the mulberry trees is Jūmonji. Setting up of the experimental plots; Since the spring of 1945 the following experimental plots were set up. Each plot was composed of 6 pots situated at random.

Quantity of manure given per 1 pot

Kind of plot		Ammonium sulphate (g)	Calcium superphosphate (g)	Potassium sulphate (g)	Calcium carbonate (g)
A-exp.	P_2O_5-given	200	100	50	100
	P_2O_5-not given	200	0	50	100
B-exp.	P_2O_5-given	100	100	0	100
	P_2O_5-not given	100	0	0	100

These kinds of manure were given in April—May every year. The method of training and harvest was the same. The stems which grew from the buds near the roots after the harvest during the last 10 days of June or the first 10 days of July were used for the inoculation experiment every year.

(2) The inoculation of the causal fungus to the mulberry trees

As the writer stated in the introduction of this paper, the inoculation was carried out to make clear the influence of manure upon the development of the disease in the not-growing stage as well as in the growing stage of the mulberry trees. The 10 stems (2–3 stems per one pot) were used for the inoculation in each plot. The 3 parts of each stem supplied which grew up after the summer harvest were sterilized with 70–80% alcohol and washed with sterilized water, and a peeling injury (2.5 mm×2.0 mm) was made with a knife on the bark of each part of the stem. The injured part was inoculated with the conidia of *Gibberella lateritium* (NEES) S. et H. And then the bark was restored to its original position as if it had not been injured and was painted with vaseline. The results of the inoculation were observed one or several months after.

2. Results and Discussions

N-experiment (A)-experiment. The inoculation was carried out in October—December and in March—May of the next year from 1949 to 1952. The growth of the mulberry trees is given in the table 1.

Table 1. Showing the growth of the mulberry trees in N-experiment

		Number of stem (of more than 1 m height) per 1 tree			Height (cm)		
		Dec. 1949	Dec. 1950	Dec. 1951	Dec. 1949	Dec. 1950	Dec. 1951
Kind of plot	N-little	18.9	19.3	22.7	107.0	142.8	132.5
	N-much	37.2	37.7	35.0	119.8	152.1	146.2
	N-very much	38.2	37.3	40.7	135.6	166.2	156.3

Note; 'Height' was obtained from the average value of the highest 10 stems of every pot.

It is evident from the table 1 that the more $(NH_4)_2SO_4$ was given in the soil, the more the growth of the mulberry trees was promoted. This result corresponds with the results of the similar experiments concerning the influence of N-manure on the growth of mulberry trees which have been done by many investigators.

The data of the results of the inoculation experiments were divided into two groups (Table 2 and Table 3) according to the growth stages (that is to say, the growing stage and the not-growing stage) of the mulberry trees while the invasion of the causal fungus occurred, and the variances of the invaded area were analysed.

Table 2 indicates that the mulberry trees of N-little plot are less invaded than those of the other plots (N-much and N-very much plots), that is, N-manure has its influence on the development of the disease in the growing stage of the mulberry trees. On the other hand Table 3 shows that N-manure does not influence the development of the disease in the not-growing stage of the mulberry trees.

Many investigators(6, 11, 12, 15, 32, 36, 41, 42, 47, etc.) have studied the influence of N-manure on the occurrence and the development of the various diseases of plants. And it has been reported almost without exception that any plant of a N-much plot is more susceptible to the diseases than that of a N-little plot. The result of the writer's experiment, which was shown in Table 2, indicates nearly such a normal tendency. However, Table 3 shows that N-manure does not influence the development of the disease in the not-grow-

Table 2. The influence of N-manure upon the development of the disease in the growing stage of the mulberry trees

No. of exp.	Date	Index of invaded area		
	Inoculation—Measurement	N-little	N-much	N-very much
1	20/III '50—30/V '50	20.0	32.6	26.4
2	22/IV '50—30/VI '50	31.7	41.2	34.0
3	20/III '51—31/V '51	23.6	33.3	31.9
4	26/III '51— 6/VI '51	61.1	85.3	95.5
5	22/III '52—29/V '52	38.1	44.2	40.5
6	23/IV '52— 6/VI '52	57.0	59.6	61.9
Mean		38.6	49.4	48.4

Note; 'Index of invaded area' is obtained from the product of average length (mm) and average width (mm) of the real invaded areas.

Table of analysis of variance

	SS	DF	V	F
N	425.95	2	212.98	5.16*
Replicate	6 506.08	5	1 301.22	31.52**
Error	412.80	10	41.28	
Total	7 344.83	17		

D≧8.3 Difference between any two of means is significant at 5% level.

Table 3. The influence of N-manure upon the development of the disease in the not-growing stage of the mulberry trees

No. of exp.	Date	Index of invaded area		
	Inoculation—Measurement	N-little	N-much	N-very much
1	11/X '49—25/IV '50	85.3	59.6	58.3
2	24/XI '50—27/IV '51	117.7	119.9	109.5
3	12/XII '50—27/IV '51	98.5	97.3	91.5
4	23/XI '51—13/V '52	221.0	260.8	414.7
5	3/XII '51—13/V '52	191.7	157.1	141.4
Mean		142.8	138.9	163.1

Table of analysis of variance

	SS	DF	V	F
N	1 679.35	2	839.68	1.01
Replicate	99 605 38	4	24 901.35	30.04**
Error	6 632.12	8	829.02	
Total	107 916.85	14		

ing stage of the mulberry trees. This fact is very important especially from the practical point of view, because this disease is far more destructive in the not-growing stage than in the growing stage of mulberry trees as the writer stated in the previous paper.[30]

The writer must discuss here the manuring date (in this experiment manuring was done in April every year), because the question arises how long the effect of manure continues every year. USHIWODA[57,58] stated that the influence of manure on the growth of mulberry trees, which are a perenial plant, is very different from that on the growth of herbacious plants. According to him the growth of mulberry trees in spring depends not on the manure which was given in the early spring, but mainly on nutriment contained in the root of mulberry trees which had been stored in the previous year, and the manure which was given in that spring has an influence on the growth of mulberry trees in summer and autumn. At any rate, the writer investigated additionally in the following (B)-experiment the relation between the date of manuring and the influence of N-manure on the development of the disease in the not-growing stage of mulberry trees.

(B)-experiment. Inoculations were carried out on Nov. 23 and Dec. 3 of 1951, and the invaded areas were measured on May 13 of the next year. The growth of mulberry trees and the results of the inoculation experiments are shown in Table 4.

Table 4. Showing the relation between the date of manuring and the influence of N-manure upon the growth of the mulberry trees as well as the development of the disease in the not-growing stage of the mulberry trees

Date of manuring	Growth of mulberry tree				Index of invaded area			
	Number of stem (of more than 1 m height) per 1 tree		Height (cm)		23/XI '51—13/V '52		3/XII '51—13/V '52	
	N-little	N-much	N-little	N-much	N-little	N-much	N-little	N-much
Apr. 19	9.0	13.0	133.0	145.7	456.6	438.3	147.8	165.1
Jul. 10	9.0	10.3	134.1	134.3	367.7	635.9	223.5	191.8
Aug. 7	8.3	9.7	118.7	120.1	215.7	373.4	144.8	230.7

Note; 'Growth of mulberry tree' was measured in December '51.

Table 4 seems to suggest that the earlier $(NH_4)_2SO_4$ is given, the more vigorous the growth of mulberry trees is, and also that the development of the disease in the not-growing stage is not influenced by the date of manuring.

From the results of (A)-and (B)-experiments, it is concluded that N-manure has not its influence on the development of the "bud blight" of mulberry trees in the not-growing stage though the same manure influences a little the development of the disease in the growing stage of mulberry trees.

K_2O-experiment The inoculation was carried out in October—December and in March—May of the next year from 1949 to 1951. The growth of the supplied mulberry trees is given in the table 5.

Table 5. Showing the growth of the mulberry trees in K_2O-experiment

			Number of stem (of more than 1 m height) per 1 tree		Height (cm)	
			Dec. 1949	Dec. 1950	Dec. 1949	Dec. 1950
Kind of plot	A-exp.	K_2O-given	26.1	25.3	141.5	148.5
		K_2O-not given	24.5	32.3	142.1	149.7
	B-exp.	K_2O-given	18.2	17.3	124.6	134.6
		K_2O-not given	17.9	16.7	119.2	129.3

Note; 'Height' was obtained from the average value of the highest 10 stems of every pot.

The data in the table 5 suggest that the effect of K_2O on the growth of the mulberry trees is not remarkable. Many investigators[2, 4, 17, 18, 19, 20, 21, 46, 56, etc.] have studied on the effect of K_2O-manure upon the growth of mulberry trees. Some of them thought that K_2O-manure promoted a little the growth of mulberry trees. ARAMOMI[2], USHIWODA[57], etc., however, stated that the influence of K_2O manure upon the growth of mulberry trees was not always remarkable and varied with the soil in which the experiment was carried out. The result of the writer's experiment is regarded to be one of the examples that the influence of K_2O-manure on the growth of mulberry trees was not remarkable.

The data of the results of the inoculation experiments were divided into two groups (Table 6 and Table 7) according to the growth stages (that is to say, the growing stage and the not-growing stage) of the mulberry trees while the invasion of the causal fungus occurred, and the variances of the invaded area were analysed.

Table 6 and Table 7 indicate that the development of the disease is not influenced by K_2O-manure in the growing stage as well as in the not-growing stage of the mulberry trees.

Table 6. The influence of K_2O-manure upon the development of the disease in the growing stage of the mulberry trees

No. of exp.	Date	Kind of exp.	Index of invaded area	
	Inoculation—Measurement		K_2O-given	K_2O-not given
1	20/III '50—30/V '50	A	24.9	18.8
2	20/III '50—30/V '50	B	23.6	20.0
3	22/IV '50—30/VI '50	A	25.8	49.7
4	22/IV '50—30/VI '50	B	48.2	44.6
5	27/III '51—6/VI '51	A	74.2	67.0
6	27/III '51—6/VI '51	B	123.2	96.9
7	9/IV '51—30/VI '51	A	93.9	105.5
8	9/IV '51—30/VI '51	B	119.4	112.4
Mean			66.7	64.4

Note; 'Index of invaded area' is obtained from the product of average length(mm) and average width(mm) of the real invaded areas.

Table of analysis of variance

	SS	DF	V	F
K_2O	20.93	1	20.93	
Replicate	21 340.80	7	3 048.54	28.09**
Error	759.78	7	108.54	
Total	22 121.51	15		

Table 7. The influence of K_2O-manure upon the development of the disease in the not-growing stage of the mulberry trees

No. of exp.	Date	Kind of exp.	Index of invaded area	
	Inoculation—Measurement		K_2O-given	K_2O-not given
1	11/X '49—25/IV '50	A	67.4	105.1
2	11/X '49—25/IV '50	B	73.4	69.7
3	25/XI '50—28/IV '51	A	120.8	124.3
4	25/XI '50—28/IV '51	B	138.5	152.3
5	11/XII '50—28/IV '51	A	120.0	134.6
6	11/XII '50—28/IV '51	B	163.1	121.2
Mean			113.9	117.9

Table of analysis of variance

	SS	DF	V	F
K_2O	141.45	1	141.45	
Replicate	7 068.32	5	1 413.66	5.32*
Error	1 329.25	5	265.85	
Total	8 539.02	11		

P_2O_5-experiment The inoculation was also carried out in October—December and in March—May of the next year from 1949—1951. The growth of the supplied mulberry trees is given in the table 8.

Table 8. Showing the growth of the mulberry trees in P_2O_5-experiment

			Number of stem (of more than 1 m height) per 1 tree		Height	
			Dec. 1949	Dec. 1950	Dec. 1949	Dec. 1950
Kind of plot	A-exp.	P_2O_5-given	26.1	25.3	141.5	143.5
		P_2O_5-not given	25.9	28.7	139.5	147.2
	B-exp.	P_2O_5-given	18.1	16.3	125.2	129.3
		P_2O_5-not given	17.9	16.7	119.2	128.1

Note; 'Height' was obtained from the average value of the highest 10 stems of every plot.

The data in the table 8 suggest that the effect af P_2O_5 in the soil on the growth of the mulberry trees is not remarkable. Many investigators (2, 3, 16, 17, 18, 19, 46, 56, etc.) have also studied on the effect of P_2O_5-manure upon the growth of mulberry trees. Some of them concluded that P_2O_5-manure promoted a little the growth of mulberry trees. But ARAMOMI(2), USHIWODA(57), etc. stated that the influence of P_2O_5-manure upon the growth of mulberry trees was not always remarkable and varied with the soil in which the experiment was carried out. The result of the writer's experiment is regarded to be one of the examples that the effect of P_2O_5-manure on the growth of mulberry trees was not remarkable.

The data of the results of the inoculation experiments were divided into two groups (Table 9 and Table 10) according to the growth stages (that is to say, the growing stage and the not-growing stage) of the mulberry trees while the invasion of the causal fungus occurred and the variances of the invaded area were analysed.

Table 9 and Table 10 indicate that P_2O_5-manure also has not its influence on the development of the "bud blight" in the growing stage as well as in the not-growing stage of the mulberry trees.

Table 9. The influence of P_2O_5-manure upon the development of the disease in the growing stage of the mulberry trees

No. of exp.	Date	Kind of exp.	Index of invaded area	
	Inoculation—Measurement		P_2O_5-given	P_2O_5-not given
1	20/III '50—30/V '50	A	24.9	18.8
2	20/III '50—30/V '50	B	31.4	20.0
3	22/IV '50—30/VI '50	A	25.8	49.7
4	22/IV '50—30/VI '50	B	46.2	44.6
5	27/III '51— 6/VI '51	A	74.2	69.6
6	27/III '51— 6/VI '51	B	107.3	96.9
7	9/IV '51—30/VI '51	A	93.9	104.0
8	9/IV '51—30/VI '51	B	122.6	112.4
Mean			65.8	64.5

Note; 'Index of invaded area' is obtained from the product of average length(mm) and average width (mm) of the real invaded areas.

Table of analysis of variance

	SS	DF	V	F
P_2O_5	6.63	1	6.63	
Replicate	19 731.69	7	2 818.81	37.12**
Error	531.52	7	75.93	
Total	20 269.84	15		

Table 10. The influence of P_2O_5-manure upon the development of the disease in the not-growing stage of the mulberry trees

No. of exp.	Date	Kind of exp.	Index of invaded area	
	Inoculation—Measurement		P_2O_5-given	P_2O_5-not given
1	11/X '49—25/IV '50	A	67.4	71.3
2	11/X '49—25/IV '50	B	70.4	61.0
3	25/XI '50—28/IV '51	A	120.8	126.8
4	25/XI '50—25/IV '51	B	110.9	152.3
5	11/XII '50—28/IV '51	A	120.0	141.5
6	11/XII '50—28/IV '51	B	140.7	121.2
Mean			105.0	112.4

Table of analysis of variance

	SS	DF	V	F
P_2O_5	160.60	1	160.60	
Replicate	10 262.22	5	2 052.44	8.64*
Error	1 187.41	5	237.48	
Total	11 610.23	11		

3. Conclusion

From the results of the writer's experiments which were stated before, it is concluded that N-manure has not its influence upon the development of the "bud blight" of mulberry trees in their not-growing stage, though the same manure promotes a little the development of the disease in the growing stage of mulberry trees. From the practical point of view the development of this disease in the growing stage of mulberry trees is almost out of the question as the writer stated in the previous paper[30]. So it is not too much to say that we can give much N-manure to the mulberry field in order to promote the yield paying no attention to the influence of the manure upon the development of the "bud blight". Secondly, it was known that K_2O-and P_2O_5-manure had no influence upon the development of the "bud blight" in the growing stage as well as in the not-growing stage of mulberry trees.

II. THE INFLUENCE OF MANURE UPON THE FORMATION OF SPORODOCHIA AND PERITHECIA OF THE CAUSAL FUNGUS ON MULBERRY STEMS

1. The Influence of Manure upon the Formation of Sporodochia of the Causal Fungus

The condia of the causal fungus are produced mainly on the sporodochia which are formed on the diseased mulberry stems. The writer investigated the influence of manure upon the formation of the sporodochia on mulberry stems.

1. Materials and Methods

The mulberry stems or twigs which were cultured simultaneously with those which were supplied for the inoculation experiments in the previous capter were used. In a certain growth stage of the mulberry trees some mulberry stems or twigs of each plot were cut from their bases. And the mulberry stem media were made after the usual manner[29]. The causal fungus was inoculated on the mulberry stem media and was cultured in thermostats (25°C) or under the natural condition. The number of the sporodochia, which broke out epidermis or lenticels from inside and appeared on the surface of the mulberry stem media, was measured about a month after the inoculation. Each time five test tubes of medium were supplied for each plot.

Table 11. Showing the influence of N-manure upon the formation of sporodochia of the causal fungus on the mulberry stems

Kind of plot		No. of exp.	1	2	3	4	5	6	7	8	9	10	11	12	Mean
		Date of stem cutting	6/VII '46	8/VII '46	6/VII '46	8/VII '46	18/II '49	5/IV '49	5/IV '49	5/IV '49	10/V '50	20/XI '50	20/XI '50	20/XI '50	
		Age of supplied stem	1st	1st	2nd	2nd	2nd	2nd	2nd	2nd	2nd	1st	1st	1st	
	N-little	Large	17.7	4.2	5.4	3.6	0.2	0.8	0.3	0	0.6	0	0.4	0	2.8
		Small	288.0	222.4	84.8	43.7	70.1	87.1	401.7	109.6	20.4	114.4	226.4	261.3	160.8
		Total	305.7	226.6	90.2	47.3	70.3	87.9	402.0	109.6	21.0	114.4	226.8	261.3	163.6
	N-much	Large	19.1	6.8	6.6	6.2	0	3.0	0.6	0	0.2	0.4	0	0	3.6
		Small	294.5	379.0	72.8	61.7	189.0	578.0	1 140.0	1 225.0	75.5	125.4	278.6	488.8	409.0
		Total	313.6	385.8	79.4	67.9	189.0	581.0	1 140.6	1 225.0	75.7	125.8	278.6	488.8	412.6
	N-very much	Large	56.4	14.9	12.4	7.8	0.6	1.6	2.0	0.6	1.7	1.0	0.9	1.1	8.4
		Small	265.4	313.9	100.1	79.0	232.5	695.6	893.8	1 053.0	98.3	502.5	427.5	713.8	448.0
		Total	321.8	328.8	112.5	86.8	233.1	697.2	895.8	1 053.6	100.0	503.5	428.4	714.9	456.4

Table of analysis of variance (total number of sporodochia)

	SS	DF	V	F
N	5 991.92	2	2 995.96	7.58**
Replicate	21 563.68	11	1 960.33	4.96**
Error	8 697.46	22	395.34	
Total	36 253.06	35		

$D \geqq 228.7$ Difference between any two of means is significant at 1% level.

$D \geqq 168.3$ Difference between any two of means is significant at 5% level.

Table 12. Showing the influence of K_2O-manure upon the formation of sporodochia of the causal fungus on the mulberry stems

Kind of plot			No. of exp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
			Date of stem cutting	6/VII '46	8/VII '46	6/VII '46	8/VII '48	18/II '49	5/IV '49	5/IV '49	21/III '50	21/III '50	28/IV '50	28/IV '50	6/VI '50	6/VI '50	6/VI '50	
			Age of supplied stem	1st	1st	2nd	1st	2nd	2nd	2nd	2nd	2nd	2nd	2nd	1st	1st	1st	
	A exp.	K_2O-given	Large	6.0	6.3	4.6	2.9	0.5	3.6	3.4	0.7	1.4	2.3	—	5.0	2.0	1.5	3.1
			Small	157.3	122.7	41.2	448.2	75.6	289.4	438.5	300.0	69.5	100.5	—	266.5	245.0	253.0	216.0
			Total	163.3	129.0	45.8	451.1	76.1	293.0	442.0	300.7	70.9	102.8	—	271.5	247.0	254.5	219.1
		K_2O-not given	Large	10.8	6.4	4.5	3.5	1.1	1.8	7.1	7.8	2.8	2.8	—	6.5	1.5	0	4.3
			Small	206.0	139.4	149.5	456.1	160.3	883.3	622.5	290.8	35.3	116.8	—	336.0	298.5	278.5	305.6
			Total	216.8	145.8	154.0	459.6	161.4	885.1	629.6	298.6	38.1	119.6	—	342.5	300.0	278.5	309.9
	B exp.	K_2O-given	Large	9.9	18.4	7.1	37.2	0	4.9	2.6	3.6	1.5	0.8	2.3	2.0	3.5	0.5	6.7
			Small	91.1	129.1	68.0	654.2	10.5	402.1	598.8	460.3	1 071.5	153.2	328.8	476.0	320.0	461.0	373.2
			Total	101.0	147.5	75.1	691.4	10.5	407.0	601.4	463.9	1 073.0	154.0	331.1	478.0	323.5	461.5	379.9
		K_2O-not given	Large	45.2	23.8	36.0	41.2	1.2	3.0	9.0	0.7	0.4	2.0	3.9	3.0	0	1.5	12.2
			Small	213.4	130.0	168.0	784.0	15.3	605.6	732.0	910.0	758.0	72.0	226.7	774.0	580.5	536.0	464.7
			Total	258.6	153.8	204.0	825.2	16.5	608.6	741.0	910.7	758.4	74.0	230.6	777.0	580.5	537.5	476.9

Table of analysis of variance in A exp.

	SS	DF	V	F
K_2O	538.26	1	538.26	4.17
Replicate	7 125.09	12	593.76	4.59**
Error	1 550.69	12	129.22	
Total	9 214.04	25		

Table of analysis of variance in B exp.

	SS	DF	V	F
K_2O	659.60	1	659.60	3.69
Replicate	20 759.85	13	1 596.91	8.93**
Error	2 323.88	13	178.76	
Total	23 743.33	27		

Table 13. Showing the influence of P_2O_5-manure upon the formation of sporodochia of the causal fungus on the mulberry stems

Kind of plot		No. of exp.	1	2	3	4	5	6	7	8	9	10	11	Mean
		Date of stem cutting	6/VII '46	8/VII '46	6/VII '46	8/VII '48	20/III '50	21/III '50	24/III '50	27/IV '50	27/IV '50	28/IV '50	28/IV '50	
		Age of supplied stem	1st	1st	2nd	1st	2nd	2nd	2nd	2nd	2nd	2nd	2nd	
A exp.	P_2O_5-given	Large	6.0	6.3	4.6	2.9	0.7	1.4	2.3	—	—	—	—	3.5
		Small	157.3	122.7	41.2	448.2	300.0	69.5	100.5	—	—	—	—	177.1
		Total	163.3	129.0	45.8	451.1	300.7	70.9	102.8	—	—	—	—	180.5
	P_2O_5-not given	Large	3.3	5.1	4.2	3.1	3.0	3.2	0.6	—	—	—	—	3.2
		Small	156.3	137.3	39.1	693.0	479.6	232.8	76.9	—	—	—	—	259.3
		Total	159.6	142.4	43.3	696.1	482.6	236.0	77.5	—	—	—	—	262.5
B exp.	P_2O_5-given	Large	5.8	5.5	38.2	40.1	1.2	0	0.9	2.0	1.3	2.4	0.7	8.9
		Small	145.2	127.7	171.2	727.0	338.0	813.3	307.2	147.8	426.0	101.4	16.3	301.9
		Total	151.0	133.2	209.4	767.1	339.2	813.3	308.1	149.8	427.3	103.8	17.0	310.8
	P_2O_5-not given	Large	45.2	23.8	36.0	41.2	0.7	4.0	1.5	2.0	3.9	0.3	1.0	14.2
		Small	213.4	130.0	168.0	784.0	910.0	758.0	337.0	72.0	226.7	114.5	38.6	341.1
		Total	258.6	153.8	204.0	825.2	910.7	758.4	338.5	74.0	230.6	114.8	39.6	355.3

Table of analysis of variance in A exp.

	SS	DF	V	F
P_2O_5	235.34	1	235.34	3.81
Replicate	4 341.68	6	723.61	11.71**
Error	370.59	6	61.77	
Total	4 947.61	13		

Table of analysis of variance in B exp.

	SS	DF	V	F
P_2O_5	110.03	1	110.03	
Replicate	15 286.11	10	1 528.61	8.27**
Error	1 847.50	10	184.75	
Total	17 243.64	21		

2. Results

N-experiment

The mulberry stems of (A)-experiment (see the previous chapter) were supplied for this investigation. The investigations were carried on from 1946 to 1950. The results are shown in Table 11.

The result of analysis of variances of the data in Table 11 shows that the sporodochial formation on the mulberry stems of N-little plot is less than that of the other plots (N-much and N-very much plots).

K_2O-experiment

The investigations were also carried on from 1946 to 1950. The results are given in Table 12.

The result of analysis of variances of the data in Table 12 (A exp. and B exp.) shows that K_2O-manure has no influence on the sporodochial formation on the mulberry stems. But if the data concerning mulberry stems in the growing stage (No. 1, 2, 3, 4, 12, 13 and 14) alone are taken up from the table 12 and their variances are analysed (the following tables), we can know that K_2O-manure has a repressive influence on the sporodochial formation on the mulberry stems.

Table of analysis of variance in the growing stage of stems in A exp.

	SS	DF	V	F
K_2O	80.64	1	80.64	13.35*
Replicate	1 701.65	6	283.61	46.96**
Error	36.24	6	6.04	
Total	1 818.53	13		

Table of analysis of variance in the growing stage of stem in B exp.

	SS	DF	V	F
K_2O	801.06	1	801.06	15.86**
Replicate	7 418.55	6	1 236.43	24.48**
Error	303.01	6	50.50	
Total	8 522.62	13		

P_2O_5-experiment

The investigations were also carried on from 1946 to 1950. The results are shown in Table 13.

The result of analysis of variances of the data in Table 13 (A exp. and B exp.) shows that P_2O_5-manure has no influence on the sporodochial formation on the mulberry stems.

3. Conclusion

From the results of the writer's experiments which were mentioned above, it is concluded that the sporodochial formation on the mulberry stems is promoted by N-manure but is repressed by K_2O-manure only in the growing stage of the mulberry trees, and is not influenced by P_2O_5-manure.

2. The Influence of Manure upon the Formation of Perithecia of the Causal Fungus

The perithecia of the causal fungus are formed on the perithecial stroma which appears on dead mulberry stems.

1. Materials and Methods

The experiments of this section were done as the sequel of those of the previous section. Perithecial storomata forming typical perithecia appear on the mulberry stem media about after a month's culture since the inoculation of the causal fungus. The number of perithecial stromata was measured two months after the inoculation.

2. Results

The results of the measurements and the analyses of variances of the data are given in the tables 14, 15 and 16.

Table 14. Showing the influence of N-manure upon the formation of perithecial stromata of the causal fungus on the mulberry stems

	No. of exp.	1	2	3	4	5	6	7	8	Mean
	Date of stem cutting	6/VII '46	8/VII '46	6/VII '46	8/VII '46	5/IV '49	5/IV '49	5/IV '49	10/V '50	
	Age of supplied stem	1st	1st	2nd	2nd	2nd	2nd	2nd	2nd	
Kind of plot	N-little	1 300.0	200.0	2 350.0	1 460.0	558.9	257.8	212.9	778.0	889.7
	N-much	470.0	375.0	795.0	1 030.0	165.2	672.2	341.1	399.0	530.9
	N-very much	700.0	472.0	775.0	1 463.0	534.4	357.8	353.9	778.0	679.3

Table of analysis of variance

	SS	DF	V	F
N	51.69	2	25.85	1.88
Replicate	361.07	7	51.58	3.75*
Error	192.81	14	13.77	
Total	605.57	23		

Table 15. Showing the influence of K_2O-manure upon the formation of perithecial stromata of the causal fungus on the mulberry stems

Kind of plot		No. of exp.	1	2	3	4	5	6	7	8	9	10	Mean
		Date of stem cutting	6/VII '46	8/VII '46	6/VII '46	18/II '49	5/IV '49	5/IV '49	21/III '50	21/III '50	28/IV '50	28/IV '50	
		Age of supplied stem	1st	1st	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	
Kind of plot	A exp.	K_2O-given	492.0	176.3	760.0	430.0	320.0	40.9	124.8	144.8	62.6	—	283.5
		K_2O-not given	465.0	465.9	760.0	528.0	353.6	56.2	207.5	121.7	118.1	—	341.9
	B exp.	K_2O-given	635.4	295.2	590.0	331.0	331.3	31.6	78.3	341.2	121.0	224.5	298.0
		K_2O-not given	720.4	123.0	600.0	564.0	244.4	13.0	98.9	448.5	39.7	133.3	298.6

Table of analysis of variance in A exp.

	SS	DF	V	F
K_2O	1.69	1	1.69	3.67
Replicate	86.11	8	10.76	23.39**
Error	3.70	8	0.46	
Total	91.50	17		

Table of analysis of variance in B exp.

	SS	DF	V	F
K_2O	0	1	0	
Replicate	91.26	9	10.14	14.28**
Error	6.36	9	0.71	
Total	97.62	19		

Table 16. Showing the influence of P_2O_5-manure upon the formation of perithecial stromata of the causal fungus on the mulberry stems

Kind of plot		No. of exp.	1	2	3	4	5	6	7	8	9	10	Mean
		Date of stem cutting	6/VI '46	8/VI '46	6/VI '46	20/III '50	21/III '50	24/III '50	27/IV '50	27/IV '50	28/IV '50	28/IV '50	
		Age of supplied stem	1st	1st	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	
Kind of plot	A exp.	P_2O_5-given	492.0	176.3	760.0	124.8	144.8	62.6	—	—	—	—	293.4
		P_2O_5-not given	235.0	470.0	521.0	134.2	152.8	105.3	—	—	—	—	269.7
	B exp.	P_2O_5-given	418.0	348.0	600.0	119.1	604.0	77.7	89.0	233.7	18.3	108.8	261.7
		P_2O_5-not given	720.4	123.0	696.0	98.9	448.5	64.3	39.7	133.3	8.3	153.5	248.6

Table of analysis of variance in A exp.

	SS	DF	V	F
P_2O_5	0.14	1	0.14	
Replicate	43.83	5	8.77	4.30
Error	10.20	5	2.04	
Total	54.17	11		

Table of analysis of variance in B exp.

	SS	DF	V	F
P_2O_5	0.09	1	0.09	
Replicate	99.75	9	11.08	10.55**
Error	9.43	9		
Total	109.27	19		

3. Conclusion

From the results of the writer's experiments which were mentioned above, it is concluded that the formation of perithecial stromata on mulberry stems is not influenced by N-, K_2O-, or P_2O_5-manure so far as the writer's experiments were concerned.

III. THE INFLUENCE OF MANURE UPON THE FORMATION OF WOUND PERIDERM IN THE MULBERRY TREES AND THE ADDITIONAL DISCUSSION OF THE PATHOLOGICAL SIGNIFICANCE OF THE WOUND PERIDERM

The wound periderm is formed around the injured or diseased area of the bark of the mulberry trees in the growing stage. The writer investigated in the previous paper[30] the seasonal change of the formation of the wound periderm (wound cork layer) in the mulberry trees and also the formation degree of the wound periderm in the various varieties of mulberry trees, and he discussed the pathological significance of the wound periderm. In this chapter he is going to state the results of his investigations concerning the influence of manure upon the formation of the wound periderm in the mulberry trees and also to have the additional discussion of the pathological significance of the wound periderm.

Many investigators[50, 9, 10, 54, 59, 43, 60, 61, 45, etc.] have discussed the pathological significance of the wound periderm which is formed around the diseased area of various plants, and it was inferred by them on the basis of the results of their histological observations that the wound cork layer stopped the advance of the causal fungus. But the formation of the wound periderm is influenced not only by the character and condition of the host but also negatively by the invasion power or the advance speed of the parasite; that is to say, the wound periderm is formed as the result of the host-parasite interaction. BROWN[8] expresses some doubt as to whether the cork layer really functions at all or whether it is formed after the fungus has been stopped by some chemical means. So it is not too much to say that we cannot know the real pathological significance of the wound cork layer if we stand only on the result of the histological observation of the diseased area.

The witer intended to know the pathological significance of the wound periderm as he tried in the previous paper by the method of inquiring into the relation between the formation degrees of the wound cork layer around the cut injury (by a razor-edge) on the mulberry stems of various plots and the results of the inoculation experiments to the mulberry stems of the same plots. The formation degree of the wound cork layer around the cut injury depends only on the character and condition of the host and is not influenced by a parasite.

(1) Materials and Methods

The supplied mulberry trees are those cultured simultaneously with those which were used for the inoculation experiments in Chapter I.

A cut injury (about 1 cm length) was inflicted on the bark at the middle part of each mulberry stem with a razor-edge. Sections of the tissue at the middle part of the cut injury were made by free hand and were stained with Sudan III for the purpose of observing the formation degrees of the wound cork layer. Each time 10 mulberry stems were supplied for each plot.

(2) Results

N-experiment

The injury experiments were carried out in 1950 and 1951. The experi-

Table 17. The influence of N-manure upon the formation of the wound cork layer around the injury cut on the mulberry stems; the result of the investigation in 1950

Date (Injuring—Observation)	Kind of plot	Cortex I	Cortex II	Phloem I	Phloem II	Phloem III	Cambium and undifferentiated cells	Mean
7/VI—29/VI	N-little	4.63	5.67	4.89	6.85	6.83	5.15	5.67
	N-much	5.02	6.93	6.60	7.26	6.92	6.10	6.47
	N-very much	4.43	5.82	7.23	6.46	7.34	6.61	6.32
15/VI—4/VII	N-little	3.60	5.22	4.48	5.86	5.98	4.89	5.01
	N-much	3.92	5.12	5.22	5.48	5.98	4.56	5.05
	N-very much	3.76	4.56	4.64	5.40	5.63	5.20	4.87
16/VI—5/VII	N-little	4.30	6.09	5.90	6.67	6.49	6.14	5.93
	N-much	4.00	5.33	5.51	5.49	5.37	4.85	5.09
	N-very much	3.18	4.80	5.24	6.02	5.71	5.01	4.99
(Mean)	N-little	4.18	5.66	5.09	6.46	6.43	5.39	5.54
	N-much	4.31	5.79	5.78	6.08	6.09	5.17	5.54
	N-very much	3.79	5.06	5.70	5.96	6.23	5.61	5.39

Note; Cortex I and II mean the outer and inner parts of cortex. The boundary of them is the stone cell. Phloem I, II and III mean the outer, middle and inner parts of phloem. Figures in the table show the average number of the wound cork layer.

Table 18. The influence of N-manure upon the formation of the wound cork layer around the injury cut on the mulberry stems; the result of the investigation in 1951

Date of injuring	Kind of plot	Number of wound cork layer		
		5-6th day	10-11th day	16th day
31/V	N-little	0.31	2.32	4.22
	N-much	0.08	2.73	4.31
	N-very much	0.00	1.52	3.88
2/VI	N-little	0.11	2.45	2.99
	N-much	0.38	2.61	2.80
	N-very much	0.14	2.03	3.33
5/VI	N-little	0.00	1.64	3.92
	N-much	0.00	1.47	3.71
	N-very much	0.31	0.98	3.71
(Mean)	N-little	0.14	2.14	3.71
	N-much	0.15	2.27	3.61
	N-very much	0.15	1.51	3.64

Note; 'Number of wound cork layer' shows the mean value of the wound cork layers which were formed in various tissues.

ments in 1950 were carried out to make clear the formation degrees of the wound cork layer, and the experiments in 1951 were done to make clear the speed of the wound cork layer formation as well as the formation degrees of the wound cork layer. The results are given in Table 17 and Table 18.

The data (mean of each exp.) in the table 17 and those (number of wound cork layer on 16th day) in the table 18 are recomposed in Table 19,

Table 19. Analysis of variance of the wound cork layer formation in N-experiment

	No. of exp.	1 ('50)	2 ('50)	3 ('50)	4 ('51)	5 ('51)	6 ('51)	Mean
Kind of plot	N-little	5.67	5.01	5.93	4.22	2.99	3.92	4.62
	N-much	6.47	5.05	5.09	4.31	2.80	3.71	4.57
	N-very much	6.32	4.87	4.99	3.88	3.33	3.71	4.52

	SS	DF	V	F
N	0.0342	2	0.0171	
Replicate	19.2386	5	3.8477	33.31**
Error	1.1549	10	0.1155	
Total	20.4277	17		

and the variances of the formation degrees of the wound cork layer are analysed.

Table 19 shows that the differences of the formation degrees of the wound periderm between plots is not significant at 5% level.

The writer must discuss here the difference of the speed of the wound cork layer formation between N-plots, because the speed of the formation may be different between the plots and affect differently the advance of the causal fungus though the formation degrees of the wound cork layers are not different between the same plots. Table 18 seems to suggest, however, that the speed of the formation also is not different among the plots.

K_2O– and P_2O_5–experiment

The injury experiments were carried out in 1950. The results are given in the tables 20 and 21.

Table 20. The influence of K_2O-manure upon the formation of the wound cork layer around the injury cut on the mulberry stems

Date (Injuring — Observation)	Kind of plot		Cortex I	Cortex II	Phloem I	Phloem II	Phloem III	Cambium and undifferentiated cells	Mean
7/VI—29/VI	A exp.	K_2O-given	5.00	6.36	6.42	7.77	7.19	7.23	6.66
		K_2O-not given	4.74	6.25	6.30	6.80	7.04	6.01	6.19
	B exp.	K_2O-given	3.82	5.14	5.21	6.29	6.50	6.65	5.60
		K_2O-not given	3.38	5.23	4.95	6.20	5.83	5.17	5.13
15/VI—4/VII	A exp.	K_2O-given	3.32	4.56	5.31	5.37	5.90	5.40	4.98
		K_2O-not given	2.80	4.33	4.75	4.45	5.23	5.07	4.44
	B exp.	K_2O-given	3.58	4.87	4.93	4.87	5.55	5.49	4.88
		K_2O-not given	3.51	4.93	4.63	5.30	5.65	5.45	4.91
16/VI—5/VII	A exp.	K_2O-given	3.97	4.98	5.10	5.54	5.64	5.60	5.14
		K_2O-not given	3.16	4.82	4.58	5.21	5.74	5.33	4.81
	B exp.	K_2O-given	3.56	4.95	4.60	5.42	6.48	6.78	5.30
		K_2O-not given	3.15	4.61	4.19	5.22	5.05	5.40	4.60
(Mean)	A exp.	K_2O-given	4.10	5.30	5.61	6.23	6.24	6.08	5.59
		K_2O-not given	3.57	5.13	5.21	5.49	6.00	5.47	5.15
	B exp.	K_2O-given	3.65	4.99	4.91	5.53	6.18	6.31	5.26
		K_2O-not given	3.35	4.92	4.59	5.57	5.51	5.34	4.88

Note; See the note of Table 17.

Table 21. The influence of P_2O_5-manure upon the formation of the wound cork layer around the injury cut on the mulberry stems

Date (Injuring—Observation)	Kind of plot		Cortex I	Cortex II	Phloem I	Phloem II	Phloem III	Cambium and undifferentiated cells	Mean
7/VI—29/VI	A exp.	P_2O_5-given	5.00	6.36	6.42	7.77	7.19	7.23	6.66
		P_2O_5-not given	4.87	5.06	6.01	6.39	7.14	6.27	5.96
	B exp.	P_2O_5-given	4.08	5.17	5.55	6.22	7.19	6.16	5.73
		P_2O_5-not given	3.38	5.23	4.95	6.20	5.83	5.17	5.13
15/VI—4/VII	A exp.	P_2O_5-given	3.32	4.56	5.31	5.37	5.90	5.40	4.98
		P_2O_5-not given	2.87	4.51	4.78	5.50	5.67	4.78	4.69
16/VI—5/VII	A exp	P_2O_5-given	3.97	4.98	5.10	5.54	5.64	5.60	5.14
		P_2O_5-not given	4.09	5.57	6.00	5.82	6.09	6.54	5.69
	B exp.	P_2O_5-given	3.21	4.22	4.58	5.28	6.06	5.35	4.78
		P_2O_5-not given	3.15	4.61	4.19	5.22	5.05	5.40	4.60
(Mean)	A exp	P_2O_5-given	4.10	5.30	5.61	6.23	6.24	6.08	5.59
		P_2O_5-not given	3.94	5.05	5.60	5.90	6.30	5.86	5.45
	B exp.	P_2O_5-given	3.65	4.70	5.07	5.75	6.63	5.76	5.26
		P_2O_5-not given	3.27	4.92	4.57	5.71	5.44	5.29	4.87

Note; See the note of Table 17.

Mean value of each time in A aud B experiments in the tables 20 and 21 is recomposed in Table 22 and 23 respectively, and the variances of the wound cork layer formation are analysed.

Table 22. Analysis of variance of the wound cork layer formation in K_2O-experiment

	No. of exp.	1	2	3	4	5	6	Mean
	Kind of exp.	A	B	A	B	A	B	
Kind of plot	K_2O-given	6.66	5.60	4.98	4.88	5.14	5.30	5.43
	K_2O-not given	6.19	5.13	4.44	4.91	4.81	4.60	5.01

	SS	DF	V	F
K_2O	0.5122	1	0.5122	16.58**
Replicate	3.9434	5	0.7887	25.52**
Error	0.1544	5	0.0309	
Total	4.6100	11		

Table 23. Analysis of variance of the wound cork layer formation in P_2O_5-experiment

	No. of exp.	1	2	3	4	5	Mean
	Kind of exp.	A	B	A	A	B	
Kind of plot	P_2O_5-given	6.66	5.73	4.98	5.14	4.78	5.46
	P_2O_5-not given	5.96	5.13	4.69	5.69	4.60	5.21

	SS	DF	V	F
P_2O_5	0.14884	1	0.14884	1.23
Replicate	3.26414	4	0.81604	6.72*
Error	0.48566	4	0.12142	
Total	3.89864	9		

The table 22 and 23 indicate that the formation of the wound cork layer is not influenced by P_2O_5-manure, but promoted by K_2O-manure.

(3) Discussion

From the results of the writer's experiments which were stated before, it was known that the formation of the wound cork layer was not influenced by N-manure and P_2O_5-manure, but promoted by K_2O-manure. On the other hand it was known by the results of the inoculation experiments in Chapter I that the development of the "bud blight" in the growing stage of the mulberry trees was influenced by N-manure, and not influenced by K_2O-manure as well as P_2O_5-manure. From these results it can be concluded that the formation degree of the wound cork layer has nothing to do with the development degree of the disease so far as the writer's experiments in this paper is concerned.

In the previous paper[30] the writer investigated the seasonal changes of the formation of the wound periderm (or wound cork layer) and also the formation degrees of the wound periderm in the various varieties of the mulberry trees and discussed the pathological significance of the wound periderm. He concluded that the wound periderm had a very important defensive function against the invasion of the causal fungus, but it was not the only defensive factor against the invasion. The result of the investigations in this chapter seems to confirm the latter part of the previous conclusion that the wound periderm is not the only defensive factor against the invasion of the causal fungus.

IV. THE INFLUENCE OF MANURE UPON THE MAIN CHEMICAL COMPONENTS OF THE BARK OF THE MULBERRY TREES AND THE PATHOLOGICAL SIGNIFICANCE OF IT

The writer is going to state in this chapter the influence of manure upon the main chemical components of the bark of the mulberry stems and the pathological significance of it. The wound cork layer which was stated in the previous chapter is formed only in the mulberry trees of the growing stage. And the pathological significance of it may concern only the function of the defender against the invasion of the causal fungus in the growing stage of mulberry trees, but the changes of the chemical components of the mulberry stems are supposed to be connected with the various phenomena, that is, the development of the disease in the not-growing stage as well as in the growing stage, the formation degree of the sporodochia and perithecia, etc. The writer analysed, first of all, the bark of the mulberry stems of N- and K_2O-plots which are in the growing stage, because the influence of N-manure upon the development of the disease and that of N- and K_2O-manure upon the sporodochial formation were very remarkable in the growing stage of the mulberry trees. In addition the writer must state here the result of his another investigation that the influence of manure upon the formation of sporodochia on the bark (separated from the wood and pith of the stem) showed the same tendency as that on the whole mulberry stem (having the wood and pith as well as the bark).

1. The Influence of N- and K_2O-Manure upon the Main Chemical Components of the Bark of the Mulberry Trees in the Growing Stage

The first year stems or twigs of the mulberry trees, which were cultured simultaneously with those which were supplied for the experiments in the previous chapters, were cut from the bases on June I of 1950, and their bark was supplied for the analyses.

(1) General chemical component

Materials were analysed after the usual manner[55]. Analytical results were expressed by the value in 100 parts of dry matter (%) and also by the value pro unit volume of dry tissue powder[22, 23, 51]. The results which were expressed by the both methods showed the similar tendency except a few substances. The results are shown in Table 24.

Table 24. The influence of N- and K_2O-manure upon the general chemical component of the bark of the mulberry stems

Kind of expression / Plot / Chemical component	Value in 100 parts of dry matter (%)							Value pro unit volume of dry tissue powder (mg/cm^3)						
	N-experiment			K_2O-experiment				N-experiment			K_2O-experiment			
				A-exp.		B-exp.					A-exp.		B-exp.	
	N-little	N-much	N-very much	K_2O-given	K_2O-not given	K_2O-given	K_2O-not given	N-little	N-much	N-very much	K_2O-given	K_2O-not given	K_2O-given	K_2O-not given
Moisture	279.6	289.0	259.3	308.1	279.4	266.7	267.2	1 499	1 379	1 343	1 331	1 336	1 133	1 333
Crude ash	7.94	7.49	6.83	7.81	7.25	7.43	6.37	42.6	35.7	35.4	33.7	34.7	31.6	31.8
SiO_2	0.53	0.47	0.35	0.61	0.53	0.34	0.35	2.8	2.2	1.8	2.6	2.5	1.4	1.7
SO_3	0.42	0.30	0.26	0.54	0.31	0.39	0.38	2.3	1.4	1.3	2.3	1.5	1.7	1.9
Cl	0.22	0.24	0.25	0.25	0.27	0.20	0.22	1.2	1.1	1.3	1.1	1.3	0.9	1.1
P_2O_5	0.76	0.69	0.69	0.73	0.82	0.61	0.55	4.1	3.3	3.6	3.2	3.9	2.6	2.7
K_2O	2.22	1.92	1.80	2.18	1.87	2.22	1.84	11.9	9.2	9.3	9.4	8.9	9.4	9.2
Na_2O	0.37	0.26	0.41	0.42	0.48	0.42	0.40	2.0	1.2	2.1	1.8	2.3	1.8	2.0
CaO	1.92	1.82	2.05	1.98	1.83	1.68	1.29	10.3	8.7	10.6	8.6	8.7	7.1	6.4
MgO	0.23	0.31	0.56	0.51	0.58	0.45	0.57	1.2	1.5	2.9	2.2	2.8	1.9	2.8
Total N	1.43	1.68	1.91	1.74	1.71	1.86	1.83	7.7	8.0	9.9	7.5	8.2	7.9	9.1
Proteinous N	0.66	1.03	1.12	1.07	1.08	0.99	1.06	3.5	4.9	5.8	4.6	5.2	4.2	5.4
Not-proteinous N	0.77	0.65	0.79	0.67	0.63	0.87	0.77	4.1	3.1	4.1	2.9	3.0	3.7	3.8
Total carbohydrate	9.92	9.64	10.52	10.41	10.30	10.28	11.49	53.2	46.0	54.5	45.0	49.2	43.7	57.3
Crude fibres	28.09	31.59	28.48	25.01	25.11	21.44	22.32	150.6	150.7	147.5	108.0	120.0	91.1	111.4
*Water soluble total N	0.39	0.45	0.58	0.41	0.47	0.58	0.75	2.1	2.1	3.0	1.8	2.2	2.5	3.7
Amides of amino acids	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.1	0.1	0.2	0.04	0.05	0.04	0.04
Amino acids	0.13	0.14	0.14	0.19	0.18	0.20	0.20	0.7	0.7	0.7	0.8	0.9	0.9	1.0
Ammonium N	0.03	0.03	0.03	0.01	0.02	0.01	0.02	0.2	0.1	0.2	0.04	0.1	0.04	0.1

* Fresh material was supplied.

Looking over the table 24 we can find K_2O, MgO and protein to be the substances the quantity-change of which runs parallel to the resistance-change of the mulberry stems in the growing stage against the invasion of the causal fungus in N-plots and also to the formation-change of the sporodochia in N-plots and K_2O-plots.

(2) Glucose, starch, and fat by microchemical methods

Materials for starch- and fat-investigations were fixed in formalin acetic alcohol no. 2, and those for the glucose-investigation were supplied without fixing. Cross sections of the bark tissues at the middle part of the 1st year twig were made by free hand; and glucose, starch and fat were investigated by Fehling's solution method, Jodjodkalium method and Sudan III method respectively. These substances were investigated three times (each time 6–8 sections of each plot were observed). The average result of the three times investigations is shown in Table 25. Figures in the table show the formation degree; they are obtained from the average values of various formation degrees (0–none, 0.5–very little, 1–little and 1.5–much).

Table 25 seems to indicate that the accumulation of glucose is influenced by N–manure and that of starch by K_2O–manure. But as each result of three times examinations fluctuated fairly much, more experiments must be carried out before the writer comes to the conclusion.

(3) Amino acids by paper chromatography

Dry powder (0.3 g) of material of each plot was hydrolysated in 6N–HCl (4cc) at 115–120°C for 18 hours. Protein and the other high-molecular nitrogenous substances are considered to have been dissolved into amino acids after this procedure.

The method of two-dimensional chromatograms was taken using 40 cm^2 filter paper (Tôyô-roshi no. 2) and large Petri dishes into which solvents were poured. The material hydrolysate which was put as a dot on the filter paper was developed upwards on the filter paper firstly with the mixture of phenol 100: dist. water 23 (in volume) and secondly with the water saturated mixture of butanol 4: gracial acetic acid 1 (in volume). The development was done under the natural temperature in bell jars. The 0.2%–ninhydrin butanol solution was sprayed on the filter paper to know the presence of amino acids. And Rf values of the amino acids were measured. On the other hand chemicals of various amino acids were used and examined of their Rf values on the

Table 25. The influence of N- and K_2O-manure upon the accumulation of glucose, starch and fat in the bark of the mulberry stems in the growing stage

	Kind of plot / Kind of tissue	N-experiment			K_2O-experiment			
					A exp.		B exp.	
		N-little	N-much	N-very much	K_2O-given	K_2O-not given	K_2O-given	K_2O-not given
Glucose	Epidermis	1.3	1.1	1.0	1.2	1.2	1.2	1.2
	Cortex chlorenchyma	1.3	1.2	1.1	1.3	1.2	1.3	1.2
	Cortex collenchyma	1.3	1.1	1.2	1.2	1.1	1.2	1.2
	Phloem parenchyma	1.2	1.1	1.2	1.1	1.0	1.1	1.1
	Phloem medullary ray	1.1	1.0	0.9	1.1	0.9	1.1	1.1
	Cambium and un-differentiated cells	1.3	0.9	0.8	1.4	1.0	1.0	1.4
	Total	7.5	6.4	6.2	7.3	6.4	6.9	7.2
Starch	Epidermis	0.01	0.03	0	0.03	0.01	0	0
	Cortex chlorenchyma	1.5	1.3	1.3	1.1	1.0	1.3	0.8
	Cortex collenchyma	0.7	0.6	0.7	0.7	0.4	0.6	0.6
	Phloem parenchyma	0.8	0.8	1.3	1.0	0.9	1.2	1.2
	Phloem medullary ray	1.0	0.9	0.7	0.8	0.6	0.7	0.7
	Total	4.01	3.63	4.0	3.63	2.91	3.8	3.3
Fat	Epidermis	0.3	0.2	0.2	0.4	0.3	0.2	0.2
	Cortex chlorenchyma	0.5	0.5	0.5	0.6	0.4	0.5	0.6
	Cortex collenchyma	0.5	0.6	0.6	0.6	0.5	0.6	0.6
	Phloem parenchyma	0.6	0.6	0.7	0.7	0.7	0.6	0.7
	Phloem medullary ray	0.4	0.3	0.5	0.4	0.6	0.6	0.5
	Cambium and un-differentiated cells	0.4	0.4	0.7	0.4	0.5	0.5	0.5
	Total	2.7	2.6	3.2	3.1	3.0	3.0	3.1

same filter paper by the same method. The Rf values of the amino acids in the material hydrolysate were compared with those of the amino acids of chemicals in order to determine their names. The results are shown in the table 26.

Table 26. Rf values of amino acids in the bark hydrolysate of the mulberry stems

Solvent / Kind of amino acid	The mixture of phenol 100: dist. wat. 23						The water saturated mixture of butanol 4: gracial acetic acid 1					
	Chemicals	In bark hydrolysate					Chemicals	In bark hydrolysate				
		N-little	N-much	N-very much	K_2O-given	K_2O-not given		N-little	N-much	N-very much	K_2O-given	K_2O-not given
Glycine	0.26	0.24	0.23	0.24	0.23	0.22	0.18	0.17	0.17	0.16	0.16	0.17
Alanine	0.48	0.48	0.46	0.46	0.46	0.44	0.34	0.31	0.30	0.30	0.32	0.31
Serine	0.25	0.24	0.21	0.23	0.23	0.21	0.23	0.23	0.24	0.24	0.23	0.24
Valine	0.75	0.66	0.66	0.65	0.66	0.65	0.61	0.56	0.57	0.54	0.57	0.53
Leucine	0.78	0.73	0.73	0.72	0.72	0.71	0.69	0.67	0.68	0.66	0.66	0.67
Aspartic acid	0.22	0.21	0.21	0.20	0.20	0.21	0.33	0.28	0.30	0.29	0.25	0.29
Glutamic acid	0.30	0.30	0.30	0.30	0.30	0.29	0.27	0.27	0.29	0.27	0.26	0.29
Phenylalanine	0.86	0.81	0.80	0.78	0.76	0.75	0.67	0.65	0.65	0.66	0.65	0.65
Tyrosine	0.54	0.52	0.49	0.49	0.49	0.48	0.50	0.45	0.46	0.45	0.45	0.46
Histidine	0.23	0.21	0.21	0.20	0.20	0.19	0.29	0.27	0.26	0.24	0.24	0.27
Proline	0.83	0.85	0.80	0.83	0.79	0.82	0.38	0.37	0.39	0.39	0.39	0.39
Unknown A		0.33	0.31	0.32	0.32	0.31		0.20	0.22	0.21	0.20	0.21
〃 B		0.40	0.38	0.40	0.37	0.37		0.28	0.28	0.29	0.28	0.29
〃 C		0.71	0.70	0.68	0.68	0.68		0.25	0.26	0.25	0.25	0.26

Looking over the table 26, we can know the presence of the following amino acids with 3 unknown substances in the bark hydrolysate of the mulberry stems of all N- and K_2O-plots; glycine, alanine, serine, valine, leucine, aspartic acid, glutamic acid, phenylalanine, tyrosine, histidine, and proline.

2. The Pathological significance of the Change of the Main Chemical Components of the Bark of the Mulberry Trees in the Growing Stage in N- and K_2O- Experiments

SUZUKI[48,49] stated that K-NH_4 ratio in rice leaves was related to the resistance of the rice plant to the blast disease. The result of the writer's experiment concerning the ash ingredients in the bark of the mulberry stems, which were shown in Table 24, indicated that the change of the amount of K_2O (and also MgO) ran parallel to the change of the resistance of the mulberry stems to the invasion of the causal fungus in N-experiment and also to the change of the sporodochial formation in N- and K_2O-experiments. The change of K-NH_4 ratio in the bark also runs parallel to the change of the resistance of the mulberry stems and also to the change of the sporodochial formation. But the writer thinks that it is very difficult to determine experimentally the pathological significance of the ash ingredients. At any rate another investigation must be planned in future to determine the pathological significance of K_2O or K-NH_4 ratio and also MgO in the bark of the mulberry trees.

TAHARA[52] thought that the growth of *Piricularia Oryzae* BR. et CAV. was promoted when the balance of various nitrogenous substances broke in rice leaves, for instance, when the density of not-proteinous N rose in comparison with that of protein. SAKAMOTO[40] stated that the increase of susceptibility of rice leaves to the blast disease owing to N-manure depended on that accumulation of ammonia in the leaves which had the injurious influence upon the function of the cells of the rice leaves. However, the results of the writer's experiment which were shown in Table 24 indicates that the amount of not-proteinous nitrogen or free ammonia in the bark did not always run parallel to the change of the resistance of the mulberry trees to the invasion of the causal fungus in N-experiment and also to the change of the sporodochial formation in N- and K_2O-experiments.

ŌTANI[37,39] states that the change of the susceptibility of the rice leaves to the blast disease depends on the change of the amount of soluble organic N compounds (especially α-amino acids and basic amino acids) in the rice leaves. He[37,38] regarded these compounds as the nitrogen sourse of the growth or the production of enzyme of *Piricularia Oryzae* BR. et CAV. which invaded in the rice leaves. TANAKA and KATSUKI[53] also stated that the increase of amids in the rice plant under the susceptible condition promoted the growth of *Piricularia Oryzae* BR. et CAV. ALTEN and ORTH [1] studied the relation between amino acids contained in the tubers and the leaves of the potato and the susceptibility of the potato to *Phytophthora infestans* DE BARY. They found out that both the concentration of arginine in the tubers and the leaves of the potato and the resistance of the tubers to the invasion of the causal fungus were raised by K-manure. On the other hand they recognized that arginin (and also sulphur-containing amino acids) checked the germination of the sporangia of the causal fungus. Many other investigators[5, 7, 13, 14, 24, 25, 26, 27, 28, 31, 33, 34, 35, 44, etc.] have also studied the influence of amino acids to the growth or the spore formation of various fungi. These experimental results suggest that the influence of amino acids to the growth or the spore formation of fungi is not definite and varies with the kinds of amino acids as well as those of fungi.

The writer investigated those kinds of amino acids which were produced as the results of protein hydrolysis (or in free state) in the bark of the mulberry stems by the paper chromatography in the previous section. He is now going to inquire into the influence of these amino acids to the growth and the sporulation of *Gibberella lateritium* (NEES) S. et H. The fundamental components of the medium which was supplied for this experiment are as follows——K_2HPO_4 5 g, $MgSO_4$ 0.2 g, sucrose 10 g, water 1 l and agar 20 g. Various amino acids of chemicals were added to the fundamental components in the proportions of 1%, 0.1% and 0.01% per the amount of water. The cultural experiment was done in test tubes three times at about 25°C. Each time 5 test tubes of medium were supplied for each amino acid. The average results of these experiments are shown in the table 27. The data in the table 27 indicate that the effect of amino acids to the growth of *G. lateritium* (NEES) S. et H. is not definite and varies with the kinds of the amino acids, but the effect of amino acids to the spore formation of the same fungus is very remarkable and promotes the spore formation without exception so far as the writer's experiments are concerned. It was known by chemical analyses in the previous section that N-manure promoted the amount of protein in the bark but K_2O-manure diminished it a little. It was also known in Chapter II of this paper that N-manure promoted the sporodochial formation of *G. lateritium* on the mulberry stems but K_2O-manure diminished it in the growing stage of the mulberry trees. These experimental results seem to suggest that

Table 27. The effect of various amino acids to the growth and the sporulation of *G. lateritium* (NEES) S. et H.

Amino acid: Kind	Amino acid: % added	Growth (diameter of hyphal colony, cm)	Spore formation: Pionnotes	Spore formation: Sporodochia	Total
Glycine	1	2.09	++(+)	++	++++(+)
	0.1	2.14	++	+++	+++++
	0.01	2.37	+(+)	+	++(+)
l-Alanine	1	1.12	++	++++	++++++
	0.1	1.65	+	+++	++++
	0.01	2.38	+	+(+)	++(+)
Serine	0.1	1.82	++(+)	+	+++(+)
	0.01	2.40	+	±	+
dl-Valine	1	2.15	+++	+(+)	++++(+)
	0.1	2.16	+(+)	++(+)	++++
	0.01	2.16	+	++	+++
l-Leucine	1	1.70	+++	—	+++
	0.1	2.15	+(+)	+	++(+)
	0.01	2.41	+	±	+
l-Aspartic acid	0.01	2.26	+	+	++
l-Glutamic acid	0.1	2.34	++(+)	++	++++(+)
	0.01	1.93	+	+++	++++
dl-Phenylalanine	1	2.00	+	++(+)	+++(+)
	0.1	2.36	+	++(+)	+++(+)
	0.01	2.23	(+)	+(+)	++
l-Tyrocine	0.1	2.10	+(+)	++	+++(+)
	0.01	2.75	(+)	+	+(+)
l-Histidine	0.1	2.38	++	++(+)	++++(+)
	0.01	2.65	+(+)	+	++(+)
l-Proline	1	2.63	+	++++	+++++
	0.1	2.81	+	+++	++++
	0.01	2.64	+	+	++
(Control)	0	2.36	±	(+)	(+)

Note; (1) pH values of the media before the inoculation of the fungus were pH 5.2—7.0 except those of aspartic acid (0.1% : pH 3.0—3.1 and not coagulable) and glutamic acid (1% : pH 3.0—3.1 and not coagulable). (2) 1% plots of serine, tyrocine and histidine were not established from the beginning. (3) 'Growth' was measured when 5 days had passed since the inoculation, and 'spore formation' was measured when 20 days had passed since the inoculation.

the degree of the spore formation of *G. lateritium* (NEES) S. et H. can be explained by the amount of protein (or amino acids as the product of protein hydrolysis). It may be doubtful that the differences of the spore formation on the mulberry stems among N- or K_2O-plots depend wholly upon the differences of the amount of protein (or amino acids as the product of protein hydrolysis) in the bark of the mulberry stems among the same plots. But the writer can say without a mistake that the differences of the amount of protein (or amino acids as the product of protein hydrolysis) influence more or less the spore formation of the causal fungus on the mulberry stems.

Summary

This paper dealt with the pathological studies of the "bud blight" of mulberry trees, especially of the influence of manure upon the development of this disease and the formation of sporodochia and perithecia of the causal fungus. The mulberry trees which were cultured in the round pots (75 cm or 95 cm diameter, 120 cm depth, bottomless, and buried in the soil) one tree in one pot, were supplied for these investigations, and the following results were obtained.

1. N-manure has no influence on the development of the "bud blight" of mulberry trees in the not-growing stage (autumn, winter and early spring) though the same manure promotes a little the development of the disease in the growing stage of mulberry trees. This result is very interesting from the pathological and also practical points of view, because the development of this disease in the not-growing stage of mulberry trees is far more conspicuous and destructive than that in the growing stage of mulberry trees. K_2O-manure and P_2O_5-manure have no influence upon the development of this disease in the growing stage as well as in the not-growing stage of the mulberry trees.

2. The formation of sporodochia of the causal fungus on the mulberry stems is promoted by N-manure, but is repressed by K_2O-manure only in the growing stage of the mulberry trees and is not influenced by P_2O_5-manure. On the other hand the formation of perithecial stromata of the causal fungus on the mulberry stems is influenced neither by P_2O_5-manure nor by N- and K_2O-manure.

3. The formation degree of the wound periderm (or wound cork layer),

which is formed around the injury on the mulberry stem in the growing stage, is promoted by K_2O-manure, but is not influenced by N- and P_2O_5-manure. Inquiring into the relation between the formation degree of the wound cork layer and the development degree of the disease in the growing stage in N- and K_2O-experiments, the writer could know the following fact that the formation degree of the wound cork layer had nothing to do with the development degree of the disease. This result seems to confirm the latter part of the following writer's conclusion, which was stated in the previous paper[30], that the wound periderm has a very important defensive function against the invasion of the causal fungus but is not the only defensive factor against the invasion.

4. The influence of N- and K_2O-manure upon the main chemical components of the bark of the mulberry stems was investigated. K_2O, MgO and protein were found as the substances, the quantity-change of which ran parallel to the resistance-change of the mulberry stems to the invasion of the causal fungus (in N-experiment) and also to the change of the sporodochial formation (in N- or K_2O-experiments). The writer discussed especially on the influence of the amount-change of the protein in the bark upon the sporodochial formation of the causal fungus. The kinds of amino acids which were produced by the hydrolysis of the protein in the bark (or in free state) were investigated by the paper chromatography; and glycine, alanine, serine, valine, leucine, aspartic acid, glutamic acid, phenylalanine, tyrosine, histidine and proline were found. The writer confirmed by another investigation that these amino acids promoted without exception the sporodochial formation of the causal fungus. From the results of these experiments the writer concluded that the differences of the amount of protein (or amino acids as the products of the protein hydrolysis) in the bark of the mulberry stems among N- or K_2O-plots influenced more or less the difference of the spore formation of the causal fungus on the same mulberry stems.

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Explanation of plate

Fig. 1 Showing the formation of sporodochia on the mulberry stem media. A: N-little plot, B: N-much plot, C: N-very much plot.

Fig. 2 Showing the formation of perithecial stromata on the mulberry stem medium.

Fig. 3 Showing the culture of the mulberry trees (Dec. 1951).

Plate