PHYSIOLOGICAL INVESTIGATIONS ON RED RASPBERRY PLANTS INOCULATED WITH RED RASPBERRY MOSAIC

A thesis

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By

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Table of Contents

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I. Introduction

Virus diseases of the raspberry were first recognized in 1895 by Green (17) although it is said that Detmers (12) in 1891 described a disease that was very likely mosaic but was then ascribed to bacteria. In 1922 Rankin and Hockey (28) divided the virus diseases into two groups, "curl" and "mosaic". Bennett (3) in a more recent study has reported at least five distinct virus diseases of the raspberry. They are, red raspberry mosaic, mild mosaic, yellow mosaic, curl and streak. The first is the one dealt with in these studies.

Bennett (3) also states that temperature has a very decided influence in masking symptoms of red raspberry mosaic and mild mosaic. At temperatures higher than 28^{o} C., no symptoms of mottling are shown. Plants with symptoms masked were therefore easily obtained when desired.

Numerous publications, summarized by Smith (33) dealing with symptoms, methods of dissemination and transmission, and other pathological phases have appeared but the writer has found very little in the literature on the effect of red raspberry mosaic virus on physiological activities of the host. It appeared worth while then to make comparative studies of healthy and mosaic plants and also plants in which the symptoms of red raspberry mosaic had been masked. Mosaics are responsible for the so called "running out" of several varieties many of which have been abandoned. Furthermore such troubles not only cause serious reduction in yields and quality of fruit but account for considerable decline in acreage.

II. Materials and Methods

In all of these studies the Latham variety was used. Healthy plants of this variety were inoculated with the virus responsible for the disease described by Bennett (3) as red raspberry mosaic and by Cooley (8) as green mottle mosaic. The symptoms produced vary considerably with the variety and with the environmental conditions. The leaves present a typical mosaic mottling characterized by yellowish green areas alternating with raised dark green areas, when the plants are grown at a relatively cool temperature. Typical leaves of this sort are shown in figures 1 and 2. Under ordinary summer temperature in Michigan the Mosaic-infected plants show no symptoms and the disease is said to be masked.

In the fall of 1932 twenty-five Latham plants inoculated with red raspberry mosaic were received from Dr. W. H. Rankin of the New York Agricultural Experiment Station at Geneva. These plants were grown in the greenhouse during the winter and transferred to the field in the following spring. While in the field they were covered with white muslin cages to prevent any possible infection from other sources. Additional diseased plants were secured through the use of bark grafting, a method suggested to the writer by Harris of the East Malling Research Station, England. Since the results with this method have yielded more than ninety per cent infection the writer early gave up attempts to transmit the disease by insect vectors. During this time many methods were tried, as insect vectors were difficult to find during the earlier periods of this study. This difficulty was due to the hot, dry weather and other environmental conditions. Various inoculation methods for virus transmission were given a trial especially the carborundum method. Every attempt in this direction failed. It has been suggested that the virus is destroyed by oxidation when juice from infected plants is used for inoculation.

The symptoms of the disease do not appear in the grafted plants until a new set of canes is sent up from the roots. In a comparatively short time these will appear when the plant is cut back. Dr. Harris, in a letter to Dr. Hibbard, states that in England symptoms do not appear on the grafted plants till the year after grafting, but that in St. Catherines, Ontario, where he (Harris) had made many grafts during 1934, red raspberry mosaic appeared within two months after grafting. No explanation was given to account for the difference obtained in the Canadian material. Plants for this study, in which the symptoms were masked, were secured by selecting diseased plants and exposing them to proper temperature conditions.

III. Carbohydrate Studies

Differences in the biochemical reactions in the healthy, mosaic, and masked plants might be expected. In other plants such as tobacco, potato, tomato, peaches, etc., investigators have noted differences in carbohydrate and nitrogen relationships besides a number of other features. The first studies made with raspberries were those on carbohydrates. Leaves of approximately the same age were secured from healthy, mosaic and masked plants. Two series of experiments were conducted, one in the greenhouse and one in the field. The leaves were gathered late in the morning and dried in the oven at 100° C., for one hour and then at 60° C., until constant weight was reached. The samples were then ground to pass a 40 mesh screen and further treatments made according to the routine suggested by the committee on chemical methods of the A. S. P. P. (30). Bertrand's (4) permanganate titration method was substituted in the place of that of Shaffer and Hartman.

The results of these analyses are shown in Table I. Although the total carbohydrates in the leaves of mosaic-infected plants varied little or none from those in healthy ones, masked plants under field conditions, showed less carbohydrates than plants from the greenhouse. Much less emphasis is centered on these total carbohydrate values than on the figures for the different carobhydrate fractions.

It may be said, however, that there is little unanimity of results as shown by various investigators. Dunlap (15) obtained an increase in total carbohydrates in two year raspberry canes but a decrease in one year canes. He does not state what form of carbohydrate is responsible for the increase or the decrease that he finds in the two cases. He did not analyze his material into the various carbohydrate fractions. Among a number of other mosaic-infected plants such as tobacco, squash, tomato, pepper, cucumber, etc., he found a decrease of carbohydrates. Rosa (32) reported an increase of carbohydrates in yellow blighted tomatoes. True and Hawkins (38) found an increase of carbohydrates in blighted spinach leaves. Brewer, Kendricks and Gardner (7) obtained a decrease in carbohydrates in tomato mosaic. Bailey (2) found a decrease in starch and soluble carbohydrates in

Table I

Percentage of Carbohydrates in leaves of healthy plants, mosaic-infected plants and plants in which symptoms were masked.

Date	Hour	Plant and Habitat	Sugar		Total	Starch		
			Simple	Sucrose	Sugar	Soluble	Insol.	Total
July 20	10 A.M.	Healthy, greenhouse	0.64	0.74	1.38	1.00	.73	1.73
" 20	10 "	" "	.64	.73	1.37	.95	.71	1.66
Aug. 21	10 "	" "	.69	1.19	1.88	.52	.97	1.49
" 21	10 "	" "	.64	1.19	1.82	.50	.92	1.42
" 21	10 "	" field	.70	1.22	1.92	1.07	.68	1.75
" 21	10 "	" "	.67	1.22	1.89	1.07	.70	1.77
" 26	10 "	" "	.65	1.39	2.04	.37	.56	.93
" 26	10 "	" "	.64	1.43	2.07	.38	.55	.93
July 20	10 "	Mosaic, greenhouse	.59	1.20	1.79	.50	.85	1.35
" 20	10 "	" "	.60	1.18	1.78	.50	.85	1.35
Aug. 21	10 "	" field	.57	1.72	2.29	.47	.87	1.34
" 21	10 "	" "	.57	1.75	2.32	.50	.86	1.36
" 21	10 "	Masked, greenhouse	.75	1.29	2.04	.65	.79	1.44
" 21	10 "	" "	.75	1.29	2.04	.65	.80	1.45
" 26	10 "	" field	.57	1.45	2.02	.47	.59	1.06
" 26	10 "	" "	.57	1.45	2.02	.47	.67	1.14

diseased material of tobacco, tomato and petunia. Bunzel secured less carbohydrates in sugar-beet leaves infected with curly top, according to Dunlap's (15) interpretation but Brewer et al (7) interpret these findings as showing little or no effect on carbohydrates.

Turning now to a consideration of total sugars we find according to the table that these are higher in field-grown plants than in those grown in the greenhouse. This was to be expected. In the field, mosaic-infected plants produced more sugars than any other group, masked plants were intermediate, and healthy ones contained the least. A further study of the table reveals the fact that glucose is more abundant in greenhouse plants than in field grown ones, while sucrose is more prevalent in field grown plants than in those grown in the greenhouse. Healthy plants contain greater amounts of glucose than either mosaic-infected or masked plants. Sucrose on the other hand is more abundant in mosaic-infected and masked plants. These results are in agreement with those of a few other investigators working with other plants. For example Barton-Wright and McBain (1) found larger amounts of sucrose than glucose in the crinkle-infected potato. The authors suggested a slowing up of translocation due to an accumulation of sucrose. As the hexoses were reduced this naturally was the form translocated. Sucrose was not translocated. True and Hawkins (38) reported a reduction in hexoses and an accumulation of sucrose in the leaves of blighted spinach plants. Dunlap (15) also found less hexoses in the leaves of mosaic-infected tobacco plants. The writer's tables show also a decrease in glucose in mosaic-infected raspberries.

In regard to starch the table reveals the following facts. Healthy plants show higher values than do masked or mosaic-infected plants, therefore the diseased condition shows a decrease in starch. Though there is not much difference in starch values between greenhouse and field specimens the latter are usually lower. Both masked and mosaic-infected plants in the field show a decrease in the soluble fraction as well as the insoluble form when compared with healthy plants. However, the greater losses are registered in the soluble amylodextrin fraction than in the amylocellulose or insoluble form. This reduction in the amount of starch in the diseased plant is also in agreement with the findings of other workers. Brewer et al (7) found a decrease in sucrose and starch in mosaic-infected tomatoes. Cook (8) reported an inverse relationship between starch and the severity of sugar cane mosaic. Bolas and Bewley (5) observed a decrease in starch formation in the yellow mosaic of the tomato when the plant had been infected for some time but not in the early stages.

One is tempted to criticize experiments of this type that are conducted in the greenhouse because of the different environmental conditions to which the plants are subjected. However, the results indicated in the table show good agreement when a comparison of diseased and healthy plants is made whether in the greenhouse or in the field. The writer feels that as these observations are the first of their kind as far as the raspberry is concerned, similar studies of a more detailed nature, perhaps, should be conducted over a period of years. Further, the data found in table I were obtained from an analysis of leaves from many plants selected on the particular dates indicated. These were mixed together after drying and two-gram samples taken for carbohydrate analysis. An alternative method consists in analyzing the leaves in the separate set or groups and then finding the averages. The data then, are capable of statistical treatment and the degrees of significance determined.

The carbohydrate metabolism in the mosaic-infected plants, as indicated in the preceeding account, has been attributed by most workers to the interference in transport rather than to inability to produce carbohydrates. In plants where one effect of the virus is to cause a yellowing of the photosynthetic tissue it is quite probable that carbon assimilation is reduced but for many of the mosaic diseases this explanation is not valid, for in many plants there is very little yellow colored tissue while in some cases the green color is more intense. It was thought that a study of the carbohydrate changes in a raspberry plant during a twenty-four hour period might give information as to whether production or transport was being checked.

For this experiment field grown plants were used and samples of leaves were collected early in the morning before sunrise and again the same day just before dark. The samples were selected in such a way that material from the same plants was being analyzed in each case. The fourth and fifth leaves back from the tip are usually fully expanded and in this case were considered mature. By taking, then, the fourth leaf of one cane and the fifth leaf of another cane in the morning it is possible to secure a sample in the evening from the same canes which is comparable in every respect to those taken previously. The samples were analyzed by the same method indicated above and the results are given in table II.

The values shown in the column headed P. M. are for the samples secured before sunrise and represent the carbohydrate fractions in the leaves after a period of eleven hours during which no photosynthesis had taken place. Obviously these changes are chiefly related to translocation and utilization. The figures indicated in the column headed A. M. are for the samples gathered in the evening and represent the carbohydrate fractions in the leaves as the result, chiefly, of photosynthesis.

A study of the table shows that healthy plants produce more simple sugars during the day time than either the masked or mosaic-infected plants. This indicates a greater efficiency in sugar production. During the night there is a greater loss of these sugars in healthy plants than in the diseased ones indicating efficient transport and more rapid transformation. When the sucrose figures are considered all groups of plants show similar amounts, with slightly more occurring in the mosaic-infected plants in the daylight period. From this it may be concluded that sucrose is maintained at a fairly constant level. During the night, however, the sucrose content increases in all groups, with the greatest increases occurring in the diseased plants. One can see in these results on simple sugar and the disaccharide sucrose evidence of further support to the theory that glucose is the first sugar formed and that sucrose is derived from the hexoses and furthermore is the sugar of transport.

Table II.

Changes occurring during day and night periods in the percentage of carbohydrates in the leaves of healthy, mosaic-infected plants and plants in which the symptoms are masked.

Plant	Simple Sugar		Ratio		Sucrose		Ratio		Soluble Starch		Ratio		Insoluble Starch			
	A.M.	P.M.	A. M.	P.M.	A. M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Healthy	2.00	1.60	100	100	3.52	4.18	100	100	2.09	1.77	100	100	0.88	0.69	100	100
Healthy	2.10	1.59			3.53	4.18			1.99	1.75			.90	.67		
Mosaic	1.49	1.10	72	69	3.58	4.35	101	103	2.15	1.72	105	97	.89	.89	100	130
Mosaic	1.49	1.13	72	70	3.60	4.37	102	103	2.12	1.72	104	97	.93	.95	104	139
Masked	1.79	1.72	87	107	3.52	3.70	100	89	1.81	1.73	88	97	1.45	1.26	163	182
Masked	1.79	1.75	87	107	3.52	3.63	100	88	1.80	1.80	88	102	1.40	1.26	157	182

Soluble starch is more abundant during the daytime period in the mosaic-infected plants. During the night it is somewhat lower. In all three groups the soluble starch has decreased during the night showing that it has been utilized in some biochemical reaction. The insoluble starch fraction in the mosaic-infected plants is the same for day and night period. The healthy and masked plants show significant differences between day and night periods.

To summarize, it is readily seen that sucrose remains quite constant during the day, and that also, as is shown in table I, the diseased plants contain more of this sugar than the healthy ones.

The increase in sucrose during the night is in part at the expense of the simple sugars. The P. M. values for simple sugar are considerably lower than the A. M. values while the P. M. values of sucrose are higher than the A. M. values. For the mosaic-infected plants this accounts for approximately half of the increase in sucrose. The other half is accounted for by the transformation of the soluble starch fraction (dextrin) into sucrose for as may be seen in the table the P. M. values of soluble starch are that much less than the A. M. values. A similar line of agrument is applicable in the case of the healthy and masked plants. The data in the column headed insoluble starch are difficult to interpret. Had the values been higher in the night time for healthy plants and the same or lower for the masked and mosaic-infected plants, it would seem to prove that the diseased plants were inefficient in transforming the sugars and soluble starches into permanent structure. Further study is contemplated along this line. The results should be regarded as introductory to further work of a more detailed nature.

Support for the idea that sucrose is increased at the expense of glucose and starch is found in the studies of Barton-Wright and McBain (1) who attributed the increase of sucrose in potato crinkle mosaic to a loss of hexoses and starches. They showed also that the sucrose was produced from glucose and fructose (hexoses). The results reported here in tables I and II do not support the idea of checked or retarded translocation since a considerable increase in sucrose occurred in the healthy plants as well. Cook (8) also found no interference in translocation in the mosaic-infected sugar cane.

IV. Ensyme Studies

Experimental procedures in enzyme extractive purification, and tests for activity, have been very greatly modified in the past few years as far as work on animal tissues is concerned. The work on plant tissue extracts has received but little attention. Present laboratory methods are those of ten or fifteen years ago and can be considered only roughly quantitative. Better methods of extraction, time of sampling, purification, conditions necessary for optimum activity and better control of the pH of the reacting medium, etc., should be carefully considered. It has been found, for example, that diastase not only is a mixture of enzymes, but that there are difficulties in separating them. Again, three methods of determining diastatic activity have been suggested but usually only one is taken. In the three methods however the changes do not always take place in the same order or at the same rate and unless a comparison by means of the three methods is made the degree of activity by any one method might not represent actual conditions. Sometimes there is a rapid liquification of starch paralleled by a slow maltose formation: at other times there is a very rapid formation of maltose but a slow disappearance of products which give a blue color with iodine.

The differences in carbohydrate metabolism between the healthy and diseased plants suggested a possible connection with enzyme activity or inactivity. Only qualitative or rough quantitative tests were possible in the two experiments conducted at two different seasons. Enzyme extracts were secured from leaves of similar age selected from the three different groups of plants. In one series the usual water extraction method was used. The leaves were gathered in the late morning period. In the second experiment the enzymes were extracted by a method described by True and Hawkins (38) in which glycerine was the extracting medium. In both experiments a preliminary grinding of the tissues with clean quartz sand was necessary. The glycerine extraction was continued for 24 hours and at a temperature of 10^{0} C. The extract was then squeezed through cheesecloth and made up to mark in a 250 cubic centimeter volumetric flask. A one per cent Lintner's starch solution was made up fresh each time a test was conducted. A test consisted in placing varying concentrations of the enzyme solution in a series of test tubes and then adding 5 cubic centimeters of a one per cent starch solution. One-half a cubic centimeter of toluol was placed in each tube. The tubes were closed with a stopper, shaken, and placed in an incubator at 37^{0} C., for 48 hours. At the end of this time the tubes were removed from the incubator and immersed in cold water and to each was added ten cubic centimeters of cold distilled water and one-tenth cubic centimeter of a one per cent iodine solution. The tubes were then shaken and the enzymatic activity determined by the observation of changes in color. The data may be found in table III.

That tube containing the lowest dilution of enzyme, in which no blue color was found, on treatment with iodine was arbitrarily taken as zero. Then the tube into which a small quantity of boiled enzyme had been introduced and which on treatment with iodine showed the blue color was taken to represent the other end of the scale or 100. The other tubes were graded according to color on a percentage basis. From the table it is obvious that the diastatic activity of the leaves of mosaic-infected plants was greater than that of the masked or healthy ones. When considered with the carbohydrate changes already described above the increased diastatic activity explains why there was such a small amount of soluble starch or dextrin and why there was an increased amount of sucrose in the mosaic-infected plants. In the case of the "masked" plants it appears that diastatic activity has not been modified. Masking effects in the raspberry may be in the some phases comparable to the effects of a latent virus in the metabolism of the potato. Barton-Wright (2) reported that potatoes containing a latent virus had in no way modified carbohydrate metabolism. In that case no changes in enzyme action would be expected. These experiments will be repeated on a quantitative basis and definite conclusions must be reserved, for the future, but out present results indicate a significant difference.

Proof that diastatic activity is increased in mosaic infected tobacco has been shown by Dunlap (15). Sreenivasaya (34) found that the diastatic activity of spiked leaves of sandal was much greater than

Table III.

Diastatic activity of extracts from leaves of healthy, masked and mosaic-infected plants as determined by the color produced by the addition of iodine to a starch substrate. Zero indicates greatest activity and 100 indicates no activity.

Plant	Quantity of enzyme extract					
	0.5cc.	0.4cc.	0.3cc.	0.2cc.	0.1cc	Control
Healthy	20	40	60	80	100	100
Healthy	20	40	60	80	100	100
Masked	20	40	60	80	100	100
Masked	20	40	60	80	100	100
Mosaic	0	20	50	60	100	100
Mosaic	0	20	50	60	100	100

that of the healthy leaves. True and Hawkins (38) however were unable to detect any difference between the diastatic activity of the leaves of healthy and diseased spinach. Raspberries then appear to be not unlike other plants infected with mosaic in their power to quickly convert starch and also glucose into sucrose.

V. Photosynthesis and Respiration Studies

The rate of photosynthesis in leaves, or their efficiency in the food manufacturing process, has been determined for many types of plants under varying climatic conditions, experimental procedures etc. It has been shown that not only do external conditions modify this process, but internal conditions also, such as water and chlorophyll content, age, variations in the ability to react (protoplasmic fatigue), improper nutrient conditions, translocation and utilization of assimilated materials. In spite of all these modifying conditions it is not impossible to obtain a fair approximation of a normal leaf's efficiency. The object of this work was to compare the photosynthetic efficiency of healthy raspberry leaves and those infected with mosaic. The method of Heinicke and Hoffman (19) as applied to apple leaves was selected as a guide for these studies. In principle it is like many others dating back even to the time of Kreussler's work in 1885, but it has the advantage of being useful in cases where the leaves are still attached to the plant. These authors have also been very explicit in their descriptions of the method, and in pointing out the various steps and the need for care and practice in developing technique. This is important as it is then possible to duplicate procedures, a state of affairs which has usually been impossible to accomplish in experiments of this type.

The report that follows deals with plants grown in the greenhouse. Results obtained in this way are comparable to those found in field plants. The carbohydrate studies reported above have shown that normal healthy plants exhibit the same general type of metabolism in the greenhouse as in the field. The field plants, however, produce approximately twice as much material. Similar studies by other workers have also been conducted in greenhouses and no marked variation in the type of carbohydrate metabolism has been thus far reported. First studies are naturally made where experimental facilities are available and where complicated apparatus can be set up without fear of mistreatment. Arrangements for field study have already been made for the coming growing season. Three series of experiments were carried through. One in the spring of 1936, a second in the summer of the same year and the last in the spring of 1937.

Photosynthetic efficiency was estimated by determining the difference in Co_2 content between a continuous stream of normal air and a similar stream of air that had passed over leaf tissue confined in a specially constructed photosynthetic chamber. The photosynthetic chambers were in the form of cylinders made of celluloid as shown in figure 3. They were large enough to enclose the entire end of a raspberry cane. Thus a considerable amount of photosynthetic tissue can be used and crowding of leaves avoided. There was also a minimum of temperature variation so prevalent in experiments where single leaves or portions of leaves are used in cup chambers or other small containers.

The cylindrical chambers were 20 inches high and 30 inches in circumference. A celluloid cover was cemented to one end with a mixture of celluloid in acetone. All joints at this end were sealed tight. At the other end the cylinder was attached to a metal rim to which was fastened a long metal rod that could be used for a support when stuck in the ground. Cellophane fastened to this rim by an elastic band, and loosely tied around the exit and inlet air tubes and stem, closed up the chamber. The absorption units with slight modification were the same as those pictured and described by Heinicke and Hoffman (19) on page four of their publication. Their general method of procedure as to calibration: taking of samples, regulating air supply, titrations, etc., was followed. The running time of an experiment was always for four hours as this was a convenient time interval for the operator and was of sufficient length to produce recognizable quantities of Co_2. Similar runs for the same time periods were made in darkened chambers or during the night in regular chambers for information on respiratory activity in leaves.

Leaf areas had to be determined and this was accomplished without injury to the leaf or plant by means of a simplified and portable photoelectric device described by Hibbard, Grigsby and Keck (21). This device has been used for three years and has a few special features not yet noted in the descriptions of several devices recently published. It is operated on a 6 volt battery, has a multiple point, low intensity light source, and a specially constructed cone shaped light chamber and needs no correction for transmission of light through the test leaf.

The device can be made portable and an automobile battery can be used as the source of power. Figure 4 is from a photograph of the apparatus.

The data for photosynthesis and respiration may be found in tables IV, V, and VI. The figures given for photosynthesis are actually, apparent photosynthesis, and represent the milligrams of Co_2 per 100 square centimeters of surface per hour, over and above the quantity of Co_2 liberated through the operation of respiration and other metabolic processes of the plant. These figures are below the column headed A. M. The figures below the column headed P. M. are for the quantity of Co_2 liberated per 100 square centimeters per hour, during runs at night or in black hooded chambers. These figures represent respiration. Greenhouse temperatures between night and day are usually of wide variation but a system of regulation was possible and in these experiments the greatest variation in temperatures were not more than 5^{o} F. The canes used were in their first season of growth. A study of all three tables shows that the apparent photosynthesis is reduced in the mosaic plants. In other words the diseased plants are less efficient in photosynthesis than the healthy ones and their rate of activity is reduced. Respiration on the other hand showed a greater activity in the mosaic-infected plants. The respiratory activity of the masked plants is intermediate, less than the diseased but more than the healthy.

A further study of the tables shows that photosynthetic activity varies with the time of the year. The spring or May values are lower than the summer values. Two explanations are possible.

Table IV

Apparent photosynthesis and respiration of healthy and mosaic-infected plants in the spring of 1936, expressed as milligrams of Co_2 per 100 sq. cm. per hour. Average of 4-hour determinations during the day or night.

Date	Plant	Day	Night	Ratio	
				Day	Night
May 4	Healthy	6.50	4.78	100	100
4	Mosaic	4.15	5.28	63	110
11	Healthy	17.79	5.23	100	100
11	Mosaic	9.97	6.07	77	115
13	Healthy	15.31	4.62	100	100
13	Mosaic	9.09	6.10	59	132
15	Healthy	6.52	2.78	100	100
15	Mosaic	3.82	3.56	58	128

Table V.

Apparent photosynthesis and respiration of healthy, mosaic-infected and masked plants expressed as milligrams of Co_2, per 100 sq. cm., per hour. Average of 4-hour determinations during day or night. Summer of 1936.

Date	Plant	Day		Day Av.	Night	Ratio	
		A.M.	P.M.			Day	Night
July 8	Healthy	11.70	10.75	11.22	4.06	100	100
8	Masked	13.09	11.79	12.44	4.61	110	113
8	Mosaic	10.50	9.69	10.09	5.05	89	121
9	Healthy	8.08	8.08	8.08	4.41	100	100
9	Masked	8.45	7.71	8.08	4.07	100	92
9	Mosaic	7.70	6.81	7.25	4.47	88	101
10	Healthy	12.12	13.22	12.67	4.07	100	100
10	Masked	12.13	12.13	12.13	4.03	95	99
10	Mosaic	11.62	12.07	11.84	4.53	93	111
14	Healthy	31.64	24.90	28.27	4.60	100	100
14	Masked	29.10	22.44	25.77	5.46	91	118
14	Mosaic	27.49	19.49	23.49	6.06	83	131
15	Healthy	17.22	15.68	16.45	5.54	100	100
15	Masked	17.94	17.37	17.60	7.46	107	134
15	Mosaic	13.24	12.10	12.67	8.97	77	161
16	Healthy	16.71	18.02	17.36	7.19	100	100
16	Masked	20.02	19.86	19.94	8.54	114	110
16	Mosaic	16.51	17.14	16.82	12.07	96	167
17	Healthy	16.90	15.94	16.42	6.41	100	100
17	Masked	20.07	17.40	18.73	7.55	114	117

Table V (continued)

Date	Plant	Day A.M.	Day P.M.	Day Av.	Night	Ratio Day	Ratio Night
July 17	Mosaic	15.00	14.82	14.91	8.90	84	138
Aug. 18	Healthy	17.32	15.50	16.41	5.48	100	100
18	Masked	17.83	17.17	17.50	8.02	106	146
18	Mosaic	15.96	15.00	15.48	9.24	94	168
23	Healthy	13.49	12.37	12.93	4.64	100	100
23	Masked	12.16	12.16	12.16	4.64	94	100
23	Mosaic	11.38	10.73	11.05	5.00	85	107

Table VI.

Apparent photosynthesis and respiration of healthy and mosaic-infected plants in early spring, 1937. Results expressed as milligrams of Co_2, per sq. cm., per hour. Average of 4-hour determinations during day or night.

Date	Plant	A. M.	P. M.	Day Av.	Night	Ratio Day	Ratio Night
March 29	Healthy	5.54	8.92	7.23	3.00	100	100
29	Mosaic	2.43	5.21	3.82	4.43	52	144
30	Healthy	4.17		4.17	1.32	100	100
30	Mosaic	1.38		1.38	2.73	33	206
April 1	Healthy	7.91	5.54	6.72	3.41	100	100
1	Mosaic	4.19	2.86	3.52	3.58	52	105
2	Healthy	5.50	5.50	5.50	1.17	100	100
2	Mosaic	4.23	4.49	4.36	1.35	79	115

First, it might be a case of difference in vigor, the summer plants being the most vigorous or, secondly, it might be a group of external conditions, chiefly light and temperature, that cause the difference. The former explanation does not appear to be as reasonable as the latter, since the type of growth in the raspberry is indeterminate, and the groups of leaves on the ends of the canes are in similar growth stages. Seasonal variations in photosynthetic rate do occur according to Dastur and Desai (10). Heinicke and Hoffman reported (19) variations from day to day. Bolas and Melville (6) observed a seasonal variation, the rate of photosynthesis being high in summer and then declining towards winter. Variation is the rule rather than the exception.

Stone (36) working with mosaic-infected potatoes found a reduction in photosynthetic efficiency, but noted that the severity of the disease had little effect upon the degree of reduction. Dufrenoy (14) claimed that mosaic inhibited photosynthetic efficiency. Barton-Wright (2) found that in young potato plants suffering from mosaic there was no difference in carbohydrate metabolism or the ability to make carbohydrates, but as the plants grew older there was a decline in the amount of carbohydrates formed. There seems to be no doubt in the case of the raspberry of a decrease in activity. As to a relationship between severity of the disease and photosynthetic activity it would appear that there is one. The degree of reduction or decrease of activity seems to be proportional to symptom expression. In the masked plants the reduction is not so great as in the mosaic-infected ones. Whitehead (40) obtained similar results in potato plants infected with leaf roll. Dunlap (15) gives data that show increased rate of respiration in young raspberry plants and a decrease in older ones. His results are based on Co_2 evolved per hour in an average sized leaf, the area of which was unknown.

VI. Transpiration

A comparative study of the transpiration rate of healthy, diseased and masked plants was undertaken in the greenhouse. All records here were obtained by the standardized hygrometric paper method. This was first suggested by Stahl (35) and later was standardized by Livingston and his co-workers (23). There have been some modifications by Livingston himself and by other workers in this field in more recent times. The modifications by Meyers (25) were followed in the main. By using this method the leaves did not have to be detached from the plant and one can make tests on leaves under different conditions while they are growing naturally in benches or pots in the greenhouse. The method of obtaining results is more simple and quicker than experiments with potometers or water culture set-up. It is preferable, especially in experiments of a preliminary nature, to the more elaborate methods which have been described. The writer believes that much more information should be collected not only from plants in the greenhouse but from field specimens. These reported figures, however, are indicative of the effect of a virus on plant transpiration. Up to the present, few studies of this kind have been made and none at all on the raspberry.

Results, shown in table VII, are recorded as grams of water

Table VII

Grams of water vapor transpired from leaves of healthy, and masked plants per hour per 100 square centimeters of leaf area.

Time	Healthy (H)		Masked (M)		Diseased (D)		Ratio D/H		Ratio M/H	
	Bench	Pot	Bench	Pot	Bench	Pot	Bench	Pot	Bench	Pot
March 13	1.95	-	1.95	1.08	2.03	1.14	104	-	100	55
" 20	1.37	-	2.01	1.08	2.00	0.76	145	-	145	78
" 21	-	-	-	1.30	1.78	1.11	166	-	-	122
" 31	1.01	-	-	.83	1.77	.95	176	-	-	82
April 8	1.55	-	1.87	1.67	1.57	.95	101	-	120	107
" 12	-	-	-	-	1.63	1.25	140	-	-	-

vapor lost in one hour per 100 square centimeters of leaf area. The relation between the color of the hygrometric paper and the amount of water taken up is determined in the following way. The time, in seconds, necessary for a definite area of paper pressed on the leaf surface, to attain the same pink color as a similar piece of paper in a saturated atmosphere and used as a reference standard, was found. Now, by using the formula $G = \frac{(0.06784 \times 3600)}{T}$ the amount of water vapor can be determined. The calibration of the reference standard showed that the pink color was equivalent to the absorption of 0.0678 grams of water vapor per 100 square centimeters of paper surface. Thus the color was a measure of water content of the paper and this was related to the time. In the equation G equals the grams of water vapor lost per hour and T is the time of color change in seconds. The number of seconds in one hour is 3600, the figure indicated in the numerator of the equation above.

The figures in the table show that the mosaic-infected plants have a greater transpiration rate than the healthy ones and that the results from the masked plants are intermediate. Heuberger and Norton (20) observed a greater rate of transpiration in mosaic-infected tomato plants. Harrison (18) reported no increased transpiration in mosaic-infected bean leaves but water losses from the pods of diseased plants were greater than from those of healthy plants.

The data also show that the loss of water vapor is more rapid in plants growing in the soil in an open bench where the soil is two feet deep, than in pots where the soil mass is restricted and not more than four or five inches deep. In the latter the supply of moisture is less than in the bench and the plant roots quickly use up the supply. When plants have grown in pots for some time the majority of the roots grow near the sides of the pot and on the outside of the soil mass. The water here is soon used up and a decrease in transpiration occurs.

After it had been determined that the transpiration rate in diseased plants was greater than in the healthy an explanation was sought in the distribution and number of stomata on the leaves of the different types of plants. The raspberry lamina is of a delicate structure, its surface is of a convoluted or wavy nature and its only support are the prominent veins. To assist in maintaining its rigidity the surface is impregnated with silicon. When leaves are ashed considerable amounts of silicon are found. There was great difficulty therefore in stripping off suitable pieces of epidermis for the purpose of fixing the stomata on the leaf in absolute alcohol so that stomatal studies could be made later. The collodion film method first suggested by Newirth (26) and later improved by Long and Clements (24) was not successful. The chief thing that militated against these methods was the fact that the surface of the leaf was covered by a mat of long, tenuous interlocking hairs. It was difficult also to see the stomata because of the dense, dark, cell contents. The stomata are small and therefore quite numerous and some method had to be devided to avoid these difficulties.

First it seemed necessary to use some clearing agent. A lactic-acid-phenol preparation as reported by Davis (11) proved very good. The method of counting and studying stomata consisted first in selecting suitable comparable leaves, detaching them from the parent plant and immersing them in absolute alcohol to kill and fix them in the form in which they were gathered. The leaves were next immersed in the clearing fluid in which they remained for twenty-four hours and after clearing were washed with water so as to be free of clearing fluid. The tissue was stained with an aqueous solution of iodine in potassium iodide. This reagent turned the starch in the guard cell a dark blue color and it was not difficult then to find the stomata and count them. Slides were made and studied under high magnification of the microscope. Stomata were found only on the lower face of the leaf and on the margin of the leaf, they occur in larger numbers than in the center portion of the leaf. Stomatal counts are found in table VIII. They were calculated on the basis of so many per square millimeter of leaf area. Counts were taken at random in different regions of the blade, some near the mid-rib, others near the tip of the leaf, some at the base and others on the margin, etc. Counts on ten different microscopic fields were usually obtained.

A comparison was made of old leaves and young leaves. The number of stomata is different for the two types indicated. The younger leaves possess per unit area a greater number of stomata than older ones, whether one considers the healthy or mosaic-infected plants. The diseased leaves have twice as many as the healthy. A determination of the size of the opening between the guard cells was attempted but because of the uneveness of the surface and the abundance of hairs present on the surface of the leaf no satisfactory results were obtained. This information is needed because it will

Table VIII

Number of stomata per sq. mm. of leaf area in healthy and mosaic-infected plants.

	Healthy						Mosaic				
	Random Counts				margin		Random Counts				margin
	old	old	old	young	young	old	old	old	young	young	old
1	14	14	11	21	21	16	20	25	51	45	30
2	5	10	12	13	15	14	23	30	53	30	33
3	5	11	14	14	13	21	34	22	27	52	29
4	5	9	8	15	14	14	32	26	32	46	36
5	11	13	7	13	16	15	26	34	39	38	31
6	8	8	5	21	11	14	30	28	37	29	34
7	11	10	14	11	16	16	32	31	44	49	27
8	8	9	11	16	18	12	25	24	50	52	30
9	13	12	9	16	15	13	27	23	49	48	31
10	14	11	7	11	16	15	29	30	35	36	35
Average	9.4	9.7	9.8	15.5	15.0	15.0	26.8	25.5	41.7	40.5	31.6

help to decide the question of whether or not the greater rate of transpiration is due to the greater number of stomata. Harrison (18) reported in the case of bean mosaic that there was a reduction in transpiration, and accounted for this reduction by demonstrating a decrease in the number of functioning stomata. In fact he found many stomata closed, although the number did not vary in the two sorts of plants. The increased number of stomata on the raspberry mosaic-infected leaf may be accounted for by the fact that the leaf is smaller than a healthy one and therefore more stomata are collected in a unit of area than on a well expanded healthy leaf. It is a well known fact that the diseased leaves are somewhat dwarfed.

VII. Chlorophyll

A comparative study of the chlorophyll content of the leaves of the three sorts of plants was made. In appearance, the diseased condition is accompanied with mottling of the leaves. There are blistered areas in which the tissue is a darker green than the normal and there are other areas in which the tissues are a pale green. In these studied the entire leaf was taken. To separate the green portions from the pale green in any one leaf was uncalled for at this time. The masked plants, on casual observation, did not look very different from the healthy but as one can see from the table they contained more chlorophyll.

Peterson (27) in 1931 found a reduction of chlorophyll in mosaic-infected tobacco. Chloroplasts in leaves of mosaic-infected plants have been studied and the changes noted but little or no knowledge is obtainable on the chlorophyll content and its possible variation in diseased and healthy plants. In connection with the diseased plant's reduced photosynthetic activity, one might expect a reduction in chlorophyll content. However, reduced photosynthetic activity might just as well be due to accumulation of products or some other condition.

The chlorophyll content of leaves was determined by the method described by Ulvin (39). The colorimetric method of chlorophyll determination was selected. The results are given in milligrams of chlorophyll per 100 square centimeters of leaf area and are noted in table IX.

A study of this table shows that the mosaic-infected leaves contained approximately 12 per cent more chlorophyll than the healthy. The leaves of the masked plants also possessed about 11 per cent more chlorophyll. These results do not agree with those of Peterson (27) and were unexpected and a little surprising. The experiments were repeated in a different season and the same general results were obtained. The percentage increase is not great but is significant. A possible explanation is advanced as a result of some cytological studies of diseased leaf tissue. The mosaic-infected leaves have a pale green color and in some spots are almost yellow, but alternating with these areas are other numerous, raised or blistered areas that are very dark green in color. A microscopical study of sections in these blistered areas show two or three extra layers of palisade cells, all of which contain numerous plastids. These extra cells are apparently plentiful enough to give the diseased leaves more

Table IX

Fresh weight, total chlorophyll and chlorophyll per 100 sq. cm. in leaves of healthy masked and mosaic-infected plants.

Plant	Fresh Wt. Gm.	Area $Cm.^2$	Wt./100 Cm^2	Chlorophyll mg.	Chlorophyll 100 Cm^2	Average Chlor/100 Cm^2	Ratio
Healthy	8.127	324	2.54	8.94	2.76		
Healthy	5.132	322	1.60	7.96	2.47	2.61	100
Masked	4.152	204	2.07	6.30	3.15		
Masked	4.044	196	2.02	5.59	2.79	2.97	111
Mosaic	3.301	144	2.20	4.78	3.18		
Mosaic	3.358	162	2.09	4.78	2.98	3.08	112

chlorophyll than healthy ones. Bennett (3) states that the severely affected varieties of red raspberries "show a fine-grained pattern of mottling consisting of small yellow spots surrounded by tissue of deeper green color. Leaves in general are deeper green than are leaves of healthy plants." Until further work is done no definite conclusion, perhaps, should be drawn though the two tests are indicative of a small increase in chlorophyll in the mosaic-infected leaves.

VIII. Leaf Ash Studies

Rao and Sreenivassaya (29) made a study of the ash of healthy and "spiked" sandal and found less calcium and potassium in "spiked" tissue than in healthy, but the writer has been unable to find any further literature on ash analysis in healthy and mosaic-infected tissues. Only one set of tests was made with raspberry plants and further work should be done to clear up discrepancies and establish tentative conclusions. Leaves of healthy, masked, and mosaic-infected red raspberry plants were collected separately and ashed. The ashing temperature was approximately 550° C. The same method was followed as that used successfully in another project, not yet reported.

The elements (Ca, Fe, My, P. and K) in the ash were determined by the spectrographic method and the tests were made under the direction of Professor D. T. Ewing of the Chemistry Department of this Institution. The data may be found in table X. The per cent of ash is calculated on the dry weight basis. It may be observed from a study of the table that the afternoon figures are lower than the morning figures, indicating that possibly a diurnal variation in salt content exists.

Table X

Dry weight, weight of ash, per cent ash and minerals present in ash of leaves from healthy, masked and mosaic-infected plants.

Plant		Dry Wt.	Wt. Ash	% Ash	*Element 100% of Ash				
					Ca.	Mg.	P.	Fe.	K.
Healthy	A.M.	4.834 gm.	0.463 gm	9.57	10.7%	4.5%	3.7%	3.4%	16.0%
"	P.M.	3.383	.323	8.40	15.6	6.1	3.6	2.4	15.3
Masked	A.M.	2.716	.269	9.90	6.3	4.7	3.0	3.4	19.0
"	P.M.	3.117	.284	9.11	2.9	4.3	3.1	2.4	18.0
Mosaic	A.M.	3.111	.290	9.32	10.5	5.0	5.3	3.7	17.3
"	P. M.	2.687	.232	8.63	7.2	3.2	3.5	2.4	17.3

*Large quantities of silicon were found in all plants.

Potassium is more abundant than any other element and iron is the least plentiful. Calcium follows potassium as the next most abundant. When diseased and healthy plants are compared it is obvious that there is less calcium and more potassium in mosaic-infected leaves. The difference is not great and may not be significant but if potassium or calcium play any important roles in photosynthesis as some think, these studies do not bear out such conclusions. One would expect to find greater quantities of these elements in plants where photosynthesis occurs most rapidly or in this case in the healthy plants. Since magnesium is an integral part of the chlorophyll molecule, and since the chlorophyll content of diseased plants is appreciably higher, one might expect a higher content of magnesium, but there is hardly a difference between the healthy and diseased in this respect. Wolf's (43) numerous tables do not include data on the raspberry and the writer has not been successful in finding in the literature any figures on the elements present in the respberry plant. An abundance of silicon was found. In fact it interferred at first in the preparation of samples for spectrographic analysis, but the quantity obtained was not determined. More study should be given to the ash content of the raspberry leaf and several tests should be conducted before definite conclusions are drawn.

IX. Discussion

It has been known for many years that when a raspberry planting becomes infected with mosaic the plants gradually decline in productivity and the planting is usually described as having "run out".

The data presented in this paper may be interpreted in the main as offering an explanation for this "running out" of plantings of susceptable varieties.

It is shown that production of carbohydrates is reduced by the presence of the virus and that its influence upon photosynthesis is evident. Naturally this leads to a decrease in the supply of reserves stored in the roots to be used in the succeeding crop of fruit and new canes. Progressive dwarfing of the canes produced each year in an infected planting bears out these observations. The formation of fruit of poor quality and flavor is also attributable to a reduction in photosynthesis. In addition to a reduction in decrease in carbohydrate manufacture, it has also been possible to show that respiration is greatly increased. A high respiratory rate indicates that the products of photosynthesis are being used up much more rapidly in the mosaic-infected plants. The virus, perhaps, is wasteful of the stored energy of the diseased plant. This rapid release of energy together with the decrease in rate of formation of respirable material soon leads to starvation and eventually death. The scarcity of starch in diseased leaves is one incident at least of starvation conditions. Starch may be considered as one of the reserve forms of carbohydrate and in this case the simple materials are not converted into starch or if starch is formed it is soon hydrolyzed to produce respirable simple sugars when synthesis is slowed up or inactive. That something of this sort has taken place is indicated by the poor fruiting habits of the mosaic-infected plants.

The higher rates of transpiration and the increased number of stomata per unit area in diseased leaves indicate that the water relations of the plant are seriously affected by the presence of the virus. It is a well known fact that the fruits from mosaic-infected plants are seedy, dry and tend to crumble when harvested. This condition may be brought about by the inability of the plants to maintain a proper water balance. Under favorable conditions, the poor control of water balance may not be evident nor act as a detrimental factor, but when seasonal conditions and temperatures are unfavorable and the supply of moisture is limited, then mosaic-infected plants exhibit their inefficiency in water conservation by producing fruit of poor quality.

The results gathered so far in this study bring to one's attention certain important differences in the physiology of the three sorts of plants. They constitute sign posts or guides in the study of the causes underlying these differences and possibly point out a method of correction or control. Enzymatic activity or inactivity seem to play the major role and this phase is to be stressed in future work.

No method of controlling mosaic diseases other than roguing or producing disease-resistant forms has been found. Broadly speaking, once a plant is infected it can not be cured. Claims have been made that plants occasionally recover from attack. Storey (2) reported recovery of sugar cane from the streak disease and Robbins (31) has described the recovery of sugar-beets from mosaic. In many of these reported cases, it has been shown that the cure was an instance of a masking of the symptoms. Wilbrink (42) has described a method of cure for diseased sugar-cane cuttings by treatment with hot water. Kunkel (22) found that a heat treatment was a cure for certain virus disease of the peach. Masking in the raspberry is related to the temperature at which the plants are grown, and a study of the possibility of curing red raspberry mosaic by a heat treatment might well be made.

Summary

This paper reports the results of investigations dealing with the physiological activities of healthy, masked, and mosaic-infected raspberries. The studies were confined to the Latham variety infected with the red raspberry mosaic.

Analyses of the leaves for various carbohydrate fractions were made. A decrease in simple sugars and starches occurs in the mosaic and masked plants. Sucrose occurred in increased amounts in mosaic-infected plants.

Diastatic activity was studied and appears to be accelerated in plants exhibiting the mosaic symptoms.

Photosynthetic activity and respiratory rate were determined by the Co_2 absorption method. Photosynthesis was decreased in plants showing marked symptoms of disease.

Respiration proceeds at a higher rate in diseased tissue regardless of whether the symptoms are masked or evident.

In experiments where standardized hygrometric paper was used to determine the quantity of transpiration, it was found that a higher rate of water loss was exhibited by the leaves of mosaic-infected plants.

An increase in the number of stomata per unit area of leaf tissue probably accounts for the higher rate of transpiration in the diseased leaf.

Chlorophyll determinations show a greater amount of chlorophyll per unit area of leaf in the mosaic-infected plants.

Ash analyses of the leaves of the different plants were made by the spectrographic method. This one test indicated a high amount of silicon, a greater per cent of ash in the morning material than that in the material gathered in the evening, a lower calcium content and a slightly higher per cent of potassium in the diseased tissues.

Literature Cited

1. Barton-Wright, E., and Alan McBain. Studies in the physiology of the virus diseases of the potato: A Comparison of the Carbohydrate metabolism of normal with that of crinkle potatoes together with some observations on carbohydrate metabolism in a carrier variety. Ann. Appl. Biol. 20: 526-548. 1933.

2. Barton-Wright, E. Recent Advances in Botany. P. Blakiston's Son and Co., Inc., Philadelphia. 1932.

3. Bennett, C. W. Virus diseases of raspberries. Mich. Agr. Exp. Sta., Tech. Bul. 80. 1927.

4. Bertrand, G. Le dosage des sucres reducteurs. Bull. Soc. Chim., ser. 3. 35: 1285-1299. 1906.

5. Bolas, Bernard and W. F. Bewley. Aucuba or yellow mosaic of the tomato. A note on metabolism. Nature 126: 471. 1930.

6. Bolas, B. D. and R. Melville. The influence of environment on the growth and metabolism of the tomato plant. I Methods, technique and preliminary results. Ann. Bot. 47: 673-688. 1933.

7. Brewer, P. H., James B. Kendrick and Max W. Gardner. Effect of mosaic on carbohydrate and nitrogen content of the tomato plant. Phytopath 16: 843-851. 1926.

8. Cook, M. T. Photosynthesis of the sugar cane mosaic plant. Jour. Dept. Agr., Porto Rico 10: 239-242. 1926.

9. Cooley, L. M. The identity of raspberry mosaic. Phytopath. 26: 44-55. 1936.

10. Dastur, R. H. and B. L. Desai. The relation between water content, chlorophyll content and the rate of photosynthesis in some tropical plants at different temperatures. Ann. Bot. 47: 69-88. 1933.

11. Davis, W. H. Lactophenol. Bot. Gaz. 77: 343-344. 1924.

12. Detmers, F. Diseases of the raspberry and blackberry. Ohio Agric. Exp. Sta. Ann. Rpt. 10: 128-129. 1891.

13. Dickson, B. T. Studies on mosaic. Phytopath 11: 446-458. 1921.

14. Dufrenay, Jean. La retention de l'eau par la cellule vegetale: Maladies d'hypotome et maladies d'hypertomie. Rev. Path. Compt. et Hyg. Gen. 31: 212-223. 1931.

15. Dunlap, A. A. The total nitrogen and carbohydrates and the relative rates of respiration in virus affected plants. Amer. Jour. Bot. 17: 348-357. 1930.

16. ______________. Carbohydrate variation accompanying the mosaic disease of tobacco. Amer. Jour. Bot. 18: 328-336. 1931.

17. Green S. B. Cane rust of raspberries (anthracnose) Minn. Agric. Exp. Sta. Ann. Rept. 1894: 230. 1895.

18. Harrison, Arthur L. The physiology of bean mosaic. N. Y. State Agr. Exp. Sta. Tech. Bul. 235. 1935.

19. Heinicke, A. G. and M. B. Hoffman. The rate of photosynthesis of the apple under natural conditions. Cornell Agr. Exp. Sta. Bull. 577. 1933.

20. Heuberger, J. W. and J. B. S. Norton. The mosaic diseases of tomatoes. Md. Agr. Exp. Sta. Bul. 345: Part I, 447-486. 1933.

21. Hibbard, R. P., Buford H. Grigsby and G. W. Keck. A low light intensity photoelectric device for the determination of leaf areas. (In print.)

22. Kunkle, L. O. Heat treatment for the cure of yellow and rosette of peach. Phytopath I, 25: 24. 1935.

23. Livingston, B. E. and E. B. Shreve. Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World 19: 287-309. 1916.

24. Long, F. L. and F. E. Clements. The method of collodion films for stomato. Amer. Jour. Bot. 21: 7-17. 1934.

25. Meyer, Bernard S. The measurement of the rate of water-vapor loss from leaves under standard conditions. Amer. Jour. Bot. 14: 582-591. 1927.

26. Neuwirth, F. Beitrag zur Kenntnis des Oheflâche der Rübe. Zeitsch. Zuckerind. Czech. Republ. 54 : 341-349. 1929-30.

27. Peterson, P. D. Plastid pigment and chlorophyllase contents of tobacco as influenced by three types of mosaic. Phytopath 21: 119. 1931.

28. Rankin, W. H. and J. F. Hockey. Mosaic and leaf curl (yellows) of the cultivated raspberry. Phytopath 12: 253-264. 1922.

29. Rao, D. A. Roma and M. Greenivasaya. Contributions to the study of Spike Disease of Sandal. Part IV. Chemical composition of healthy and spiked sandal stems. Jour. Indian Inst. Sci. 11A: 241-243. 1928. (Authors' summary in Biol. Absts. 4: 341. 1930.)

30. Report of the Committee on Methods of Chemical Analysis for the American Society of Plant Physiologists. Pl. Physiol. 2: 91-97, 195 - 204, 297-402. 1927.

31. Robbins, W. W. Mosaic diseases of sugar beets. Phytopath, 11: 349-365. 1921.

32. Rosa, J. T. Chemical changes accompanying the western yellow blight of tomato. Pl. Physiol. 2: 163-169. 1927.

33. Smith, Kenneth M. Recent advances in the study of plant viruses. P. Blakiston's Son and Co., Philadelphia. 1934.

34. Sreenivasaya, M. Masking of spike disease symptoms in Santalum album. Nature 126: 957. (1930)

35. Stahl, E. Einige Versuche uber Transpiration und Assimilation. Bot. Zeit. 52: 117-146. 1894.

36. Stone, Winona E. Growth, chemical composition and efficiency of normal and mosaic potato plants in the field. Jour. Agr. Res. 52: 295-309. 1936.

37. Storey, H. H. (In Barton-Wright (2))

38. True, R. H. and L. A. Hawkins. Carbohydrate production in healthy and blighted spinach. Jour. Agr. Res. 15: 381-384. 1918.

39. Ulvin, Geo. B. Chlorophyll production under various environmental conditions. Pl. Physiol. 9: 59-82. 1934.

40. Whitehead, T. Respiration of healthy and leaf roll potatoes. Nature 128: 967. 1931.

41. Wilcox, R. B. Observations on masking of raspberry mosaic by high temperature. Phytopath 16: 80. 1926.

42. Wilbrink, G. Warmwaterbehandlung van stekken als geneesmiddel tegen de serchziekte van het suikerriet. Arch. Suikerindus. Nederland - Indië, Meded. Proefst. Java Suikerindus. 1: 1 - 15. 1923.

43. Wolf, E. V. Aschenanalysen von landwirtschaftlichen Produkten usw. Berlin: P. Parey I Teil 1871. II. Teil 1880.

Figure 1. Typical leaf of the Latham variety infected with red raspberry mosaic.

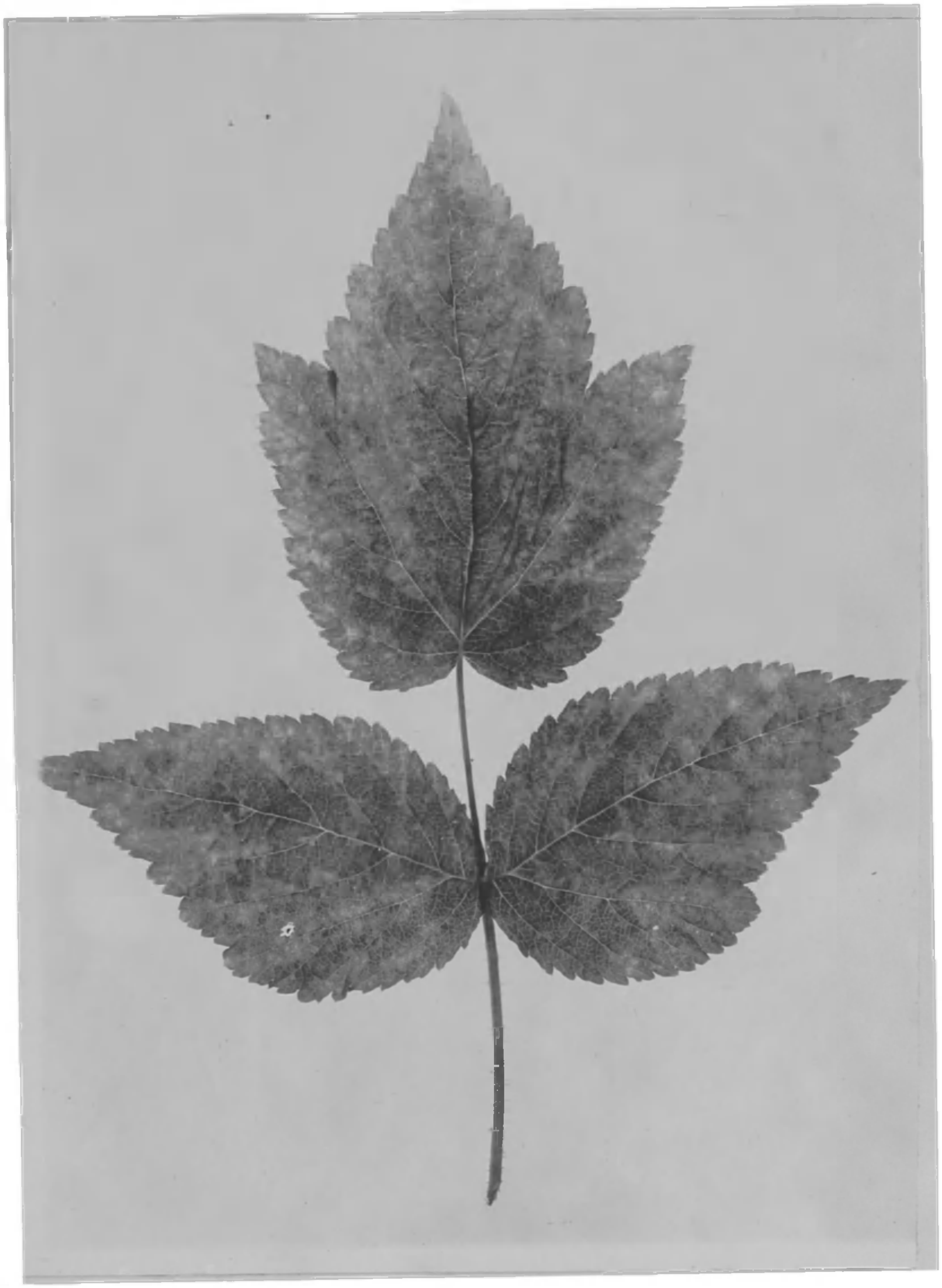
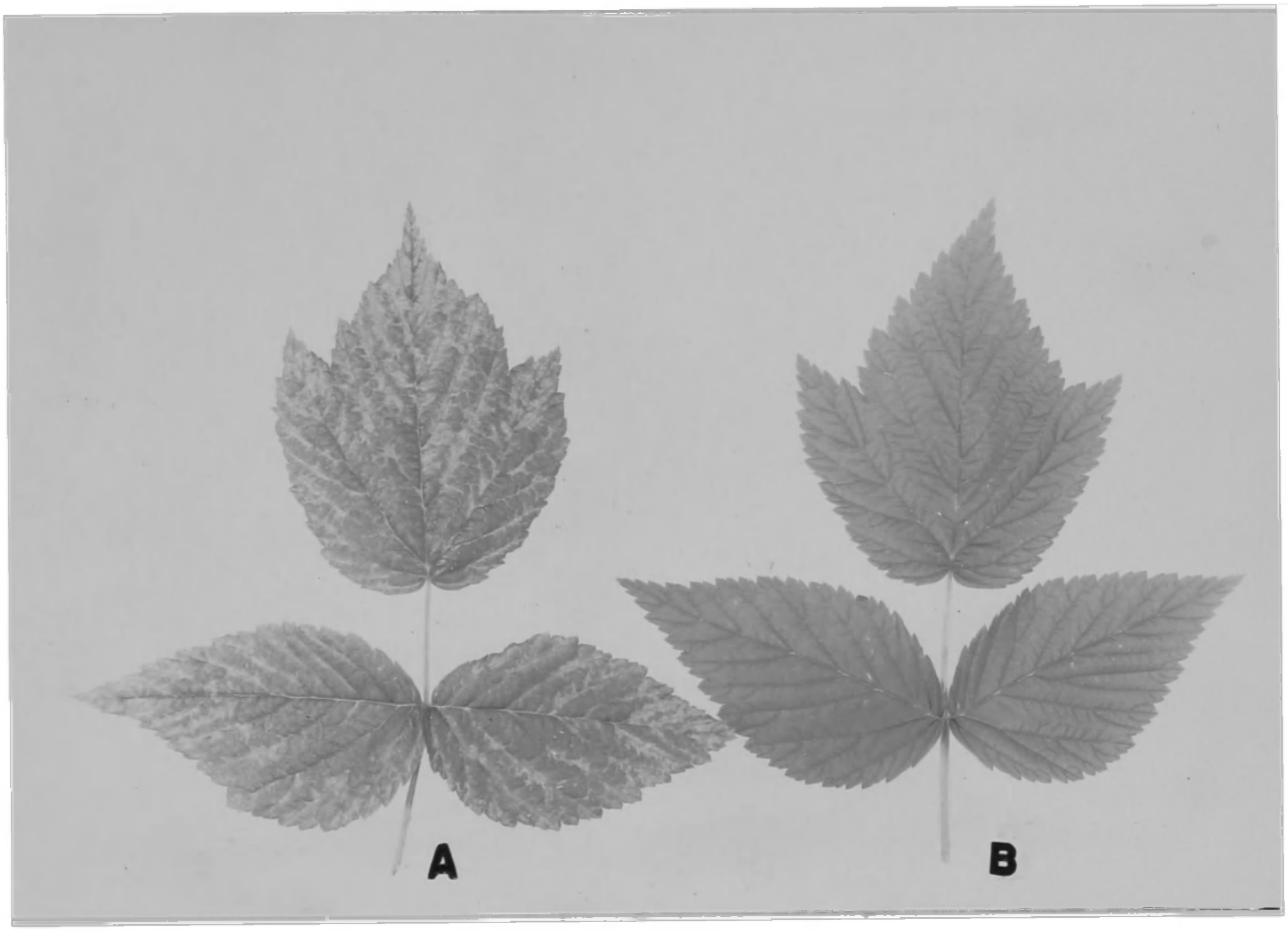


Figure 2. Healthy and mosaic infected leaves of the Latham variety.

A. Mosaic infected. B. Healthy



A
B

Figure 3. Absorption unit and assimilation chambers as set up for operation. A. Celluloid assimilation chamber.

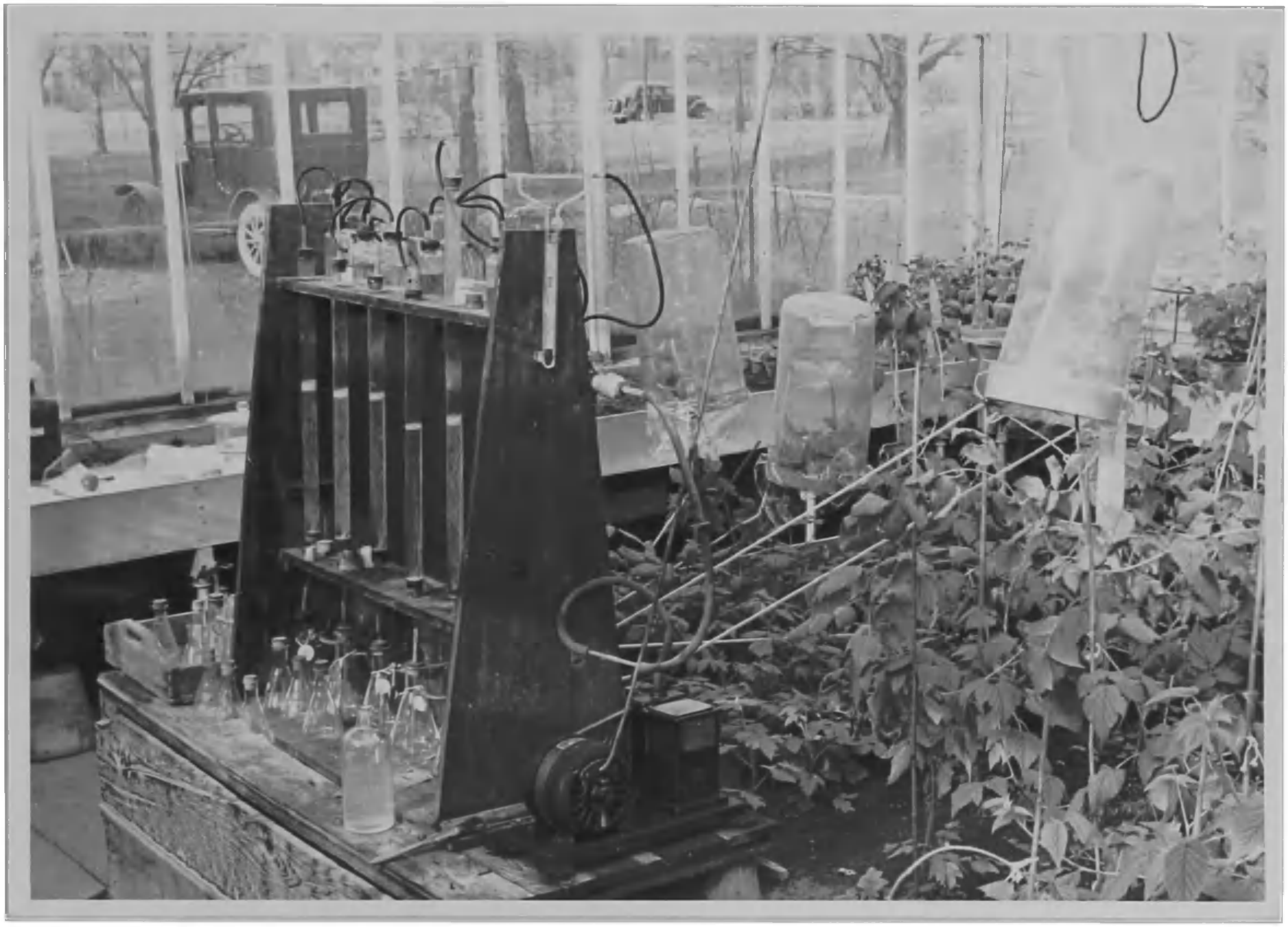


Figure 4. Photoelectric leaf area apparatus.