PHYSICO-CHEMICAL PROPERTIES OF THE VIRUS OF BROAD BEAN MOSAIC

By

Daiki MURAYAMA

Contents

I. Introduction

In Japan the mosaic disease of broad bean was noticed for the first time by Dr. FUKUSHI in Tottori in 1928. In the summer of 1935, mosaic diseases made their appearance on the pea and broad bean plants which were grown in the experimental plots of the University Farm in Sapporo.

Recent investigations appear to indicate that the mosaic diseases of pea, broad bean and red clover are all intertransmissible and these diseases may probably be caused by the same agency and that the virus may overwinter in the red clover. (DOOLITTLE and JONES

[Jour. Facul. Agr., Hokkaido Imp. Univ., Sapporo, Vol. XLIV, Pt. 1, October, 1938]

(1925)[6], BÖNING (1927)[1], CHAMBERLAIN (1937)[3], MURPHY and PIERCE (1937)[20], PIERCE (1937)[28], and FUKUSHI (1937)[10]).

It is considered to be most significant to investigate the physical properties of the virus of broad bean mosaic in order to make certain regarding the relations above mentioned and to identify it with other legume viruses hitherto reported. The present work was undertaken to obtain some ideas on the physical properties of the virus of the mosaic disease of broad bean. It was carried out during the period from 1935 to 1937.

The writer wishes to acknowledge gratefully his indebtedness to Dr. S. ITO and Dr. Y. TOCHINAI, Professors of Hokkaido Imperial University, and to Dr. UEMURA, Director of Morioka Imperial College of Agriculture and Forestry, for their kind advices. Thanks are also due to Dr. FUKUSHI, Assistant Professor of Hokkaido Imperial University, for his constant encouragement in the course of the present investigation and for reading this manuscript and also to Prof. HASEGAWA for kind help rendered in the work on H-ion concentrations and to Prof. KOSAKA and Assistant Profs. YAMAMOTO and OKUMA in the greenhouse work at Morioka College of Agriculture and Forestry.

II. Review of literature

The earliest report concerning the mosaic disease of broad bean plant was made by DICKSON[4], who read a paper on mosaic diseases of plants before the meeting of the Canadian Division of the American Phytopathological Society which was held in 1920. Cytological studies of mosaic broad bean plants were published by DICKSON (1922)[5], NELSON (1923)[21], and SCHAFFNIT and WEBER (1927)[31]. In 1927, BÖNING[1] described, in detail, the external and internal symptoms and the results of transmission experiments of the mosaic disease of broad bean plant. The relation of aphids to the transmission of broad bean and other legume mosaics was also reported by VAN DER MEULEN (1928)[34] and MERKEL (1929)[19]. In 1930, FUKUSHI[9] reported the occurrence of this disease in Japan. In 1934, IMAI[13] stated that the broad bean mosaic was readily transmitted by the agency of three species of aphids, Aphis laburni, Acyrthosiphon pisi and Rhopalosiphum persicae and that it was successfully transmitted through plant juice. The relation of broad bean

mosaic to other legume mosaics has been reported by many investigators (REDDICK and STEWART (1919)[29], ELLIOTT (1921)[7], KURIBAYASHI (1926)[18], BÖNING (1927)[1], VAN DER MEULEN (1928)[34], MERKEL (1929)[19] IMAI (1933, 34)[12,13], SAITO (1935)[30], JOHNSON and JONES (1936, 37)[14,15], HARTER (1936)[11], CHAMBERLAIN (1936)[2], ZAUMEYER and WADE (1935, 36)[35,36,37] PIERCE (1935, 37)[27,28], STUBBS (1936, 37)[32,33], OSBORN (1934, 35, 37)[22,23,24,25], MURPHY and PIERCE (1937)[20] and FUKUSHI (1937)[10].

III. Symptoms of the disease

The most characteristic symptoms of this disease are the mottling of the leaves due to the presence of light-green areas of various shapes and sizes and the dwarfness of the plant, as in the other mosaic diseases of plants. The symptoms closely resemble those of the "Marmoriermosaik" of BÖNING. The symptoms are more or less various, depending upon the age of the plant when inoculated and the environmental conditions under which the plants are grown.

The first noticeable symptoms of the mosaic disease usually appear on the growing leaves as a slight upward curling and distortion and the leaves become at the same time more or less lighter green. As these leaves increase in size, the slight clearing of the veins or numerous clear spottings make their appearance. Later dark-green, irregularly shaped areas appear adjoining the light green portions which develop along the veins. As these leaves grow larger, the dark-green areas develop rapidly into large, irregular swellings, whereas the yellowish green portions are markedly reduced in their growth and remain unthickened. The mosaic-infected leaves are smaller and narrower than healthy ones, exhibiting a slight waving and curling of the edges and wrinkling. The leaflets also show extreme malformation; sometimes the apex of the leaflet is divided into two portions or tapers towards the distal end. In the most advanced stages of the disease the green areas occupy only small areas and larger portions of the leaflet become pale yellow. The mosaic patterns also appear on the stipules. The symptoms usually appear on the young shoots and leaves which developed after infection, while the older leaves which have almost completed their growth before infection appear entirely normal. On the stems of the infected plants, elongated dark brown specks are often produced, form-

ing fine interrupted streaks. The plants infected at a very early stage of their growth become severely stunted. The blossoms of the affected plants are few in number. In particularly severe cases the plants often develop small, misshapen blossoms: in such cases the pods are small and may produce only few viable seeds.

IV. Materials and methods

Materials

Most of the experimental work was carried out in the greenhouse at Morioka Imperial College of Agriculture and Forestry, though a part of the work was performed at Hokkaido Imperial University. As for the source the virus was obtained from mosaic-infected broad bean plants grown in the Experimental Farm of Hokkaido Imperial University in 1935. The plants used for inoculation were vigorously growing young broad bean plants (*Vicia Faba* L.) of Issun-Soramame variety. The seeds were sown in sawdust in wooden boxes. Being sprinkled every day, these seeds began to germinate in about a week in the greenhouse. Then the germinated young plants were immediately transplanted into humus soil in four-inch pots. When the potted plants were ascertained to be healthy and the fifth or sixth leaf was just beginning to grow or when the plants reached 10 to 25 cm. in height, they were used for inoculation. The number of plants inoculated in each experiment was ten and great care was taken to use healthy plants of nearly the same age and uniform size.

Methods

All glass wares, mortar, scalpel and needles to be used were thoroughly washed and sterilized by boiling for about half an hour in the water bath or by KOCH's steam sterilizer. Fresh, young leaves and stems of mosaic-diseased broad bean plants were thoroughly ground in a sterilized mortar and filtered off through two thicknesses of thin cotton cloth. The filtrate was used for inoculation.

Inoculations were performed by pricking each plant with the needle. A large drop (approximately 0.5 c.c.) of the inoculum was placed with a sterile scalpel on a leaf axil and with a sterile needle about 30 pricks were made through the fluid. Three inoculations were made on each plant on the axils at the basal part of the stem.

Between different inoculations the needles and scalpel were flamed, while the hands were washed with soap several times. All the inoculations were performed in the afternoon and then the floor of the greenhouse was watered so as to prevent a too rapid drying.

V. Experimental results

A. Effects of dilution

In order to obtain some idea concerning the effect of dilution upon the infective power of the juice of mosaic broad bean plant, the following experiments were carried out.

Young stems and leaves of the mosaic-infected broad bean plants were ground in a sterilized mortar and the expressed juice was filtered through two thicknesses of thin cotton cloth. Using sterilized graduated cylinders and pipettes, the desired dilutions were obtained. The inoculations were made beginning with the highest degree of dilution to avoid accidental infection by contaminated hands. The results of the experiments are shown in summarized form in the following table.

TABLE I. The effect of dilution upon the virus in the extract from mosaic broad bean plants

TABLE Ia

No. of experiment	Date of inoculation	Degree of dilution	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
1	Sep. 4 '35	1 10,000 1 : 5,000 1 : 1,000 cont.*	10 10 10 10	0 0 0 10	7-12	100%
2	Oct. 6 '35	1 : 1,000 1 : 500 1 : 100 cont.	10 10 10 10	0 0 5 6	10-14 10-12	50 60
3	Oct. 26 '35	1 : 300 1 : 100 1 : 50 cont.	10 10 10 7	0 2 7 6	8-10 8-16 8 19	20 70 86

* Undiluted mosaic juice.

TABLE Ia (*Continued*)

No. of experiment	Date of inoculation	Degree of dilution	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
4	Feb. 1 '36	1 : 200	10	4	10-20	40
		1 : 150	10	5	10-20	50
		1 : 100	10	3	10-17	30
		cont.	10	6	10-20	60
5	Mar. 2 '36	1 : 300	10	3	13-20	30
		1 : 250	10	2	8-13	20
		1 : 200	10	4	8-13	40
		cont.	10	8	8-20	80
6	Mar. 5 '36	1 : 300	10	6	10-20	60
		1 : 250	10	2	10	20
		1 : 200	10	2	10	20
		1 : 50	10	7	10-20	70
		cont.	10	8	10-21	80
7	July 24 '35	1 : 500	10	1	19	10
		1 : 400	10	2	15	20
		1 : 300	10	2	12-15	20
		1 : 200	10	8	10-21	80
		1 : 10	10	10	10-14	100
8	July 25 '36	1 : 500	10	1	14	10
		1 : 400	10	1	16	10
		1 : 200	10	3	10-12	30
		1 : 10	10	6	9-11	60
9	Sep. 26 '36	1 : 500	10	0		
		1 : 400	10	2	17-18	20
		1 : 300	10	1	11	10
		1 : 200	10	1	11	10
		1 : 100	10	1	11	10
		1 : 10	18	10	10-18	56
10	Oct. 9 '36	1 : 700	10	1	20	10
		1 : 400	10	3	20-22	30
		1 : 300	10	2	22	20
		1 : 200	10	4	20-22	40
		1 : 100	10	2	14	20
		1 : 10	15	9	14-22	60
11	Oct. 16 '36	1 : 500	10	1	17	10
		1 : 400	10	5	15-17	50
		1 : 300	10	5	15-17	50
		1 : 200	10	9	17	90
		1 : 10	10	10	13-18	100
12	Nov. 4 '36	1 : 700	10	2	20-25	20
		1 : 600	10	4	20-24	40
		1 : 500	10	1	20	10
		1 : 400	10	6	21-25	60
		cont.	5	5	20-25	100

TABLE Ia (*Continued*)

No. of experiment	Date of inoculation	Degree of dilution	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
13	Nov. 12 '36	1 : 600	10	1	20	10
		1 : 500	10	2	18–21	20
		1 : 400	10	2	18–22	20
		cont.	5	3	18–20	60
14	Feb. 26 '37	1:1,000	10	0		
		1 : 800	10	0		
		1 : 700	10	0		
		1 : 500	10	1	21	10
		1 : 10	10	7	10–11	70
15	July 20 '37	1:1,000	10	0		
		1 : 900	10	1	12	10
		1 : 800	10	0		
		1 : 500	10	0		
		cont.	10	9	10–14	90

TABLE Ib

Degree of dilution	1:10,000	1:5,000	1:1,000	1:900	1:800	1:700	1:500	1:400
Total number of plants tested	0*/10	0/10	0/40	1/10	0/20	3/30	7/90	21/70
Percentage of infection %	0	0	0	10	0	10	7.8	30
Degree of dilution	1:300	1:250	1:200	1:150	1:100	1:50	1:10	Undiluted mosaic juice
Total number of plants tested	19/70	4/20	35/80	5/10	13/50	14/20	52/73	61/77
Percentage of infection %	27	20	44	50	26	70	71	79

* The numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

As shown in the summarized table, the dilution of the virus of broad bean mosaic at the rate of one part of mosaic juice to five hundred parts of water materially decreases the infectivity and in some cases the infective power was lost at the dilution near 1:500,

according to the concentration of the virus in the extracted mosaic juice. The virus lost its infective power at dilutions greater than 1:1,000.

B. Resistance to heat

The expressed juice of mosaic-diseased broad bean plants was placed in pieces of glass tubing about 5.8 mm. in diameter and 80 mm. long, approximately 3 c.c. of juice being contained in each tube, and plugged with rubber-stoppers at both ends. The tubes containing the virus extract were immersed in hot water in the constant temperature tank, the temperatures of which were regulated within 0.2°C. around the desired temperature. After 10 minutes the tubes were removed and plunged into cold water immediately. The contents of the tubes were used as inoculum. The experimental results are summarized in the following table.

TABLE II. Effect of temperatures upon the virus of broad bean mosaic

TABLE IIa

No. of experiment	Date of inoculation	Temperature in degrees °C.	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
1	Oct. 12 '35	70	10	0		
		60	10	0		
		50	10	0		
		cont.*	7	2	11-14	29%
2	Nov. 11 '35	60	10	1	18	10
		50	10	5	10-17	50
		40	10	8	12-17	80
		30	10	5	16-18	50
		cout.	3	1	15	33
3	Dec. 30 '35	63	10	0		
		60	10	1	22	10
		57	10	0		
		cont.	10	10	8-12	100
4	Feb. 2 '36	57	10	2	12-16	20
		55	10	0		
		53	10	1	17	10
		50	10	3	12-15	30
		cont.	10	8	10-15	80

* Unheated mosaic juice.

TABLE IIa. (*Continued*)

No. of experiment	Date of inoculation	Temperature in degrees °C.	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
5	Mar. 7 '36	63	10	1	14	10
		60	10	0		
		57	10	2	11 15	20
		55	10	6	10-14	60
		cont.	10	7	10-14	70
6	Mar. 10 '36	63	10	0		
		60	10	0		
		57	10	1	15	10
		50	10	6	10-16	60
		cont.	10	8	11-16	80
7	Dec. 5 '36	63	10	0		
		60	10	0		
		57	10	1	17	10
		55	10	1	18	10
		cont.	10	10	11-13	100
8	Apr. 20 '37	57	10	0		
		55	10	0		
		cont.	10	10	14-16	100
9	May 11 '37	63	10	0		
		60	10	0		
		57	10	0		
		55	10	2	17	20
		cont.	10	7	12-15	70
10	June 10 '37	63	10	0		
		60	10	1	9	10
		57	10	0		
		55	10	0		
		cont.	10	8	8-9	80
11	June 18 '37	63	10	0		
		60	10	0		
		57	10	0		
		55	10	1	12	10
		cont.	10	10	10-12	100
12	June 24 '37	63	10	0		
		60	10	0		
		57	10	2	10	20
		55	10	0		
		cont.	10	8	10-12	80

TABLE IIa (*Continued*)

No. of experiment	Date of inoculation	Temperature in degrees °C.	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
13	July 24 '37	63	10	0		
		60	10	1	11	10
		58	10	1	13	10
		56	10	0		
		cont.	10	9	10–14	90
14	Aug. 6 '37	62	10	0		
		60	10	0		
		58	10	0		
		56	10	0		
		cont.	10	8	10–15	80

TABLE IIb

Temperature in degrees °C.	70	63	62	60	58	57	56	55	50	40	30	Unheated mosaic juice
Total number of plants tested	0*/10	1/90	0/10	4/120	1/20	8/100	0/20	10/80	14/40	8/10	5/10	106/130
Percentage of infection %	0	1.1	0	3.3	5	8	0	12.5	35	80	50	82

* Tne numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

As shown in the summarized table, the virus under consideration was weakened at temperatures higher than 50°C. and nearly or entirely lost its virulence at 63°C. in ten minutes. Sometimes the virus was inactivated even at 50°C.

C. Longevity *in vitro*

Young stems and leaves of mosaic broad bean plants were ground and the extracted juice was kept for varying lengths of time in tightly plugged sterile test tubes with or without the addition of an antiseptic at room temperature or in an incubator. After the desired period of time, the juice which had been kept in this manner was shaken in order to mix up the supernatant fluid with the precipitate and was then used as inoculum. The results are shown in the following table.

TABLE III. The resistance to aging *in vitro* of the broad bean mosaic virus

TABLE IIIa

No. of experiment	Date of inoculation	Age of virus in days	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection	Remarks
1	Nov. 10 '36	25	10	0			without the addition of preservative and kept at room temperature (at 23—1°C.)
	Nov. 5 '36	20	10	1	18	10%	
	Oct. 28 '36	12	10	3	21-24	30	
	Oct. 16 '36	cont.[a]	10	10	19-25	100	
2	Nov. 19 '36	15	10	4	26-27	40	without the addition of preservative and kept at room temperature (at 23—1°C.)
	Nov. 4 '36	cont.	5	5	20-25	100	
3	Dec. 3 '36	21	10	1	18	10	without the addition of preservative and kept at room temperature (at 22—1°C.)
	Nov. 27 '36	15	10	2	18-20	20	
	Nov. 12 '36	cont.	10	4	15-17	40	
4	Jan. 21 '37	23	10	0			without the addition of preservative and kept in the incubator (at 28°C.)
	Jan. 18 '37	20	10	0			
	Jan. 13 '37	15	10	1	13	10	
	Jan. 8 '37	10	10	1	14	10	
	Dec. 29 '36	cont.	10	10	13-15	100	
5	Feb. 1 '37	11	10	1	16	10	without the addition of preservative and kept in the incubator (at 25°C.)
	Jan. 26 '37	5	10	0			
	Jan. 21 '37	cont.	5	2	15-19	40	
6	Mar. 9 '37	11	10	1	15	10	without the addition of preservative and kept in the incubator (at 28°C.)
	Mar. 3 '37	5	10	4	9 30	40	
	Mar. 1 '37	3	10	1	8	10	
	Feb. 26 '37	cont.	10	7	11-13	70	
7	Apr. 10 '37	15	10	0			without the addition of preservative and kept in the incubator (at 28°C.)
	Apr. 5 '37	10	10	1	17	10	
	Apr. 2 '37	7	10	3	9-24	30	
	Mar. 31 '37	5	10	3	11-26	30	
	Mar. 26 '37	cont.	10	8	10-14	80	

TABLE IIIa (*Continued*)

No. of experiment	Date of inoculation	Age of virus in days	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection	Remarks
8	May 8 '37	25	10	0			without the addition of preservative and kept in the incubator (at 28°C.)
	May 3 '37	20	10	0			
	Apr. 13 '37	cont.	10	8	13-17	80	
9	May 31 '37	20	10	0			without the addition of preservative and kept in the incubator (at 28°C., May 11-15, at 26°C., May 16-31)
	May 26 '37	15	10	0			
	May 21 '37	10	10	0			
	May 11 '37	cont.	10	7	12-15	70	
10	May 30 '37	10	10	1	17	10	without the addition of preservative and kept in the incubator (at 26°C.)
	May 27 '37	7	10	0			
	May 20 '37	cont.	10	10	14-16	100	
11	June 14 '37	20	10	0			added an equal weight of distilled water, without the addition of preservative and kept in the incubator (at 26°C.)
	June 9 '37	15	10	0			
	June 4 '37	10	10	0			
	May 30 '37	5	10	1	15	10	
	May 25 '37	cont.	10	10	12-15	100	
12	June 12 '37	14	10	0			without the addition of preservative and kept in the incubator (at 26°C.)
	June 8 '37	10	10	0			
	May 29 '37	cont.	10	9	15-17	90	
13	July 5 '37	25	10	0			added a drop of toluol and kept in the incubator (at 26°C.)
	June 26 '37	16	10	0			
	June 20 '37	10	10	0			
	June 10 '37	cont.	10	8	8-9	80	
14	July 5 '37	17	10	0			added a drop of toluol and kept in the incubator (at 26°C.)
	June 29 '37	11	10	0			
	June 26 '37	8	10	0			
	June 18 '37	cont.	10	10	10-12	100	

TABLE IIIa (*Continued*)

No. of experiment	Date of inoculation	Age of virus in days	Degree of dilution	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection	Remarks
15	July 23 '37 July 20 '37 July 15 '37	8 5 cont.		10 10 10	0 1 5	 15 11-14	 10 50	without the addition of preservative and kept in the incubator (at 26°C.)
16		In days	Degree of dilution					without the addition of preservative and kept in the incubator (at 26°C.)
	July 28 '37	8	1 : 10 cont.[b]	10 10	0 9	 11-14	 90	
	July 23 '37	3	1:1,000 1 : 800 1 : 500 1 : 10 cont.	10 10 10 10 10	0 0 0 0 9	 12-14	 90	
	July 20 '37	cont.[a]	1:1,000 1 : 900 1 : 800 1 : 500 1 : 10 cont.	10 10 10 10 10 10	0 1 0 0 9 9	 12 11-14 10-14	 10 90 90	
17	Oct. 16 '37 Oct. 12 '37 Oct. 9 '37 Oct. 6 '37	10 6 3 cont.		10 10 10 10	0 3 5 6	 12-14 13-16 14-18	 30 50 60	without the addition of preservative and kept at room temperature (25-7°C.)

a. Fresh mosaic juice.

b. Undiluted mosaic juice.

TABLE IIIb

Age of virus in days	25	21	20	15	12	10	6	3	Fresh mosaic juice	Remarks
Total number of plants tested	0* / 10	1 / 10	1 / 10	6 / 20	3 / 10	0 / 10	3 / 10	5 / 10	25 / 35	without the addition of preservative and kept at room temperature
Percentage of infection %	0	10	10	30	30	0	30	50	71	

Age of virus in days	25	23	20	15	14	11	10	8	7	5	3	Fresh mosaic juice	Remarks
Total number of plants tested	0 / 10	0 / 10	0 / 40	1 / 40	0 / 10	2 / 20	3 / 60	0 / 10	3 / 20	9 / 50	1 / 10	76 / 95	without the addition of preservative and kept in the incubator (at 25-28°C.)
Percentage of infection %	0	0	0	2.5	0	10	5	0	15	18	10	80	

Age of virus in days	8		3					Fresh mosaic juice						Remarks
Degree of dilution	1:10	undiluted	1:1,000	1:800	1:500	1:10	undiluted	1:1,000	1:900	1:800	1:500	1:10	undiluted	
Total number of plants tested	0 / 10	9 / 10	0 / 10	0 / 10	0 / 10	0 / 10	9 / 10	0 / 10	1 / 10	0 / 10	0 / 10	9 / 10	9 / 10	without the addition of preservative and kept in the incubator (at 26°C.)
Percentage of infection %	0	90	0	0	0	0	90	0	10	0	0	90	90	

Age of virus in days	25	17	16	11	10	8	Fresh mosaic juice	Remarks
Total number of plants tested	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	18 / 20	added a drop of toluol and kept in the incubator (at 26°C.)
Percentage of infection %	0	0	0	0	0	0	90	

* The numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

The virus lost its power of infection after about 21–25 days' aging *in vitro* without any antiseptics at room temperature, while in the incubator at 25–28°C. its virulence was lost after about 15–20 days' aging. The virus survived no longer as a result of adding a drop of toluol in the test tube containing the mosaic juice but was inactivated after 8 days' aging. The virus lost its infective power soon when the extract had been diluted.

D. Longevity in dried plant tissues

From 10 to 20 grams of young leaves and stems of mosaic-diseased broad bean plants were cut off, placed in a paper bag and preserved at room temperature for various lengths of time. The material was weighed at the beginning and the end of the period. The dried tissues were weighed and ground in a sterilized mortar, adding the same amount of distilled water as had been lost. Then the juice was filtered off and used as inoculum.

TABLE IV. The resistance to drying in the plant tissues of the virus of broad bean mosaic

TABLE IVa

No. of experiment	Date of inoculation	Duration of drying	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
1	Sep. 9 '36	15	10	0		
	Sep. 4 '36	10	10	0		
2	July 19 '37	25	10	0		
	July 14 '37	20	10	3	15-19	30%
	July 9 '37	15	10	8	14-16	80
	June 24 '37	cont.*	10	8	10-12	80
3	Sep. 20 '37	30	10	0		
	Sep. 15 '37	25	10	0		
	Sep. 5 '37	15	10	0		
4	Oct. 31 '37	25	10	0		
	Oct. 26 '37	20	10	0		
	Oct. 21 '37	15	10	4	16-18	40
	Oct. 6 '37	cont.	10	6	14-18	60

* Fresh mosaic juice.

TABLE IVa. *(Continued)*

No. of experiment	Date of inoculation	Duration of drying	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
5	Dec. 10 '37	25	10	4	17-20	40
	Dec. 6 '37	21	10	6	15-20	60
	Nov. 30 '37	15	10	4	14-19	40
6	Dec. 22 '37	25	10	3	16-24	30
	Dec. 17 '37	20	10	5	17-24	50
	Dec. 13 '37	16	10	4	14-23	40
7	Feb. 21 '38	30	10	1	12	10
	Feb. 16 '38	25	10	5	10-16	50
8	Feb. 28 '38	28	10	0		
	Feb. 25 '38	25	10	0		
	Feb. 22 '38	22	10	3	11-18	30
9	Mar. 23 '38	30	10	0		
	Mar. 21 '38	28	10	3	10-17	30
	Mar. 18 '38	25	10	8	10-22	80

TABLE IVb

Month of inoculation	June to October[a]					November to March							
Duration of drying	30	25	20	15	Fresh mosaic juice	30	28	25	22	21	20	16	15
Total number of plants tested	0[b]/10	0/30	3/20	12/30	14/20	1/20	3/20	20/50	3/10	6/10	5/10	4/10	4/10
Percentage of infection %	0	0	15	40	70	5	15	40	30	60	50	40	40

a: Except Experiment 1.

b: The numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

In Experiment 1 the preserved materials were so rotted owing to the moist atmosphere that the infectivity was lost even after 10 days. In the experiments carried out in winter, the materials were not so completely dried, the room temperature being low, so that the

materials were still yellowish green after 25 days and remained infectious, whereas in summer the plant tissues dried rapidly assuming a dark color, and lost their infectivity in 20 to 25 days.

E. Effects of ethylalcohol

Two methods were used for preparing the inoculum. In experiments 1 and 2, 10 c.c. of the extracted juice was put into a sterilized graduated cylinder and 40 c.c. of distilled water and 50 c.c. of absolute ethylalcohol (Merck Brand) were added. In this way 50 per cent alcohol solution was obtained. Likewise to each 10 c.c. of the extract in the graduated cylinder, were added 50 and 60 c.c. of distilled water and 40 and 30 c.c. of absolute ethylalcohol, respectively, to secure 40 and 30 per cent alcohol solution. In experiments 3 and 4, to each 20 c.c. of the mosaic juice were added 20, 16, and 12 c.c. of absolute ethylalcohol and then 4 and 8 c.c. of distilled water into the second and third, respectively, to obtain 50, 40 and 30 per cent alcohol solution. Each graduated cylinder was well shaken and allowed to stand for half an hour. In the first part of these experiments, where alcohol was added to the virus solution, a light flocculent precipitate occurred which was separated from the supernatant fluid by being allowed to stand. The precipitate was placed in a sterilized Petri dish and used as inoculum. In experiments 3 and 4, the solutions were not separated into two parts, so they were used as inoculum. The results of the experiments are shown in the following table. (Table V.)

From the results shown in the table it is considered that the virus of the broad bean mosaic is sensitive to alcohol and that it is destroyed in alcohol of a strength of 50 per cent in half an hour in most cases, although it occasionally tolerates a strength of 30 to 40 per cent.

F. Effects of formaldehyde

Seven c.c. of the mosaic juice was put into a sterilized graduated cylinder by means of pipette, to which 3 c.c. of 10 per cent formaldehyde (Merck Brand) was added. Thus 3 per cent formaldehyde solution was obtained. Likewise by mixing 7 c.c. of the extract, 1 c.c. of distilled water and 2 c.c. of 10 per cent formaldehyde in a graduated cylinder, 2 per cent formaldehyde solution was prepared.

TABLE V. The effect of ethylalcohol upon the virus in the extract from mosaic broad bean plants

TABLE Va

No. of experiment	Date of inoculation	Percentage of alcohol	Duration of treatment	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
1	Mar. 26 '37	50%	30 min.	10	0		
		40	do.	10	1	18	10%
		30	do.	10	6	9-18	60
		cont.*	do.	10	8	8-19	80
2	Apr. 20 '37	50	30 min.	10	0		
		40	do.	10	1	20	10
		30	do.	10	3	14-20	30
		cont.	do.	10	10	14-17	100
3	May 20 '37	50	30 min.	10	1	18	10
		40	do.	10	2	18	20
		30	do.	10	4	16-18	40
		cont.	do.	10	10	16-17	100
4	July 15 '37	50	30 min.	10	0		
		40	do.	10	0		
		30	do.	10	2	10-12	20
		cont.	do.	10	5	7-11	50

* Untreated mosaic juice.

TABLE Vb

Percentage of alcohol	50	40	30	Untreated mosaic juice
Total number of plants tested	1*/40	4/40	15/40	33/40
Percentage of infection %	2.5	10	37.5	82.5

* The numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

By similar method 1 per cent formaldehyde solution also was secured. The standard inoculum was prepared by mixing 7 c.c. of the extract and 3 c.c. of the distilled water. Each graduated cylinder was then

shaken well and allowed to stand for half an hour. To avoid the deleterious effects of formaldehyde upon the plant 15 c.c. of distilled water was added to each inoculum before inoculation.

TABLE VI. The effect of formaldehyde upon the virus of broad bean mosaic

TABLE VIa

No. of experiment	Date of inoculation	Percentage of formaldehyde	Duration of treatment	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
1	May 29 '37	3%	30 min.	10	0		
		2	do.	10	0		
		1	do.	10	0		
		cont.*	do.	10	9	16-18	90%
2	June 4 '37	3	30 min.	10	0		
		2	do.	10	0		
		1	do.	10	0		
		cont.	do.	10	9	12-14	90
3	June 16 '37	3	30 min.	10	0		
		2	do.	10	0		
		1	do.	10	0		
		cont.	do.	10	10	10-14	100

* Untreated standard inoculum.

TABLE VIb

Percentage of formaldehyde	3%	2%	1%	Untreated mosaic juice
Total number of plants tested	0 * / 30	0 / 30	0 / 30	28 / 30
Percentage of infection %	0	0	0	93

* The numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

As shown in the above tables, the virus of broad bean mosaic was promptly destroyed within half an hour by treatment with 1 per cent formaldehyde solution.

TABLE VII. Effects of hydrogen-ion concentrations upon the virus of broad bean mosaic

TABLE VIIa

No. of experiment	Date of inoculation	pH of the fluid		No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection	Remarks
		Original pH	Final pH					
1	Sep. 24 '37	4.8	4.8	10	6	11-15	60%	with quinhydrone electrode
		5.8 (cont.)	5.8	10	6	11-14	60	
2	Sep. 25 '37	2.6	2.8	10	0			with antimony electrode
		3.7	3.7	10	3	12-17	30	
		6.3 (cont.)	6.3	10	6	10-16	60	
3	Oct. 6 '37	6.3 (cont.)	6.3	10	6	13-18	60	with antimony electrode
		8.3	7.9	10	7	12-19	70	
		8.9	8.5	10	4	13-16	40	
		9.0	8.6	10	4	14-19	40	
		9.3	8.9	10	1	14	10	
		9.5	9.0	10	0			
4	Oct. 13 '37	3.0	3.0	10	0			with quinhydrone electrode
		3.8	3.8	10	4	18 22	40	
		4.0	4.0	10	7	12-20	70	
		4.4	4.2	10	8	13-21	80	
		4.9	4.8	10	10	12-18	100	
		6.4 (cont.)	6.3	10	10	12 18	100	
5	Oct. 14 '38	5.9 (cont.)	5.9	10	10	13-18	100	with quinhydrone electrode
		8.1	7.4	10	10	13-18	100	
		9.4	9.0	10	7	15-21	70	
		10.1	9.6	10	5	14-20	50	
		10.3	10.1	10	0			
6	Oct. 15 '37	5.8 (cont.)	5.8	10	10	17-21	100	with quinhydrone electrode
		8.3	7.9	10	7	16-20	70	
		9.1	8.7	10	8	15-20	80	
		2.8	2.8	10	0			
		2.0	2.0	10	0			
		1.9	1.9	10	0			

TABLE VIIb

pH of the fluid		No. of plants		Remarks	pH of the fluid		No. of plants		Remarks
Original pH	Final pH	Inoculated	Infected	Determined by	Original pH	Final pH	Inoculated	Infected	Determined by
1.9	1.9	10	0	quin.[a]	6.3	6.3	10	6	ant.
2.0	2.0	10	0	quin.	6.3	6.3	10	6	ant.
2.6	2.8	10	0	ant.[b]	6.4	6.3	10	10	quin.
2.8	2.8	10	0	quin.	8.1	7.4	10	10	quin.
3.0	3.0	10	0	quin.	8.3	7.9	10	7	ant.
3.7	3.7	10	3	ant.	8.3	7.9	10	7	quin.
3.8	3.8	10	4	quin.	8.9	8.5	10	4	ant.
4.0	4.0	10	7	quin.	9.0	8.6	10	4	ant.
4.4	4.2	10	8	quin.	9.1	8.7	10	8	quin.
4.8	4.8	10	6	quin.	9.3	8.9	10	1	ant.
4.9	4.8	10	10	quin.	9.4	9.0	10	7	quin.
5.8	5.8	10	10	quin.	9.5	9.0	10	0	ant.
5.8	5.8	10	6	quin.	10.1	9.6	10	5	quin.
5.9	5.9	10	10	quin.	10.3	10.1	10	0	quin.

a : Quinhydrone electrode. b : Antimony electrode.

G. Effects of the hydrogen-ion concentration

The H-ion concentrations of the juice of mosaic diseased broad bean plants were determined by the quinhydrone and antimony electrode method. As the virus could not pass through any grade of filters, the experiments were performed by using unfiltered mosaic juices. The pH value of the mosaic juice fell within the limits of 5.8 to 6.4. By adding 2 to 18 c.c. of 1/10 normal HCl and 2 to 10 c.c. of 1/10 normal NaOH solution to 10 or 15 c.c. of the virus solution (diluted with an equal amount of distilled water), the H-ion concentrations were changed from pH 4.9 to 1.9 on the acid side and from pH 8.1 to 10.3 in alkalinity, respectively. Before and after the inoculation the H-ion concentrations of the virus suspensions were determined.

As shown in the table (Table VII.) the infective power of the virus of broad bean mosaic was most virulent at pH 4–8 and greatly reduced when the alkalinity or acidity of the virus suspension was increased up to pH 9 and pH 3, respectively. The virus was inactivated at pH 3.0 (quinhydrone electrode) and pH 9.5 (antimony electrode).

H. Filtrability

Filtration was performed by using a coarse Shofu L_1 and L_2 filter at a negative pressure. The filtrate was light pink in color and used for inoculation. The results of the experiments are shown in the following table.

TABLE VIII. The filtrability of the virus of broad bean mosaic

TABLE VIIIa

No. of experiment		Date of inoculation	Filter	No. of plants inoculated	No. of plants infected	Incubation period (in days)	Percentage of infection
1	a	Jan. 27 '36	Shofu L_1 cont.*	10 5	0 4	16-21	80%
	b	Jan. 27 '36	Shofu L_2 cont.	10 5	0 5	16-20	100
2		Mar. 11 '36	Shofu L_1 Shofu L_2 cont.	10 10 5	0 1 3	16 15-21	10 60
3		Mar. 16 '36	Shofu L_2 cont.	10 5	0 3	16-21	60
4		Dec. 26 '36	Shofu L_2 cont.	10 5	0 4	16-21	80
5		Dec. 28 '36	Shofu L_2 cont.	10 5	0 4	14-21	80
6		Jan. 27 '37	Shofu L_1 cont.	10 5	0 2	19-20	40
7		Feb. 5 '37	Shofu L_1 cont.	10 5	0 5	11-20	100

* Unfiltered mosaic juice.

TABLE VIIIb

Filter	Shofu L_2	Shofu L_1	Unfiltered mosaic juice
Total number of plants tested	$\frac{1}{50}$*	$\frac{0}{40}$	$\frac{30}{40}$
Percentage of infection %	2	0	75

* The numerator indicates the number of infected plants, and the denominator the number of inoculated plants.

As shown above the virus of broad bean mosaic failed to pass through the filters, Shofu L_1 and L_2 at a negative pressure. The one case of infection produced by the L_2 filtrate may very probably have been caused by an accidental infection.

VI. Discussion and conclusion

Since JOHNSON (1927, 29) [16, 17] attempted to classify the viruses affecting tobacco and potato plants on the basis of plants affected, symptoms exhibited and their physico-chemical properties, a considerable number of investigations have been performed on that line.

PIERCE (1934, 35, 37) [26, 27, 28] and ZAUMEYER &c. (1935, 36, 37) [35, 36, 37, 38] have shown that a considerable number of viruses affect legumes, the separation of which was based upon symptomatology as well as their physico-chemical properties. According to them there are more than ten viruses which affect the broad bean plant in the United States of America.

The present investigations were undertaken in order to secure further information on the nature and identity of the virus of the broad bean mosaic in Japan. As shown in the tables, the results of each experiment did not necessarily coincide in all cases. This may be attributable to the different concentrations of the virus in the original extract secured from diseased plants and used for preparation of inoculum.

A comparison of the properties of the virus under consideration with those of other legume viruses are given in the following table. (Table IX.)

The Japanese broad bean mosaic virus gave no infections at dilutions higher than 1 to 1,000, while STUBBS (1936, 37) [32, 33] reported that pea virus 2A, 2B and 2C were rendered non-infectious at dilutions greater than 1 to 1,500. Pea virus 3 which is considered to be identical with OSBORN's pea virus 2 has been reported by PIERCE (1935) [27], MURPHY and PIERCE (1937) [20] and OSBORN (1937) [24] to be inactivated at temperatures of 62–64, 60 and 64°C. for ten minutes, respectively. The virus under consideration in the present paper lost its infective power at a temperature around 63°C. for ten minutes. As to the aging *in vitro*, the virus of broad bean mosaic remained infectious for relatively a longer period than the pea viruses as reported by PIERCE (1935) [27], STUBBS (1936, 37) [32, 33],

TABLE IX. The properties of viruses of legume mosaics

Source of virus	Properties of virus										Authority
	Tolerance to dilution	Resistance to heat	Longevity *in vitro*	Longevity in dried plant tissue	Strength of alcohol destroying the virus	Concentration of formaldehyde destroying the virus	Concentration of hydrochloric acid destroying the virus	Concentration of nitric acid destroying the virus	Filtrability	H-ion concentration	
Bean mosaic virus	1:1,000	44–56°C. probably close to 46°C.	20-24 hrs.	48–72 hrs.	25%				Not filtrable through any grade of Berkefeld filter		Fajardo (1930)
Bean virus 1	1:2,000	56–58°C.	24–32 hrs.		50%	1:500		1:200			Pierce (1934)
Bean virus 2	1:1,000	56–58°C.	24–32 hrs.		50%	1:1,000		1:200			
Alfalfa virus 2 on bean	Still infectious at 1:2,000 dilution	62–64°C.	7–9 days		75%	1:100		1:200			
Red clover mosaic virus on broad bean	Still infectious at 1:20,000 dilution	70°C.	Still infectious after 34 days		40%				Not filtrable through Chamberland F, Shofu L_1, L_2 and L_3		Saito (1935)

TABLE IX. (*Continued*)

Source of virus	Properties of virus										Authority
	Tolerance to dilution	Resistance to heat	Longevity *in vitro*	Longevity in dried plant tissue	Strength of alcohol destroying the virus	Concentration of formaldehyde destroying the virus	Concentration of hydrochloric acid destroying the virus	Concentration of nitric acid destroying the virus	Filtrability	H-ion concentration	
Common bean mosaic virus	1:1,000	56-58°C.	28-32 hrs.		50%	1:500	1:200				Zaumeyer and Wade (1935)
Pea mosaic virus 2	1:1,000	56-58°C.	24-28 hrs.		75%	1:1,000	1:100				
White sweetclover mosaic virus	1:1,000	56-58°C.	28-32 hrs.		75%	1:500	1:100				
White clover mosaic virus producing systemic lesions on beans	Still infectious at 1:2,000 dilution	58-60°C.	32-48 hrs.		75%	1:500	1:100				
White clover mosaic virus producing local lesions on beans	Still infectious at 1:2,000 dilution	62-65°C.	28-32 hrs.		75%	1:200	Still infectious at 1:100				
Alfalfa virus	Still infectious at 1:2,000 dilution	62-65°C.	3-4 days		Still infectious at 75%	1:200	1:100 (35-37% hydrochloric acid solution)				

TABLE IX. (*Continued*)

Source of virus	Properties of virus										Authority
	Tolerance to dilution	Resistance to heat	Longevity *in vitro*	Longevity in dried plant tissue	Strength of alcohol destroying the virus	Concentration of formaldehyde destroying the virus	Concentration of hydrochloric acid destroying the virus	Concentration of nitric acid destroying the virus	Filtrability	H-ion concentration	
White clover virus 1 on-											Pierce (1935)
Peas		56-58°C.	5-7 days								
Broad bean		56-58°C.									
Yellow sweetclover		56-58°C.	5-7 days								
Beans		58-60°C.	5-7 days								
Pea virus 1 on-											
Peas		56-58°C.	2-3 days								
Pea virus 3 on-											
Peas		62-64°C.	2-3 days								
Broad bean		62-64°C.	2-3 days								
Bean virus 2 on-											
Beans		58-60°C.	1-2 days								
Peas			1-2 days								
Soybean virus 1 on-											
Soybean		56-58°C.	2-3 days								
Broadbean local-lesion virus on-											
Broadbean		60-62°C.	2-3 days								

TABLE IX. (*Continued*)

Source of virus	Properties of virus										Authority
	Tolerance to dilution	Resistance to heat	Longevity *in vitro*	Longevity in dried plant tissue	Strength of alcohol destroying the virus	Concentration of formaldehyde destroying the virus	Concentration of hydrochloric acid destroying the virus	Concentration of nitric acid destroying the virus	Filtrability	H-ion concentration	
Pea virus 1	1:3,000		4 days								Stubbs (1936, 37)
Pea virus 2A, Pea virus 2B, Pea virus 2C	1:1,500		24 hrs.								
Pea streak virus	Still infectious at 1:5,000 dilution	65°C.	25 hrs.								Zaumeyer (1937)
Alfalfa mosaic viruses	1:3,000	70°C.	Still infectious after 5 days								
Pea virus 2		64°C.	5 days								Osborn (1937)
Pea virus 3		60°C.	3 days (at 22°C.)								Murphy and Pierce (1937)

TABLE IX. (*Continued*)

Source of virus	Properties of virus										Authority
	Tolerance to dilution	Resistance to heat	Longevity *in vitro*	Longevity in dried plant tissue	Strength of alcohol destroying the virus	Concentration of formaldehyde destroying the virus	Concentration of hydrochloric acid destroying the virus	Concentration of nitric acid destroying the virus	Filtrability	H-ion concentration	
Severe mosaic virus of pea	1:1,000,000	60–70°C.	15–338 days	Still infectious after 338 days							Johnson and Jones (1937)
Enation mosaic virus of pea	Lost its infectivity rapidly in the extract	Lost its infectivity rapidly in the extract	Lost its infectivity rapidly in the extract	Lost its infectivity rapidly in the extract							
Vein-mosaic virus of red clover		60°C.	3 days								Osborn (1937)
Broad bean mosaic virus	1:1,000	63°C.	21-25 days (at room temperature) 15-20 days (in the incubator at 25–28°C.)	20-25 days in summer, still infectious after 25 days in winter	50%	1%			Not filtrable through Shofu L_1 and L_2	Lost its infectivity at pH 3.0 and pH 9.5	This paper

OSBORN (1937)[24], and MURPHY and PIERCE (1937)[20], whereas the red clover mosaic virus was reported by SAITO (1935)[30] to retain its infectivity still longer. In the incubator at 25–28°C. the virus under consideration lost its infectivity after 15 to 20 days' aging, while at room temperature it was rendered non-virulent after 21 to 25 days' aging. The antiseptic did not prolong the aging *in vitro* of the virus. The virus of broad bean mosaic retained its virulence in dried plant tissues for more than 20 days. The virus in question was relatively sensitive to alcohol being destroyed by 50 per cent alcohol solution in thirty minutes. It was also sensitive to formaldehyde having been inactivated by 1 per cent formaldehyde solution in half an hour. The infective power of the virus was greatly reduced when the acidity or alkalinity of the juice was increased. No infection was obtained by the filtrate of the juice from mosaic broad bean plants through Shofu L_1 and L_2 filters.

In these respects above mentioned the virus under consideration appears closely to resemble, if not to be identical with OSBORN's pea virus 2 which is identical with pea virus 3 described by PIERCE although longevity *in vitro* of our virus is considerably longer than that of the American virus.

VII. Summary

1. The virus of broad bean mosaic lost its infectivity at dilutions greater than 1–1,000.

2. The virulence of the virus was greatly diminished or quite lost after ten minutes at 63°C.

3. The mosaic juice from broad bean plants lost its infectiousness after about 21 to 25 days' aging *in vitro* at room temperature, or in the incubator at 25 to 28°C. in 15 to 20 days. The addition of antiseptics prolonged in no way the longevity of the virus. The virus was rapidly inactivated in the diluted plant extract.

4. The virus lost its virulence after 20 to 25 days' drying in plant tissues in summer, while in winter the virus resisted 25 days' drying.

5. The virus was destroyed by 50 per cent alcohol solution in half an hour.

6. The virus was completely destroyed by 1 per cent formaldehyde solution in thirty minutes.

7. The virus was inactivated when the acidity of the juice was increased to pH 3.0, and when its alkalinity was increased to about pH 9.5, a precipitation took place in the virus suspension and the infective power was lost.

8. The virus of broad bean mosaic failed to pass through the coarse porcelain filters, Shofu L_1 and Shofu L_2.

9. The virus under consideration appears closely to resemble OSBORN's pea virus 2 or pea virus 3 of PIERCE as far as the thermal inactivation point is concerned but the longevity *in vitro* of our virus is considerably longer than that of the American virus.

VIII. Literature cited

1. BÖNING, K. Die Mosaikkrankheit der Ackerbohne (*Vicia faba* L.). Ein Beitrag zu dem Mosaik der Papilionaceen. Forsch. auf dem Gebiet der Pflanzenkrankh. und der Immunität im Pflanzenreich 4:43–III, 1927.
2. CHAMBERLAIN, E. E. Pea mosaic. Host range and methods of transmission. New Zealand Journ. Sci. and Technol. 18, pp. 544–556, 1936.
3. CHAMBERLAIN, E. E. Pea mosaic. Its symptoms, economic significance, and preventive treatment. New Zealand Journ. Agr. LIV, 3, pp. 129–138, 1937.
4. DICKSON, B. T. Studies on mosaic. (abst.) Phytopath. Vol. II, p. 202, 1921.
5. DICKSON, B. T. Studies concerning mosaic diseases. MacDonald Coll. Tech. Bull. 2, 125 pp., 1922.
6. DOOLITTLE, S. P. and F. R. JONES The mosaic disease in the garden pea and other legumes. Phytopath. Vol. 15, pp. 763–772, 1925.
7. ELLIOTT, J. A. A mosaic of sweet and red clovers. Phytopath. Vol. II, pp. 146–148, 1921.
8. FAJARDO, T. G. Studies on the properties of the bean-mosaic virus. Phytopath. Vol. 20, pp. 883–888, 1930.
9. FUKUSHI, T. On the mosaic disease of broad bean (in Japanese with English summary). Journ. Plant Protect. Vol. 17, pp. 707–712, 1930.
10. FUKUSHI, T. The relation of aphids to the transmission of legume mosaics. Sapporo Society of Agr. and Forestry Vol. XXIX, No. 139, pp. 189–216, 1937.
11. HARTER, L. L. Mosaic of lima beans (abst.) Phytopath. Vol. 26, p. 94, 1936.
12. IMAI, Y. Studies on the insect transmission of virus diseases of plants, with special reference to broad bean mosaic. Unpublished graduation thesis, 1933.
13. IMAI, Y. Studies on the transmission of broad bean mosaic. Sapporo Nat. Hist. Soc. Trans. 13: 241–245, 1934.

14. JOHNSON, F. and L. K. JONES Virus diseases of peas (abst.) Phytopath. Vol. 26, p. 96, 1936.
15. JOHNSON, F. and L. K. JONES Two mosaic diseases of peas in Washington. Journ. Agr. Res. Vol. 54, No. 8, pp. 629–638, 1937.
16. JOHNSON, J. The classification of plant viruses. Wisconsin Agr. Exp. Sta. Res. Bull. 76, 16 pp., 1927.
17. JOHNSON, J. The classification of certain virus diseases of the potato. Wisconsin Agr. Exp. Sta. Res. Bull. 87, 24 pp., 1929.
18. KURIBAYASHI, K. Studies on the seed transmission of bean mosaic disease. (in Japanese). Journ. Plant Protect. Vol. XIII, No. 4, pp. 1–12, 1926.
19. MERKEL, L. Beiträge zur Kenntnis der Mosaikkrankheit der Familie der Papilionaceen. Zeitsch. für Pflanzenkrankh. u. Pflanzenschutz XXXIX, 8–9, pp. 289–347, 1929.
20. MURPHY, D. M. and W. H. PIERCE Common mosaic of the garden pea, Pisum sativum. Phytopath. Vol. 27, pp. 710–721, 1937.
21. NELSON, R. The occurrence of protozoa in plants affected with mosaic and related diseases. (abst.) Phytopath. Vol. 13, p. 41, 1923.
22. OSBORN, H. T. Incubation period of pea mosaic in Macrosiphum pisi. (abst.) Phytopath. Vol. 24, p. 15, 1934.
23. OSBORN, H. T. Incubation period of pea mosaic in the aphid, Macrosiphum pisi. Phytopath. Vol. 25, pp. 160–177, 1935.
24. OSBORN, H. T. Studies on the transmission of pea virus 2 by aphids. Phytopath. Vol. 27, pp. 589–603, 1937.
25. OSBORN, H. T. Vein-mosaic virus of red clover. Phytopath. Vol. 27, pp. 1051–1058, 1937.
26. PIERCE, W. H. Viroses of the bean. Phytopath. Vol. 24, pp. 87–115, 1934.
27. PIERCE, W. H. The identification of certain viruses affecting leguminous plants. Journ. Agr. Res. Vol. 51, pp. 1017–1039, 1935.
28. PIERCE, W. H. Legume viruses in Idaho. Phytopath. Vol. 27, pp. 836–843, 1937.
29. REDDICK, D. and V. B. STEWART Additional varieties of beans susceptible to mosaic. Phytopath. Vol. 9, pp. 149–152, 1919.
30. SAITO, K. On the viruses of mosaic diseases of bean and red clover. (in Japanese). Unpublished graduation thesis, 1935.
31. SCHAFFNIT, E. and H. WEBER Ueber das Vorkommen von intrazellularen Körpern in den Geweben mosaikkranker Rüben. Forsch. auf dem Gebiet der Pflanzenkrankh. und der Immunität im Pflanzenreich, 1927.
32. STUBBS, M. W. Viroses of the garden pea, Pisum sativum. (abst.) Phytopath. Vol. 26, pp. 108–109, 1936.
33. STUBBS, M. W. Certain viroses of the garden pea, Pisum sativum. Phytopath. Vol. 27, pp. 242–266, 1937.
34. VAN DER MEULEN, J. G. J. Voorloopig onderzoek naar de specialisatie en de infectiebronnen der mozaiekziekten von landbouwgewassen. (original not seen). Tijdschr. Planktenziekten 34: 155–176, 1928.

35. ZAUMEYER, W. J. and B. L. WADE The relationship of certain legume mosaics to bean. Journ. Agr. Res. Vol. 51, pp. 715–749, 1935.
36. ZAUMEYER, W. J. and B. L. WADE Pea mosaic and its relationship to other legume viruses. (abst.) Phytopath. Vol. 26, p. 114, 1936.
37. ZAUMEYER, W. J. and B. L. WADE Pea mosaic and its relation to other legume mosaic viruses. Journ. Agr. Res. Vol. 53, pp. 161–185, 1936.
38. ZAUMEYER, W. J. Pea streak and its relationship to strains of alfalfa mosaic. (abst.) Phytopath. Vol. 27, p. 144, 1937.

PLATE I

Explanation of Plate I

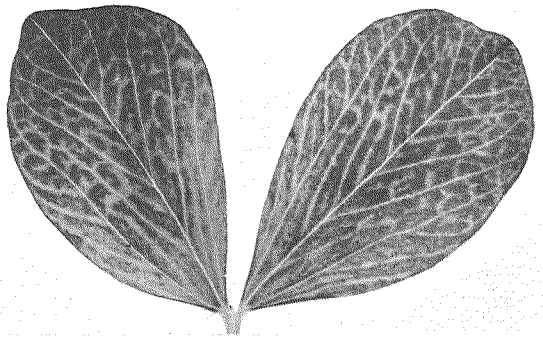
Fig. 1: Clearing of the veins.

Fig. 2, 3, 4, 5, and 6: Mottling of the leaves.

Fig. 7: Streak on the stem of infected plant (right).

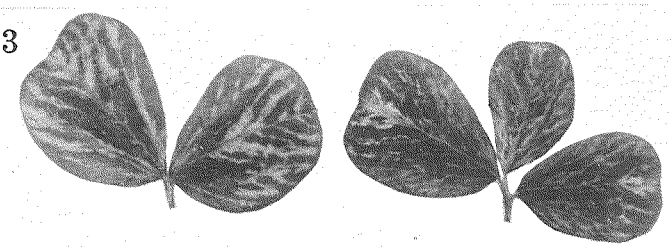
Fig. 8: Healthy (right) and infected plants.

1

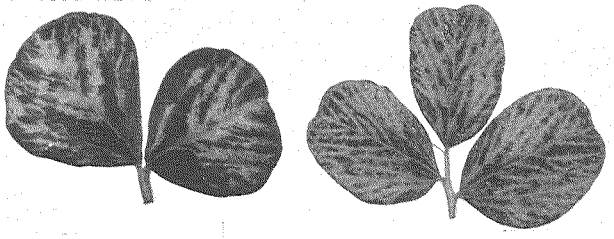


2

3

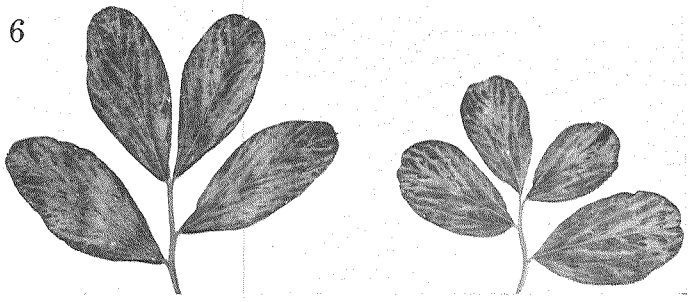


4

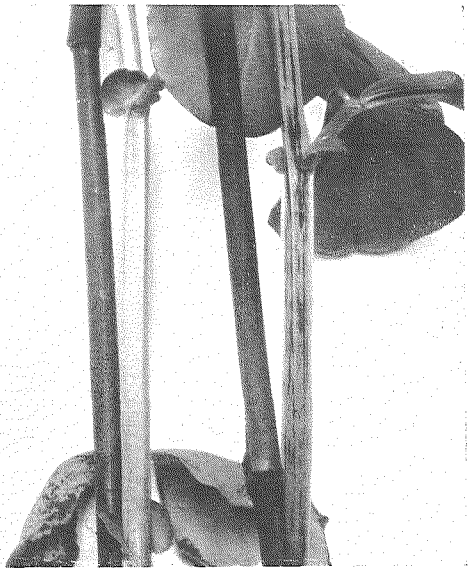


5

6



7



8