STUDIES OF PHOSPHORUS METABOLISM OF HORDEUM VULGARE L. PLANTS INFECTED WITH BROMEGRASS MOSAIC VIRUS

by

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INTRODUCTION

Viruses were recognized as causal agents of plant diseases at the beginning of the twentieth century. Tobacco mosaic virus disease was the first to be identified as a transmissible disease, the causal organism of which would pass filters too fine to let bacteria through (Iwanowski, 1892). Tobacco mosaic virus can be transmitted by merely rubbing diseased leaves against healthy ones (Mayer, 1886). Once a virus is introduced into one or more cells, it may spread through all living cells. The mosaic pattern in leaves results from degenerative changes in the mesophyll, involving changes in the chloroplasts.

Subsequent research in this field lead to the discovery of viruses which require insects for transmission. Moreover, it was also found that certain viruses induce no disease unless they are introduced into the phloem tissues. Thus the relationship between the host and the virus is highly specialized in some cases.

Esau (1961) recognized three general categories of viruses: (1) Viruses distributed throughout parenchyma, including that of the conducting tissues--these are histologically non-limited viruses; (2) Viruses limited to the phloem tissue; (3) Viruses limited to the xylem tissue. These differences are related to methods of transmission, anatomic changes in the host plant, and translocation of viruses in the plant.

Bromegrass mosaic virus (BMV) belongs to the first category, is non-limited and is capable of multiplying in any susceptible living tissue. McKinney (1944) studied the symptoms produced by BMV. The interaction results in chlorosis and necrosis. Grass hosts include wheat, rye, barley,

oats, sorghum, Johnson grass, and other perennial grasses; whereas the non-grass plants include Scotia garden beet (Phaseolus vulgaris L.), Samsun tobacco (Nicotiana tobaccum L.), garden beets, sugar beets, swisschard, and lambsquarters (McKinney, 1944).

Chiu and Sill (1963) purified BMV and studied its properties. In gross appearance, BMV particles were spherical with an average diameter of 32.8 mu. The nucleic acid content was found to be 34 per cent, based on the assumption that BMV consists only of proteins and ribonucleic acid.

Very little is known concerning the metabolic effects of BMV on host plants. Hence, the present study was undertaken to evaluate the changes in total phosphorus, inorganic phosphorus, reducing and non-reducing sugars, and other organophosphorus compounds in barley infected by the BMV virus.

REVIEW OF LITERATURE

Bawden (1964) opined that the changes accompanying infection are due to disturbances in cellular metabolism, the exact nature of which is unknown. In effect, viruses become part of their host cells. The composition of viruses is known to consist of protein and nucleic acids and none has been found to contain enzymes. The nucleic acid is infective, self-replicating, and initiates the synthesis of the appropriate protein with which it combines to give completed virus particles; whereas the virus protein protects the nucleic acid from inactivation. It has been found that leaf extracts which contain ribonuclease and other substances readily inactivate virus nucleic acid, whereas the leaf proteases do not hydrolyze virus protein.

Among the mineral elements, phosphorus plays a central role in metabolism (Glass, 1951). It is a component of phospholipids, which are important in the formation of cell membranes and contribute to regulate permeability. Phosphorus provides the linkage between the nucleotides which compose ribose and deoxyribose nucleic acids and is therefore essential for growth and heredity. Phosphates are also important natural buffers which help to regulate hydrogen ion concentration of protoplasm and the surrounding fluids. It participates in the formation of numerous intermediate compounds and coenzymes which are essential to the metabolism of carbohydrates, as well as to many oxidation-reduction reactions and other intracellular processes. Thus organic phosphorus compounds play an important role in the synthesis of proteins in phospholipid metabolism, in the synthesis and metabolism of nucleic acids, and in photosynthesis.

Changes in Phosphorylated Compounds

Changes in phosphorylated compounds have far-reaching effects in many metabolic processes. According to Diener (1963) synthesis of viral nucleic acid is the most basic process of virus replication and, therefore, the synthesis of this anomalous nucleic acid is by itself a disturbance of the metabolism of phosphorylated compounds in the host.

Porter and Weinstein (1957) found decreased levels of non-nucleic acid organic phosphorus in tobacco leaves infected with cucumber mosaic virus as compared with healthy leaves. Total phosphorus, inorganic phosphorus, and organic phosphorus contents were not materially affected by virus infection, but both RNA and DNA phosphorus contents were higher in infected than in healthy leaves (Porter and Weinstein, 1957, 1960).

Elbertzhagen (1958) found the total phosphorus content to be unaffected in tobacco leaves infected with TMV or with potato virus X, although the nucleic acid phosphorus content was higher in diseased than in healthy ones.

Changes in Enzymic Activity

The enzymes of the host plant are also affected. Increase in the activity of oxidases and peroxidases has been reported by many workers (Woods, 1900; Bunzel, 1913). Polyphenol oxidase has been found to be increased by infection with several different viruses in many different host plants (Martin et al., 1938; Hampton and Fulton, 1961). Farkas et al. (1960) found that tobacco mosaic, cucumber mosaic, or tobacco necrosis viruses increased polyphenol oxidase activity. They attribute necrotic

local lesions to the oxidation of polyphenols. Farkas et al. (1960) also reported the increased activity of the enzyme dehydrogenase, which helps to prevent oxidation of the phenolic compounds.

Peterson and McKinney (1938) reported changes in chlorophyllase produced by three strains of viruses. Increase in chlorophyllase activity was found in infected plants, whereas chlorophyllase activity and chlorophyll content were found to be directly proportional in healthy leaves. Chlorophyll, carotene, and xanthophyll decreased by the same proportion in the infected plants. However, Elmer (1925) found double the concentration of carotene with tobacco mosaic virus and less amounts of both chlorophyll and xanthophyll.

Zaitlin and Jagendorf (1960) reported a direct relationship between nitrogen nutrition and photochemical activity, with isolated chloroplasts. Spikes and Stout (1955) found that chloroplasts from sugar beet leaves infected with beet yellows virus had only half the photochemical activity of those from uninfected leaves. This effect was common for both yellows and the mosaic types.

Carbon:Nitrogen Ratio

Virus infections usually change the carbon:nitrogen ratio in leaves--some viruses increase it, others decrease it (Jodid et al., 1920; Campbell, 1925). Dunlap (1930) suggested a classification based on C:N ratio. (1) Those causing mosaic-type diseases, which increased nitrogen content and decreased carbohydrates; and (2) those causing yellow-type diseases, which increased carbohydrate content and decreased nitrogen.

Quanjer (1913) found an accumulation of carbohydrates with phloem necrosis in potato plants. This has been attributed to the collapsed sieve tubes which prevented normal movement of sugars.

Murphy (1923) showed the presence of starch in infected leaves by the iodine test, whereas uninfected leaves lost their starch. This holds good for many viruses causing necrosis. Holmes (1931) suggested staining at different times of the day to know the rate at which starch accumulates during daylight and the rate at which it disappears in darkness.

Wynd (1943) found that a general effect of infections by mosaic and yellow-type viruses was to alter the permeability of cytoplasm so that soluble substances diffused more slowly than in uninfected cells.

The translocation rate of sugars is unchanged in sugar beet yellows by infection. Hence, the rise in carbohydrate content of the leaf, possibly due to increased resistance to the movement of sugars through the leaf lamina (Watson and Watson, 1953). The sugar of translocation in healthy potatoes is sucrose and in plants with leaf roll is hexose (Barton-Wright et al., 1941). Mannitol was detected in the spike disease of sandal, and absent in healthy sandal (Sreenivasaya, 1930).

Among the few mosaic diseases that have been studied, beet mosaic (Watson, 1953) and potato crinkle (Barton-Wright, 1941) had little or no effect on carbohydrate content of leaves, but leaves of Arran Victory potatoes with paracrinkle had more carbohydrate than uninfected leaves. Potato plants with crinkle had more nitrogen and protein than uninfected leaves (Barton-Wright, 1941), and beet mosaic virus increased the nitrogen content of all parts of beet plants without nitrogenous fertilizers, but not of those that were fertilized.

Martin et al. (1938) found no differences between the total nitrogen and total protein of healthy plants and plants infected separately with three strains of tobacco mosaic virus. As a proportion of the total nitrogen, the variation is much greater than this, from 10 per cent in well fertilized plants to 60 per cent in plants receiving abundant phosphorus but no nitrogen (Holden and Tracey, 1948). However, the virus will multiply in detached leaves given no extra supply of nitrogen; in such conditions there can be no increase in total nitrogen (Bawden, 1964).

Photosynthesis and Respiration

Photosynthesis has been found to be decreased by potato leaf roll and by infection with the mosaic type viruses, tobacco mosaic, potato X and tobacco etch (Owen, 1956, 1957). Respiration increased after infection with several yellow-type and many mosaic type viruses (Yarwood, 1953).

According to Diener (1963) after a period of active photosynthesis the sites of local infection contained less starch than neighboring non-infected tissues; whereas, after a period of darkness infected tissues contained more starch than non-infected ones. Thus infection decreased the rate of starch synthesis and translocation. Starch was retained in infected areas, because of the inactivation of enzymes which transformed starch into sugars.

Owen (1955) found 10 per cent increase in respiration in directly inoculated leaves within an hour. The rate remained unchanged for a few days, during virus multiplication, then became equal to uninfected plants. With the decrease in photosynthesis as much as 20 per cent and increase in respiration by 40 per cent, the plants became stunted. However, the stunted

effect was overcome by supplying gibberellic acid in the case of tobacco etch virus (Chessin, 1957).

Goss et al. (unpublished) found increased respiration in BMV infected barley plants two days after inoculation, which decreased for two days, increased again just before symptoms appeared, and finally remained above that of healthy plants throughout the experimental period. According to them the increase did not appear to be due to uncoupling of phosphorylation by the virus and probably not to increased available substrate.

The following conclusions were drawn by Diener (1963) regarding physiological dearrangements associated with virus infection: (a) Decreased photosynthetic activity; (b) increased rate of respiration; (c) accumulation of soluble nitrogen compounds, particularly amides; (d) increased activity of polyphenol oxidase and accumulation of oxidized polyphenol derivatives; and (3) decreased activity of growth regulating substances.

MATERIALS AND METHODS

The winter barley variety Reno was selected for this study. The seedlings were raised for about two weeks in one-half Hoagland (1938) nutrient solution in 3 litre jars. During the experimental period the nutrient solution was changed weekly.

Plant Inoculation

Fifteen-day-old barley plants were mechanically inoculated on the upper leaf surface with BMV. The inoculum consisted of carborundum and crude sap from plants infected with BMV and diluted by distilled water. Healthy control plants were wiped with distilled water containing carborundum. The leaves were harvested on the first, second, third, fourth, fifth, eighth, and fifteenth day after inoculation, by cutting with blade for the determination of reducing and non-reducing sugars, phosphorus and P^{32}.

Sample Preparation and Extraction for the Determination of Sugars

The sap from weighed samples of freshly harvested plants, both from infected and healthy controls, was extracted by means of mortar and pestle. During the process of extraction, crystals of ice were used to make up the required volume. The extract was transferred to graduated centrifuge tubes kept in an ice bath, the temperature of which was maintained near $2^{\circ}C$. After centrifuging for 5 minutes at 3400 r.p.m. with 1640g. the supernatant extract was used for paper chromatography.

Chromatographic Separation

Sheets of Whatman No. 1 filter paper were used as illustrated below. Twenty-five micro liter micropipettes were used to spot plant extract samples on the space shown in Fig. 1. The identification of the sugars in the extract was accomplished by the use of known standards.

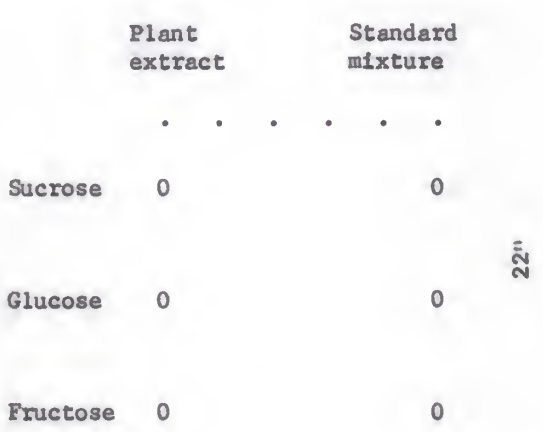


Fig. 1

Among the solvents used, Ethyl acetate, acetic acid, water (3:1:3) was found to be the best (top layer, Laidlaw and Reid, 1952). The solvent was run twice in order to get the best separation.

Reagents

The chromatograms were developed to identify individual sugar spots

(Trevelyan et al., 1950):

1. The dried chromatograms were dipped into a solution of acetone saturated with silver nitrate and were air dried.
2. These were then drawn through methanol containing 4 per cent sodium hydroxide. Spots appeared within 2 to 3 minutes.
3. The sugar spots were clarified with sodium thiosulphate solution.
4. Finally the chromatograms were drawn through a solution containing 1:1 ethyl ether and acetone, and dried.

Estimation of Sugars

The developed chromatogram was superimposed over the unspotted chromatogram. In so doing the area of the reference spot corresponded closely with the area of the unknown. Thus the individual sugar spots were located and cut out for elution with water in test tubes. Elution was continued overnight. The quantitative estimation of sugars was done by the method described by Hall (1956).

Estimation of Total Phosphorus

The total phosphorus was determined by a modification of the stannous chloride method. Twenty-five mg. of dried plant material (30 mesh) was used for acid digestion (Goss, 1962, 1964) and the content of phosphorus was determined by means of standard curves prepared, using a colorimeter (Goss, unpublished).

Detection of Organic and Inorganic Phosphorus

A similar chromatographic procedure was adopted for estimating organic and inorganic phosphorus P^{32}. Thirty and sixty mc of P^{32} was added to each jar of 3 litre capacity in Experiments 1 and 2, respectively. The time given for P^{32} incorporation within the plant was approximately 18 hours. The solvents used were EAW (3:1:3) in Experiment 1 and BAW (Benson et al., 1950) Butanol, Acetic acid, water (74:19:50) in Experiment 2.

The thoroughly dried chromatogram sheet was sprayed with Hanes and Isherwood solution (1949) and exposed to a germicidal lamp. This resulted in the appearance of blue colors for the organic phosphate and yellow-green for inorganic phosphate.

Autoradiography

The chromatograms were placed in contact with X-ray film, and the developed film was later superimposed over the chromatogram. The radioactive spots were cut from the chromatogram and the amount of radioactivity present determined for both inorganic and organic phosphates, using a scaler and thin end-window GM tube.

RESULTS AND DISCUSSION

Symptom Development

Normally symptoms developed on the barley plants on the fourth day after inoculation and became prominent on the fifth. Young leaves exhibited the first symptoms at the base. Non-infected plants remained green throughout.

Effect on Individual Sugars

The experiment was conducted twice and the results are presented in Tables 1 and 2. It is apparent that there is a considerable numerical difference regarding the content of sugars. Experiment No. 1 was conducted during the cloudy weather of winter, whereas experiment No. 2 was conducted during the spring season. The differences may be attributed to the changes in light and temperature in the greenhouse. However, there is an element of similarity between these experiments. In Experiment 2, there is an increase in the content of sucrose on the second day after inoculation; this may be due to increased synthetic activity within the host cells. Many workers have reported an increase in the rate of respiration following virus infection (Yarwood, 1953). This in turn follows increased synthetic activity. The first evidence towards this was furnished by Gottlieb and Garner (1946), in wheat infected with stem rust and supplied with radioactive phosphorus.

Marked accumulation of glucose, fructose, and sucrose reported by Yarwood and Jacobson (1955) in barley infected with powdery mildew, and sucrose synthesis from glucose, supplied by infiltration of glucose solution,

Table 1. Mg/g of fresh weight sap extract.

Experiment 1

Days	Healthy			BMV-Infected		
	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
1	0.320	0.612	1.34	0.28	0.22	0.98
2	0.272	0.984	1.58	0.40	0.12	1.50
3	0.880	1.14	1.36	0.61	1.60	1.60
4	0.832	1.60	1.83	0.36	1.60	1.32
5	0.800	1.216	1.88	0.98	1.36	1.00
8	0.760	1.25	1.48	0.93	1.60	1.90
15	0.200	1.36	1.93	0.24	1.14	2.20

Table 2. Mg/gm of fresh weight sap extract.

Experiment 2

Days	Healthy			BMV-Infected		
	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
1	1.566	2.052	2.970	0.62	2.10	1.4
2	1.476	1.305	2.655	.990	2.484	3.600
3	1.710	2.214	2.565	1.476	2.106	2.736
4	1.386	1.872	3.847	1.710	2.052	2.655
5	1.242	.648	1.957	1.476	1.710	1.404
8	.648	1.242	1.863	1.386	3.600+	5.400
15	.648	1.305	1.863	1.305	1.305	3.078

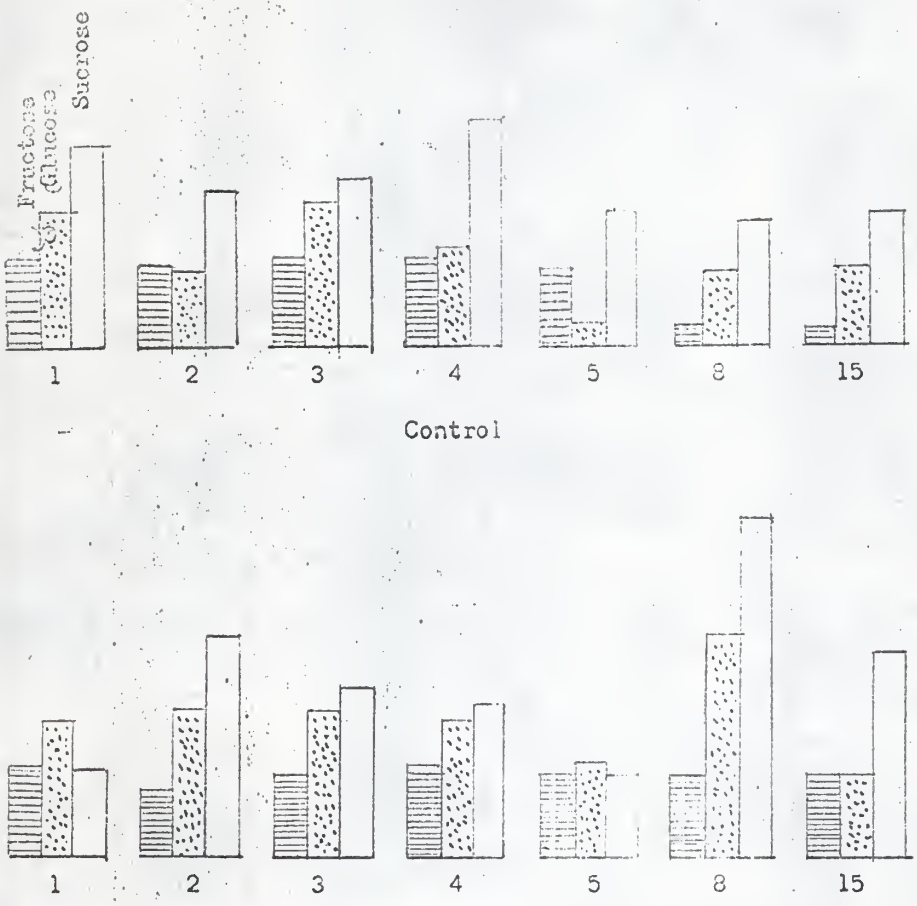


FIGURE 2

Changes in fructose, glucose, & sucrose in BMV infected barley leaves

was more rapid in mildewed than in healthy plants. In some plants the trend toward greater synthetic activity of the infected tissue reaches the point of renewed growth (Daly and Seyre, 1957).

The symptoms appeared on the fourth day in the present work and became prominent on the fifth. It can be seen (Table 2, Fig. 2) that there is a gradual decrease in sucrose on the third, fourth, and fifth days, the period when the multiplication of the virus is at its peak. Farkas (1957) opined that the accumulated products of synthetic activity, particularly carbohydrate, are gradually dissipated during the phase of "peaceful coexistence," i.e., the period of active proliferation of the parasite. However, on the eighth and fifteenth day there appears to be an increase in the sucrose content showing the trend toward accumulation, probably due to decreased rate of translocation; whereas the content of fructose and glucose remained approximately the same, except on the eighth day. These results are still inconclusive and need further verification. The rise and fall of sucrose content may also be due to other experimental errors.

Total Phosphorus

Results of the quantitative determination of total phosphorus are shown in Table 3. Comparison of the two experiments made indicates negligible differences owing to virus infection.

Table 4 shows that the level of P^{32} accumulation in non-infected and infected plants was not much affected by BMV except on the eighth and fifteenth day in the experiments.

Since the levels of total phosphorus were not affected, virus infection

Table 3. Per cent of total phosphorus.

	Experiment 1		Experiment 2	
Days	Healthy	Infected	Healthy	Infected
1	.83	.80	.80	.81
2	.83	.84	.81	.80
3	.80	.81	.80	.80
4	.85	.76	.80	.79
5	.79	.79	.80	.68
6	.80	.80	.80	.80
8	.81	.75	.80	.80
15	.72	.80	.80	.80

Table 4. P^{32} accumulation c.p.m./25 mg. dry wt.

	Experiment 1			Experiment 2		
	30 mc/3 l			60 mc/3 l		
Day	Healthy	Infected	I/H	Healthy	Infected	I/H
1	872.5	813.8	.93	3239.5	3216.0	.99
2	635.8	548.8	.86	3317.5	2938.5	.89
3	656.1	543.8	.83	1714.5	2025.0	1.18
4	444.4	610.0	1.37	3034.0	2996.0	.99
5	1155.8	704.9	.61	3467.5	3476.5	1.00
8	1837.8	934.2	.51	3511.5	728.5	.21
15	1162.2	1005.0	.86	5368.5	1386.7	.26

Table 4 (cont.).

Experiment 3

60 mc/3 l

Day	Healthy	Infected	I/H
1	320.5	490.4	1.2
2	549	330	0.6
3	495.5	709.5	1.4
4	584.0	600.5	1.2
5	350	216.0	0.6
8	595	415.5	0.6
15	630	442.0	0.7

did not appear to affect the uptake of phosphorus from nutrient medium. Similar results were obtained by Porter and Weinstein (1957-1960) in the case of cucumber mosaic virus-infected tobacco plants. However, P^{32} accumulation is less in later stages of virus infection.

Inorganic and Organic Phosphorus

It is apparent from Tables 5 and 6 (Fig. 3) that inorganic phosphorus was not greatly affected due to infection during the first five days. However, the level has decreased in infected plants on the eighth and fifteenth day; this effect being marked in Experiment No. 2. The changes observed in both the experiments reveal a little bit more on the fifth day, when the symptoms become prominent.

Table 5. Inorganic phosphorus fraction.

Experiment No. 1

30 mc P^{32} c.p.m./paper disk

Days	Healthy			Infected			I/H
	A	B	Total	A	B	Total	
1	3.6	39.5	43.1	3.3	32.3	35.6	.83
2	-	22.7	22.7	-	24.2	24.2	1.06
3	-	33.8	33.8	-	29.9	29.9	.88
4	-	42.1	42.1	-	35.3	35.3	.83
5	2.8	54.7	57.5	4.2	56.4	60.6	1.05
8	6.9	103.9	110.8	4.6	62.7	67.3	.61
15	10.4	88.5	98.9	6.5	44.2	50.7	.51

Table 6. Inorganic phosphorus fraction.

Experiment No. 2

60 mc P^{32} c.p.m./paper disk

Days	Healthy			Infected			I/H
	A	B	Total	A	B	Total	
1	28.5	450.6	479.1	26.5	336.6	363.1	.76
2	43.7	344.5	378.2	44.4	206.5	250.9	.66
3	53.0	86.0	139.0	31.1	149.2	180.3	1.29
4	181.1	302.9	484.0	104.7	254.0	358.7	.74
5	200.6	171.2	371.8	149.4	363.0	512.4	1.37
8	118.0	134.5	252.5	29.2	20.0	49.2	.19
15	409.5	228.4	637.9	53.3	121.0	174.3	.27

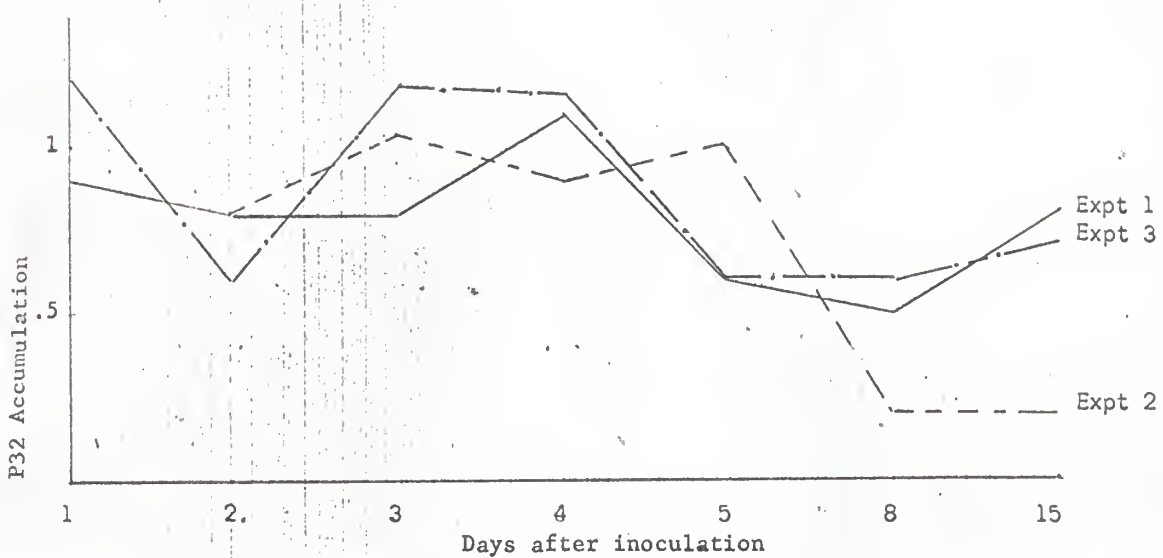
Table 7. P^{32} organic labeled phosphorus. C.p.m./paper disk.

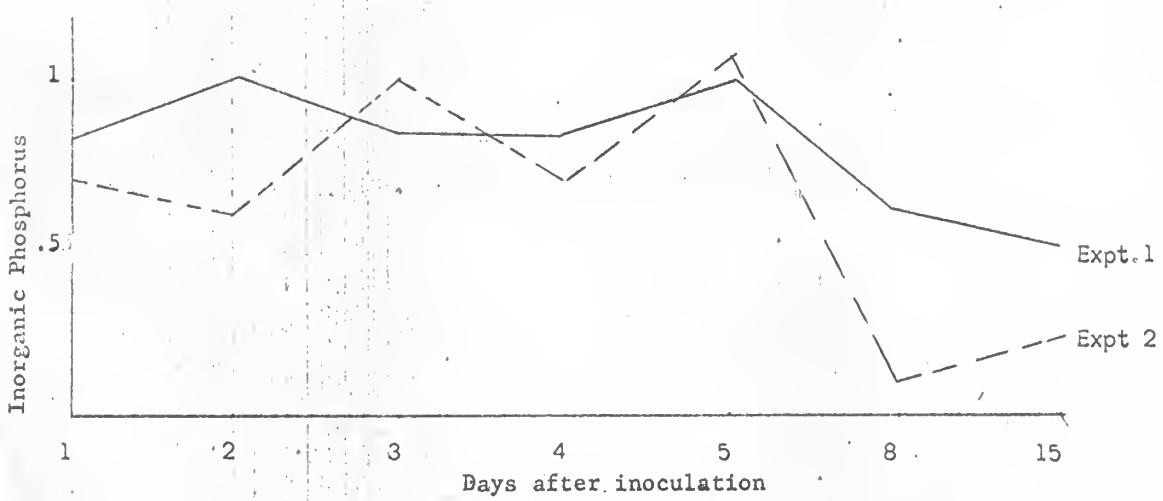
Experiment No. 2 with EAW

Days	Healthy	BMV-Infected	I/H
1	24.8	14.2	.24
2	18.0	12.2	0.67
3	5.6	10.0	1.78
4	25.2	19.9	.8
5	17.9	11.3	.62
8	17.3	3.1	.17
15	56.6	17.0	.30

Experiment No. 3 with BAW

Days	Healthy	BMV-Infected	I/H
1	23.7	68.5	3.0
2	61.0	31.6	0.51
3	31.2	69.2	2.2
4	92.6	47.2	0.50
5	69.9	19.3	0.30
8	61.3	53.6	0.80
15	35.0	60.0	0.50


P32 Accumulation
1
.5
Expt 1
Expt 3
Expt 2
1
2
3
4
5
8
15
Days after inoculation


Inorganic Phosphorus
1
.5
Expt 1
Expt 2
1
2
3
4
5
8
15
Days after inoculation

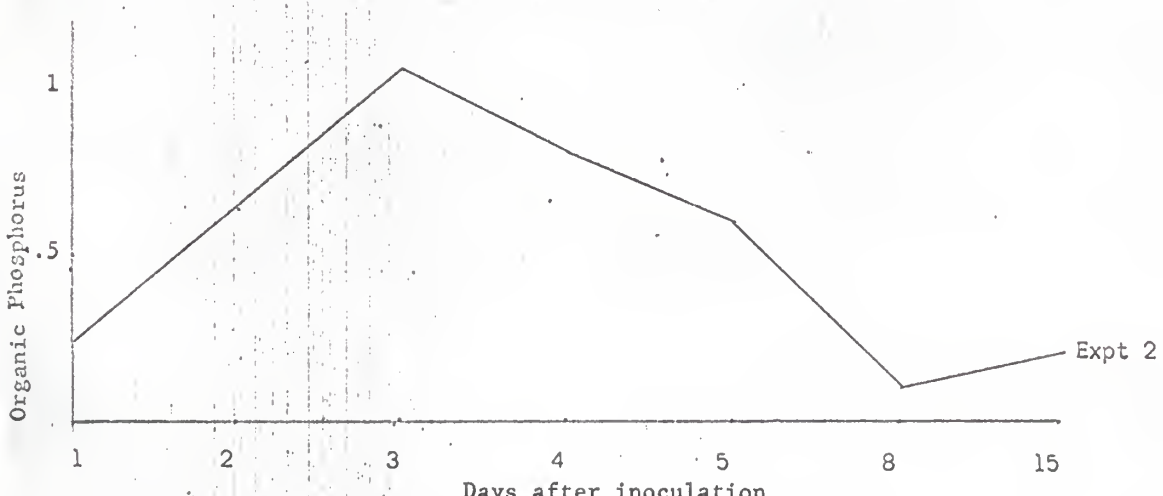

Organic Phosphorus
1
.5
Expt 2
1
2
3
4
5
8
15
Days after inoculation

FIGURE 3

The amount of organic labeled phosphorus present (Table 7, Fig. 3) shows a rise on the third day after infection, the lowest being on the eighth and fifteenth day. The presence of low amounts of organic and inorganic phosphorus on the eighth and fifteenth day may be due to a stimulated utilization of organic phosphorus compounds, as energy sources or component parts in the synthesis of more complex molecules such as nucleic acids, proteins, and nucleoproteins (Porter and Weinstein, 1957-1960), or due to low uptake.

The present data are in accord with the findings of Porter and Weinstein (1957-1960). More or less similar results were obtained by them with cucumber mosaic virus-infected tobacco plants.

SUMMARY

Bromegrass mosaic virus infected and non-infected barley plants were grown in half-Hoagland nutrient solution in the greenhouse, and their leaves analyzed for individual sugars, total phosphorus, inorganic and organic P^{32}. Infected plants exhibited virus symptoms on the fourth day after inoculation. The severity of symptoms increased on the fifth day, and became chronic on eighth.

Virus infection may have induced a stimulation of host metabolism as revealed by sucrose content from aqueous leaf extracts of infected barley plants, its consequent utilization during the peak period of virus multiplication, and a tendency toward accumulation. This may be due to decreased rate of translocation. However the data is preliminary and need further verification.

The data indicate that total phosphorus was not much affected. Virus infection, therefore, did not appear to affect the uptake of phosphorus from the nutrient medium.

The probable presence of lower amounts of organic and inorganic phosphorus after the virus inoculation period may possibly be due to a stimulated utilization of phosphorus as an energy source or for component parts in the synthesis of more complex organic molecules such as nucleic acids, proteins, and nucleoproteins.

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STUDIES OF PHOSPHORUS METABOLISM OF *HORDEUM VULGARE* L. PLANTS INFECTED WITH BROMEGRASS MOSAIC VIRUS

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Analytical results are presented with regard to individual sugars, total phosphorus, organic and inorganic phosphates. It is apparent from the results that major physiological changes were induced in barley plants by infection with BMV. The experimental plants were approximately of the same age. Therefore it is reasonable to evaluate the changes produced by the virus infection.

The present study is an evaluation of some physiological changes which accompany bromegrass mosaic virus infection. Infected and non-infected leaves of barley plants were analyzed on first, second, third, fourth, fifth, eighth and fifteenth day after inoculation, for individual sugars, total inorganic and organic phosphorus. The plants exhibited symptoms on the fourth day after inoculation. The severity of symptoms increased on the fifth and became chronic on the eighth day.

Probably the most interesting effect of virus infection was changes in sugars. There was a rapid increase following infection and its utilization accompanied the incubation period. The final increase may be due to decreased rate of translocation. However these changes may be due to temperature and light variations in the greenhouse and may also be due to other experimental errors. Hence the data need further verification.

The analysis of total phosphorus reveals that the virus infection did not affect the uptake of phosphorus from the nutrient medium.

The results indicate the probable presence of lower amounts of organic and inorganic phosphates after the virus incubation period. This may possibly be due to a stimulated usage of phosphorus as energy source or for component parts in the synthesis of other complex organic molecules or to low uptake.