PHYSIOLOGICAL STUDIES ON MOSAIC-INFECTED TOBACCO PLANTS: I. RESPIRATION II. PHOSPHORUS METABOLISM III. TRANSLOCATION OF VIRUS

By

George Constantine Vayonis

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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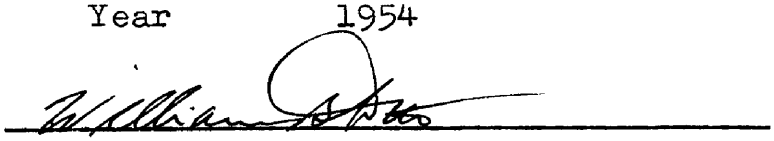
Approved 

The present study was undertaken for the purpose of investigating the effects of the mosaic virus infection on the respiration and phosphorus metabolism of Nicotiana tabacum, variety Havana, No. 38, plants. An attempt was made by the use of radioactive tobacco mosaic virus to correlate the appearance of the physiological disturbances in tissues far removed from the site of inoculation with the translocation of the initial virus inoculum.

Tobacco plants, cultured hydroponically, were used throughout the experiments. The respiratory rates of the different tobacco leaves were measured by the Barcroft differential manometers. The phosphorus metabolism of the control and diseased leaves was studied by Arney's phosphorus fractionation scheme.[1] The detection of radioactivity in the translocation study of phosphorus-32 tagged tobacco virus was carried out on fresh leaf-tissues by a Geiger-Müller tube.

The findings and conclusions of the present study can be summarized as follows:

1) The respiratory rates of leaves from tobacco mosaic virus (TMV) infected tobacco plants expressed in terms of dry weight, increased above the controls within 36 hours after the inoculation of a lower leaf. This increased respiration became apparent among the younger leaves within 12 hours after inoculation. It preceded by approximately five days the appearance of visible mosaic symptoms. Most leaves at the time when the symptoms appeared respired at lower rates than the corresponding control leaves.

2) The phosphorus metabolism of infected plants was disturbed by the disease. The total, inorganic and residual

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[1] Arney, S.E. Phosphate fractions in barley seedlings. Biochem. J., 33: II, 1078-1086, 1939.

phosphorus fractions were not consistently affected at the onset of the disease, while at later stages the first two fractions fall below the control and the third one increased above the control as the disease progressed. The phosphate esters' fraction, associated with the respiratory activities of the plant, exhibited an increased concentration in the younger leaves within 12 hours after inoculation. During the first two days after inoculation a positive correlation was detected between both the respiratory response and the phosphate esters' fraction, to the infection.

3) Radioactive tobacco mosaic virus was prepared by the incorporation of phosphorus-32 into the virus particles. Plants infected with the radioactive virus suspension exhibited systemic infection within four days after inoculation. No activity was detected in systemically infected tissues. When phosphorus-32 was used in the form of phosphate radical of approximately double the activity of the virus suspension radioactivity was detected in the growing tip within 36 hours after the rubbing of a lower leaf.

4) The data presented suggest that the respiratory abnormalities as well as the abnormalities of the phosphate esters' fraction in leaves of TMV-infected plants observed early in the development of the disease, do not follow but rather precede the appearance of virus particles.

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I. RESPIRATION II. PHOSPHORUS METABOLISM III. TRANSLOCATION OF VIRUS

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A THESIS

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PHYSIOLOGICAL STUDIES ON MOSAIC-INFECTED TOBACCO PLANTS: I. RESPIRATION II. PHOSPHORUS METABOLISM III. TRANSLOCATION OF VIRUS

I. GENERAL INTRODUCTION

Viruses are disease-producing entities clearly distinguishable from other forms of pathogens. They exhibit properties which place them in both the animate and inanimate world. They act in a manner resembling living organisms *in vivo*, while *in vitro* they are devoid of any activities characteristic to life. The ability of the viruses to multiply prodigiously upon their introduction into the "host" organism, and the general symptomatology following the infection, has given rise to the "organismic" or "self-duplication" theory. According to this theory the viruses resemble infectious agents like bacteria and other microbiological analogues of the plant and animal kingdoms. Once they are present in the "host" there is little in their behavior to suggest that they differ basically from other pathogenic organisms. This viewpoint on the viruses has found followers, particularly among the animal virologists. Researches along this line have led Beard in his review (1951) to comment that cumulative evidence points to the fact that viruses contain the fundamental materials necessary to the structure of living organisms. He even went further suggesting that viruses, at least those affecting animals and bacteria, constitute a special group of organisms. However, he did not venture to elaborate further on this

point. The "self-duplication" theory of virus multiplication offers an explanation of the nature of viruses by inference only. It does not explain how viruses act once they are within the "host" cell. It introduces the "organismic" factor which in itself offers but little explanation, and ignores completely the importance played by the cellular enviroment in the manifestation of the disease.

The development of physicochemical methods, during the last two decades, permitting the investigation of sub-microscopic particles had a pronounced impact upon the general approach to the study of viruses. Through the methods of ultracentrifugation, diffusion, stream-double refraction and the employment of the electron microscope, much has been learned about the size, shape, and state of hydration of virus particles in vitro. These methods, plus the fact that it is possible to obtain some viruses in relatively pure forms, have offered some criteria in formulating our concepts on the nature of viruses. Chemical analyses have shown that certain viruses, isolated from plants and animals, are nucleoproteins. At least some of these viruses appear to be of molecular nature. This characterization of the molecular nature of the viruses has given rise to the theory of the physiological origin of virus particles. According to this theory the viruses are the products of abnormal cellular metabolism. As such, the viruses resemble the general structure and chemistry of normal cell constituents and thus their study is more adaptable to the methods and technics of the biophysisist and protein chemist than those of the microbiologist. It is vizualized that virus diseases are caused by the interaction of inoculum virus particles with normal cellular constituents. This interaction initiates a series of abnormal metabolic activities within the infected cells which in turn give rise to newly formed virus particles. In other words, instead of reproducing directly, as do bacteria,

the viruses divert the normal metabolic cycle of their enviroment, which in turn produces virus particles in addition to the normal metabolic products.

The logical development of the theory of the metabolic origin of virus particles centers on the study of the observed abnormalities of the diseased enviroment-cell, caused by the infection. From studies of this nature, evidence should be produced shedding light on any possible mode of interaction of virus particles with normal constituents of its surroundings. It is apparent that if any abnormal metabolic cycle is postulated in infected cells, the end results will not be limited only in the formation of virus particles, but will engulf a series of other abnormalities. Though the manifestations of virus diseases are apparent in their advanced stages, the distinction of what causes the synthesis of viruses from what are the results or the after effects of the infection is not at all simple or easily resolved. In fact, one of the most important unanswered questions of the metabolic origin of virus particles lies in the mode of the initial interaction of virus inoculum with normal constituents.

The present work was undertaken to study further any possible evidence pointing toward the nature of the initial interaction of virus inoculum with normal cell constituents. Three phases closely related to the establishment of the infection were studied. These included the respiration and phosphorus metabolism of infected tobacco plants, and the translocation of virus. Each individual phase is treated separately in the following three sections.

II. RESPIRATION MEASUREMENTS IN HEALTHY AND MOSAIC-INFECTED TOBACCO PLANTS

A. Introduction and Review of Literature

Considerable effort has been exerted in the last two decades in the study of physiological disturbances accompanying the virus-infection of plants. The reasoning behind these efforts has been that the establishment of well defined patterns of abnormalities in diseased plants may lead to a better understanding of the forces acting during the virus formation. An important consideration involved in such matters is the study of the respiratory activities of diseased plants. The measurement of respiration rates of an organism offers an overall picture of the physiological activities of that organism. In the case of virus-infected plants, the respiration of the diseased plants becomes of primary importance in view of the generally accepted observation that viruses apparently do not respire in vitro. Thus any respiratory phenomenon characteristic to virus disease must consist of the direct, or indirect interaction of the viruses with the respiratory systems of the diseased organisms.

Since the first reported attempt by Thung (1928) to compare the respiration of healthy and virus-infected plants, various methods of study have been employed. On one hand, the plant material has ranged from intact plants, to entire leaves, excised leaf tissue , apical shoots, excised stems and tubers; while on the other hand the gaseous exchange has been followed by either oxygen uptake or carbon dioxide evolution measurements. The diversity of experimental material and methods used has left much to be desired.

Important factors involving genetic, physiological and morphological variabilities of the experimental material have been overlooked in many cases, thus invalidating the results. A thorough presentation of the different studies on the respiration of virus-infected plants is presented, however, in order to point out the diversity of the findings and the elimination of many of their weak points in the present study.

Thung (1928) reported that the carbon dioxide evolution of leaf-roll potato leaves was higher than that of healthy potato leaves, on the basis of fresh and dry weight. When Thung's results were graphed (Caldwell, 1934) it became apparent that the reported increased carbon dioxide evolution did not always hold true. Dunlap (1929,1930) compared the carbon dioxide evolution of naturally mosaic-infected tobacco plants grown under field conditions with that of healthy plants. He reported that the respiration rate varied depending on the age of the leaves. Young infected leaves exhibited higher carbon dioxide production than their corresponding healthy ones, while mature leaves exhibited the opposite effect. Whitehead (1931,1934) studied the course of respiration in healthy and leaf-roll infected potatoes from the immature tuber up to the development of plants. He summarized his findings as follows: "Except for a short period covering the end of dormancy of the tuber to the first unfolding of the leaves, the infected plant respires at a much higher rate than does the healthy one". J. Dufrenoy and M. Dufrenoy (1934), in direct contradiction to Dunlap's findings, reported that mosaic-infected tobacco buds utilized less oxygen than the corresponding healthy tissues, while older infected leaves exhibited higher respiratory rates than healthy leaves. Cordingley, Grainger, Pearsall and Wright (1934), on the basis of the nitrogenous fractions and carbohydrate determinations carried out on mosaic and healthy tobacco leaves

suggested that diseased leaves may be expected to respire at a lower rate than healthy leaves. Caldwell (1934), after extensive investigations with aucuba or yellow mosaic-infected tomato plants, concluded that the carbon dioxide output of infected leaves was consistently higher than that of healthy leaves. This held true when the respiration was expressed in terms of the initial fresh weight, the residual dry-matter content, or the residual nitrogen content. Lemmon (1935) using excised discs of healthy and mosaic-infected tobacco leaves reported that infected tissues always respired at much lower rates than control leaves, when the results were expressed on the basis of equal amounts of fresh weight. Kempner (1936) using the Warburg apparatus, was unable to observe any change in the respiration of mosaic-infected tobacco leaves unless necrosis had been established. Caldwell and Meiklejohn (1937) studied the oxygen uptake of healthy and aucuba-mosaic slices of tomato stems. They used the Barcroft differential manometers and reported that diseased tissues were respiring at lower rates than healthy tissues. Grigsby (1938) reported that mosaic-infected raspberries respired at higher rates than healthy ones regardless of whether or not masking of the mosaic symptoms had occurred. Woods and du Buy (1941,1942) studied the effects of tobacco mosaic virus on cellular respiration. They observed that the infection had caused an overall increased respiration, though it was claimed that the disease had complex effects upon the enzymatic systems related to respiration.

The state of our knowledge around 1940 on the effects of viruses on the respiration of infected plants is reflected in a review on this subject by Woods (1942). This author after reviewing most of the published reports related to viruses and plant respiration did not venture any conclusion. He suggested that the apparently conflicting results may partly be ascribed to possible errors in methods

of sampling and technics used in measuring respiratory rates. He fostered the use of half-leaf comparison technics in conjuction with micromethods in measuring respiration. Glasstone (1942) studied the effects of virus disease on the respiration of mosaic-infected tobacco plants from a new angle. She noted that all reported experiments were performed on tissues already diseased and she remarked that "... a study covering the entire development of the disease would be profitable." When she compared the respiration of healthy and infected whole tobacco plants during the course of the infection, she reported that the respiration ratio of diseased and healthy plants remained at the same level until the disease became systemic. Upon the spreading of the virus as it could be observed by the clearing of the veins, she observed an increase in the respiratory rate of diseased plants which was followed by a decrease until, in the older plants, the respiratory rate became approximately equal to that of healthy plants. This leveling of the respiration coincided with the appearance of the mosaic mottling. The percentage increase in respiration rates amounted to approximately 50 percent of the corresponding respiration of healthy plants. Unfortunately the experiment was not continued after the establishment of the systemic infection. However, Glasstone's data present a very interesting picture of the respiration disturbances since they express the degree of abnormality as a function of time, following the infection. Wynd (1943) carried out a detailed study of the respiration of healthy and mosaic-infected tobacco plants. He studied the respiration rates of different individual diseased leaves through the course of the infection employing the Barcroft differential manometers. The very sensitive measurements of the oxygen uptake and the distribution of TMV-particles into progressive individual leaves permitted him to check closely the respiratory abnormalities during the progress of the disease. It was

found that the rate of respiration was increased throughout the plant about four days after the inoculation of a lower leaf. The increased respiratory rates occurred simultaneously all over the plant and preceded by approximately ten days the appearance of infectious concentrations of virus. This increase amounted to about 40 percent above the control, calculated on the basis of tissue area. The respiration rate fell below normal on the 12th day after inoculation. On the basis of these findings Wynd raised the following point: "Since infectious material appears only subsequent to a disturbed metabolism, it is probable that the observed metabolic changes are cellular in nature and do not depend on any metabolic activity of the virus material." This experiment was carried beyond the time of systemic infection and filled the gap left open by Glasstone's work (1942). It provided information confirming Glasstone's findings on the increased respiration at the onset of the infection, and at the same time it showed that the time elapsed after inoculation is, qualitatively at least, a determining factor in the observed respiratory abnormalities. On the other hand, Takahashi, as late as 1947, carried out respiration studies on detached parts of mosaic-infected and healthy tobacco leaves but was unable to observe any drastic changes in respiration during the course of the infection, though the virus particles had increased considerably.

The preceding literature with the exception of one report (Takahashi, 1947), indicates that virus infection had produced pronounced effects on the respiration of diseased plants. The conflicting results as to the qualitative effects of tobacco mosaic virus on the respiration of tobacco leaves were partly intergrated by the findings of Wynd (1943). Unfortunately no one has attempted to reinvestigate these findings. This fact led Bawden (1950) to remark: "Wynd's claim that the physiology of leaves is affected before they

contain any detectable virus is clearly one of great interest, but as yet it remains unconfirmed."

The present study was undertaken for the purpose of reinvestigating the respiratory abnormalities associated with the tobacco mosaic virus infection of tobacco plants.

B. Experimental Material and Procedures

Nicotiana tabacum, var. Havana No. 38 were used as experimental plants. They were grown under greenhouse conditions at 20° C. in a subirrigation hydroponic system. The method employed was essentially that described by Wynd and Vayonis (unpublished data). The nutrient solution recommended by Spencer (1941) was used, with final concentration of the major elements in grams per 16 liters of nutrient solution as follows:

KH_2PO_4, 14.02 gms; $Ca(NO_3)_2.4H20$, 20.78 gms;

$MgSO_4.7H_2O$, 7.89 gms; and $(NH_4)_2SO_4$, 3.50 gms

The minor elements were supplied in amounts of 0.5 parts per million for boron and manganese, and 0.02 parts per million for copper. Iron was present in sufficient quantities as contaminant in the gravel. The nutrient solutions were daily made up to the mark by the addition of distilled water and every 5 days they were replaced by fresh solutions. Their pH ranged from 5.0 to 5.5 through out the study. The experimental material was selected from a large number of tobacco plants with equal number of leaves and uniform appearance. One plant was transferred per culture pot and when the plants had recovered from the transplanting further selection was carried out to insure as uniform experimental material as possible. A total of 42 vigorously growing

tobacco plants with 10 leaves each were selected for the experiment. The two lower leaves from each plant were discarded and the remaining leaves were numbered starting with the lowest-oldest leaf, number 1. During the course of the experiment as the new leaves developed they were included in the study and were given consecutively higher numbers.

Fresh tobacco mosaic virus inoculum was prepared by pressing out the cell sap of young severely mosaic-infected tobacco leaves. Twenty one of the experimental plants were inoculated by gently rubbing the upper and lower surface of leaf 1 with a cotton pad soaked in the extracted sap. The number 1 leaves of the remaining twenty one control plants were rubbed with a cotton pad soaked in distilled water. The inoculation took place in the morning of February 17th, 1952. During the complete course of the experiment the plants received artificial illumination of 16 hours from 200-watt lamps spaced 2 1/2 feet apart and 4 feet above the benches. At intervals of 1/2, 1 1/2, 2 1/2, 3 1/2, 5 1/2, 7 1/2 and 9 1/2 days, three experimental and three control plants were harvested and their respiration rates were measured.

The oxygen uptake of the different leaves was measured on composite samples of 12 discs. Each sample was prepared by removing four discs per leaf from each of the three equally numbered leaves of infected and healthy plants. The discs were cut with a sharp cork borer, nine millimeters in diameter. To minimize the sampling errors, particular care was taken in removing the discs from predetermined positions on the leaves. Two discs were cut half an inch below the tip of the leaf and a quarter of an inch away from each side of the mid-rib. The other two discs were removed at about the midpoint of the leaf, half an inch inside the margin. Only tissues lying between secondary veins were used. Leaf 1, which received the inoculum and the next immediate leaf 2 were not used in the measurements.

The next six leaves from each group of plants were employed in the beginning of the experiment, while at the end of the experiment two of the older leaves, 3 and 4, were replaced by two newly developed leaves, 9 and 10.

The Barcroft differential manometers were used in measuring the oxygen uptake. The water bath, manometers and general procedure followed were essentially those described by Wynd (1952). The water bath was kept at 31° C. and controlled within 0.010° C. throughout the complete experiment. Vessels of 5,000 cubic millimeters capacity were used with a side arm for the addition of the alkali. A center well was absent.

The vessels contained 0.2 milliliters of distilled water in order to prevent desiccation of the tissues while the measurements were taken. It was found necessary to allow at least 30 minutes for the vessels to come to equilibrium with the water bath before closing the manometers. In some instances errors in the first manometric readings can be traced to insufficient time elapsed before closing the manometers. The manometers were not shaken and were read at 15 minutes intervals. Since only six manometers were available, two complete runs were carried out with six control and six infected leaf samples. At the end of the respiration measurements, each sample of twelve discs was dried in an oven with forced air at 80° C. for 12 hours and the dried weight was obtained. It appeared desirable to calculate the oxygen uptake both in terms of area and dry weight of the tissue.

C. Experimental Results

The tobacco plants throughout the complete period of experimentation exhibited vigorous growth. The first mosaic symptoms appeared on the growing tips of infected plants

during the fifth day after inoculation. On the sixth day all inoculated tobacco plants exhibited apparent mottling of the growing tips while the control plants at no time displayed any mosaic symptom or any other visible abnormality.

It has been pointed out by previous investigators that the speed with which virus symptoms appear depends more on the physiological age of the plant, the rate of its growth and in general on its metabolic activities, than on the amount of the initial inoculum. While in the literature an average of 10 to 15 days period is mentioned as the time elapsed between inoculation and appearance of mosaic symptoms, in the present study under favorable conditions for vigorous growth a maximum of six days were required. In a preliminary experiment the time elapsed between inoculation and establishment of secondary infection symptoms was reduced to four days. The above findings suggested that observation should begin earlier in the course of the disease and shorter time intervals than those reported in the literature should be employed in measuring the respiration rates of the different leaves. The use of excised tissues in experimentation for the purpose of establishing better controlled conditions and increased sensitivity of measurements, is always open to criticism. While the absolute measurements are improved considerably, the possibility of introducing errors in the sampling and of inducing abnormal conditions are increased also. In the present experiment these difficulties became more apparent since the comparison of leaves from healthy and infected plants were carried put over a ten days period. Possible errors in sampling, however, were minimized by the use of composite three-leaves samples. Even so, a more clear picture is gained corresponding closer to the observed results when the general trend is considered instead of emphasizing individual results from individual leaves.

The data collected are presented in full detail in order that the reader personally can judge the significance and importance of the findings.

Tables I and II present the oxygen consumption of healthy and mosaic infected tobacco leaves in cubic millimeters, calculated on the basis of area and 10 milligrams dry-plant tissue, respectively. The same data for the oxygen consumption by infected leaves, calculated as percentage of the corresponding consumption by healthy leaves, in terms of area and dry weight, are presented in Tables III and IV. Figures 1 and 2 are the graphic representations of the data in Tables III and IV, respectively. The solid lines indicate the percentage respiration of infected leaves before the mosaic symptoms had appeared, while the dotted lines represent the percentage respiration after the symptoms had been established. There are differences in the results obtained when the respiratory rates are calculated on the basis of area and dry weight of the excised discs. As is indicated in Figure 1, leaves 3, 4, and 5 of the infected plants, for a period of two days after inoculation, respired at a rate below the control leaves. The remaining leaves showed either an equal or an increased respiration during the same period. There is no adequate explanation for such a distinct difference in the respiration rates between the lower and upper leaves of the infected plants at the onset of the disease, except that an error in sampling or an erroneous base of comparison between control and inoculated leaves may be responsible. However, this distinct difference was not obvious when the respiration rates were expressed in terms of the dry weight of the tissue. Figure 2 shows that the lower, older leaves at the onset of the disease respired at approximately the same rate as the control ones, while the upper leaves exhibited an increased respiration progressively. The appearance of the increased respiration followed a well

defined pattern. It exhibited a positive correlation with the general vigor of the metabolic activities of the leaf. The less actively metabolizing older leaves showed a peak, if any, two to three days after inoculation of leaf 1, while the younger leaves showed respiration rates above control within the first 12 hours. All leaves respired at rates approximately below that of the controls by the time visible mosaic symptoms had appeared on the tip leaves. This well established pattern is not evident in Figure 1, when respiratory rates were graphed in terms of area.

Leaves 9 and 10, which were absent at the time of inoculation, had developed into sufficiently large leaves by the seventh day and were included in the measurements. At that time, the mosaic symptoms were present on both leaves. Their respiration rates were not always consistent with the trends established by the remaining leaves. Unfortunately very few measurements were carried out on these leaves to establish any pattern. However, it is worth noting that in general their respiration rates were close to those of the controls with leaf 10, in Figure 2, exhibiting the latter part of the respiration curves of older leaves.

D. Discussion

The development of mosaic virus infection in tobacco plants was associated with respiratory abnormalities. These abnormalities included increased respiratory rates at the onset of the infection, varying in the time of their appearance according to the physiological state of the leaves from 12 hours to 60 hours after the inoculation of a lower leaf. After six days, when systemic mosaic symptoms had appeared, the respiratory rates of diseased plants were equal

to or below those of the controls. The above findings are in general agreement with Wynd's (1943) data, though the time sequence has been considerably shortened.

In the calculation of the results, it was found that the dry weight of the tissue gave a better basis for comparison than the area of the tissue.

In view of the need for all the leaf-tissue available in the second phase of the experiment, tests were not carried out to establish the time of the presence of infectious concentration of virus, in the various leaves. The increase in respiration, however, within 12 hours after inoculation at an average distance of 30 centimeters suggests strongly that it is not an after effect of the presence of virus but rather that it precedes the appearance of virus particles. This is supported by the findings of Commoner, Mercer, Merrill and Zimmer (1950), who were unable to detect an increase in the tobacco mosaic virus concentration at the site of inoculation before 30 hours had elapsed after inoculation. Similar results were obtained by Steere (1952), who observed a rapid multiplication of tobacco mosaic virus at the site of infection 20 hours after inoculation.

The present findings indicate that the respiratory abnormalities in mosaic virus infected tobacco plants are a function of time. They also suggest that these early abnormalities are <u>not</u> brought about by virus <u>in situ</u>, but that rather they precede the appearance of virus particles. If this statement holds true we will necessarily have to revise basically the "organismic" theory of viruses.

III. PHOSPHORUS DISTURBANCES IN MOSAIC-VIRUS INFECTED TOBACCO PLANTS

A. Introduction and Review of Literature

Tobacco mosaic virus and other plant, animal and bacterial viruses have been identified as composed primarely of nucleoproteins (Bawden, 1950; Knight, 1947). The existence of phosphorus in the nucleic acid fraction of the nucleoprotein molecule presents us with the question of the possible pathway of phosphorus in its incorporation into viruses. The fact that enzyme systems have not been proved universally to be component parts of viruses suggests that the latter have to depend for their phosphorus supply on the organic phosphorus of the host. It is not known whether there is one or more pools of phosphorus compounds from which viruses can draw their phosphorus; however, studies on the abnormalities of phosphorus metabolism in virus infected organisms will eventually shed considerable light. The most successful attempts along these lines are being carried out with phage-infected bacteria. Cohen (1951) in his excellent review has intergrated the available data related to the phage infection and the phosphorus metabolism of virus-infected bacteria. He summarizes the evidence, suggesting that the ribose and desoxyribose nucleic acids are closely related to the phage infection. He furtner states, "...that this single phenomenon of the P shunt manifested in part by the inhibition of RNA/ribonucleic acid/ synthesis could result in the total deviation of synthesis of host components to that of virus constituents." He also suggests that this inhibition of ribose nucleic synthesis is brought about at the point of glucose-6-phospnate in the respiration cycle.

Similar attempts to compare the metabolism of healthy and virus infected tissues have been carried out extensively. Anderson, C. Gemzell, L. Gemzell, Bolin and Samuels (1950) have observed an altered turnover of phosphorus-32 in the inorganic phosphate fraction and the total acid soluble organic phosphorus fraction of the brain tissues of Rhesus monkeys infected with Lansing poliomyelitis virus, during the progress and establishment of the disease. They reported positive correlation between the percentage changes of the phosphate turnover in the two fractions, the appearance of the virus, and the general pathology of the disease. They attributed the increased turnover of phosphorus to an increased metabolism within the cells, and the possible changes in the permeability of the tissues to phosphorus. Cohn (1952) also reported that influenza virus disturbed the normal phosphorus metabolism of the chorio-allantoic membrane. When he carried out the phosphorus fractionation according to the Schmidt and Thannhauser method (1945), he detected a pronounced decrease in the lipid phosphorus fraction of the membrane only 12 hours after inoculation. The total acid soluble phosphorus, total acid insoluble phosphorus, desoxyribonucleic acid and ribonucleic acid fractions were not altered to any considerable degree. Analysis carried out 24 hours after inoculation, presented a similar picture. When the phospho-lipid content of the influenza virus-infected membrane was determined every three hours during the course of the infection it was observed that the maximum decrease in rate occurred within the first 12 hours. The rate of increase in the hemagglutination titer was maximum after the first 15 hours, at a time when the rate of decrease of the phospho-lipid fraction was approximately null.

While the effects of virus infection on phosphorus metabolism of diseased tissues and bacteria have attracted considerable attention among bacteriologists and animal virologists, little attention has been paid to that aspect of the infection by plant scientists. Most of the work

on plants has been carried out on the indirect effects of the application of phosphorus fertilizers on the rate of virus multiplication, and the infectivity of newly formed virus particles. While these studies have added some light on our understanding of the physiology of virus infection, they barely contribute to our knowledge on the possible disturbances of phosphorus in infected plants. However it was shown that the phosphorus status of the infected plants affects the virus concentration as well as the rate of appearance and degree of its symptoms (Bawden and Pirie, 1952). Thus Spencer (1935) stated that the number of local lesions of tobacco leaves was increased with increased amounts of phosphorus in the substrate as long as phosphorus had beneficial effects on plant growth. Spencer (1937) further stated that the secondary symptoms of tobacco mosaic virus appeared sooner in the tip leaves of plants receiving low phosphorus than in those plants receiving an excess of phosphorus. Smirnova (1940) reported that the titer of virus in infected tomato plants was not altered when the plants were grown on phosphorus deficient medium. Bawden and Kassanis (1950a, 1950b) reported that phosphorus treatment increased the susceptibility of tobacco plants to tobacco mosaic virus. They also stated that supplements of phosphorus produced an increase in the virus concentration of expressed sap in the total virus per plant.

The effects of mosaic virus infection on the phosphorus metabolism of tobacco plants have been studied by Ryzhkov (1943). He reported that significant changes occurred in the protein phosphorus of diseased tobacco plants while the lipoid phosphorus did not undergo such changes. The amount of phosphorus in soluble proteins increased while the phosphorus of the insoluble structural protein decreased, when they were expressed on the basis of dry weight. The chemical characterization of the different fractions, however, has not been given; thus it is

impossible to correlate Ryzhkov's data with others of the same general nature. However, it is apparent that the virus infection had definite effects on phosphorus metabolism. Holden and Tracey (1948) distinguished the effects of mosaic virus in tobacco plants into local and systemic ones. In the former case, they reported that the infection had negligible effects upon the different phosphorus fractions, while in the latter case pronounced effects were detected. The total phosphorus per plant was decreased, the total phosphorus and fibre phosphorus as percentage of dry matter were increased while the percent total phosphorus on fibre and the sap phosphorus as percentage of dry matter did not exhibit any considerable change. The data of Holden and Tracey point toward the important fact that different conclusions can be drawn depending on the method of expressing the results. It was earlier stated (Vayonis, 1950) that tobacco mosaic virus had various effects upon the different phosphorus fractions of systemically infected tobacco plants. Following Arney's (1939) fractionation scheme, Vayonis reported that the residual phosphorus fraction, and the resistant phosphate esters' fraction at the time of systemic infection were related to the virus disease. He observed that the former fraction, comprised of phosphoproteins and phospholipids, in the leaves, stems and roots of diseased tobacco plants was higher than that in the corresponding healthy ones when expressed as percentage of dry matter; while the latter fraction, comprised of hexosephosphates, in the diseased tissues, was lower than that of the healthy ones expressed on the same basis.

In all the above mentioned studies the fate of the different phosphorus fractions has been considered at <u>one</u> stage during the development of the disease. In doing so, Holden and Tracey (1948) pointed out that various effects were detected upon the different phosphorus fractions

in systemically and locally infected tissues. Thus it can be visualized that some of the apparent discrepancies among the results of the various workers can be attributed to the different stages in the development of the disease at the time of the phosphorus measurements. An attempt to integrate these results is for a time-sequence study to engulf the mosaic-virus disease from its early stages up to the appearance of systemic symptoms at far distant tissues. Such a study will present an overall picture of the gradual changes and abnormalities that may accompany the disease and will furnish us with information similar to that available on respiration. The advantages of such a method can not be overemphasized and only its difficulties should be borne in mind when it is considered.

B. Experimental Material and Procedures

The same tobacco plants used in the respiration studies were employed for the phosphorus fractionations. Each set of leaves was washed free of adhering particles, by repeatedly dipping the leaves in 0.1 Normal nitric acid and washing off the acid with distilled water. The leaves were dried over forced air at 80° C. and were finely ground in a mortar. They were stored in air-tight containers until the analyses were carried out. The fractionation of the different phosphorus compounds was carried out by a modification of Arney's procedure[1] (1939). The schematic presentation of the different fractions is indicated in Figure 3.

During the course of the analyses it was found out that the labile phosphate esters' fraction gave only traces

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[1] For details on chemical procedure, see Appendix.

of phosphorus. It was thus discontinued and the labile and resistant phosphate esters' fractions were combined into one phosphate esters' fraction. The digestion of plant material for the determination of the total phosphorus in the different leaf tissues was carried out by the nitric acid-perchloric acid digestion mixture. The phosphorus content of the different fractions was determined colorimetrically with ammonium molybdate, hydroquinone and sodium succinate solutions as recommended by Kitson and Mellon (1944).

C. Experimental Results

The results are expressed as percentage of dry matter in both control and diseased plants. The effects of the infection on the metabolism of the different phosphorus fractions become apparent when the phosphorus content of the diseased leaves is calculated as percentage of the phosphorus content of the corresponding control leaves. The picture thus obtained is free of any possible enviromental changes from day to day which will become apparent when the absolute phosphorus content is considered, but it is still open to any inherent differences of the various corresponding leaves from the different plants. The possible introduction of erroneous results due to an abnormal individual plant in either the control or diseased sets was minimized by the use of composite samples out of three plants. However an error is always possible and the most assuring method of avoiding and discarding any such error in the interpretation of the results is to consider the trends in all leaves under study and compare them with each other giving emphasis on the overall picture, rather than on any individual measurement.

The total phosphorus content of healthy and virus infected tobacco leaves, expressed as percentage of dry matter, is presented in Tables V and VI. The relative changes in its concentration of diseased and corresponding healthy leaves, are assembled in Table XIII and are graphically represented in Figure 4. At the onset of the infection and up to the fifth day after inoculation there appeared to be no consistent change in the percentage disturbance of total phosphorus throughout the different leaves. However, at the time when the visible symptoms became apparent the percent total phosphorus of infected leaves tended to fall under that of the control leaves. This decrease was of the order of 10 percent and could as well be attributed to variation in experimental material. However, it was present in all different leaves, including leaves 9 and 10 which were very young at the time of inoculation.

The data on the inorganic phosphorus fraction are presented in Tables VII, VIII, XIII and in Figure 5. The virus infection had comparable effects upon the percentage inorganic phosphorus content of tobacco leaves at the onset of the infection as on the percentage total phosphorus. There appeared, however, a tendency of increasing inorganic phosphorus with the development of the disease in most leaves, reaching a peak around the fifth day after inoculation. The percentage of inorganic phosphorus in diseased leaves fell under the controls including leaves 9 and 10 during the later stage of the disease when the mosaic symptoms had well been established.

The phosphate esters' fraction exhibited an interesting response to the virus infection. As it is indicated in Tables IX, X, XIII and in Figure 6, at the beginning of the infection and up to the second day after inoculation the diseased leaves 5, 6, 7, and 8 contained higher amounts of phosphate esters than did the corresponding healthy leaves. This increased concentration of phosphate esters was followed

by a decrease under the control during the third, fourth and fifth day after leaf 1 had been inoculated. This again was followed by a second increase at the time when the mottling of the tip leaves appeared. The increased phosphate esters' fraction at the time when the infection became systemic was also apparent in both leaves 9 and 10.

The data on the residual phosphorus fraction are presented in Tables XI, XII, XIII and in Figure 7. There are no clear cut effects of the infection on the residual phosphorus fraction during the early stages of the infection. Diseased leaves 4, 6, 7, and 8 exhibited an increased residual phosphorus fraction over the control ones at the onset of the infection, while leaves 3 and 5 exhibited the opposite effect. On the ninth day after inoculation, the diseased leaves contained higher amounts of residual phosphorus than the corresponding control leaves, including leaves 9 and 10.

D. Discussion

The mosaic infection had differential effects upon the various phosphorus fractions of tobacco leaves. The nature and degree of these effects varied with the stage in the development of the disease. The total and inorganic phosphorus fraction at the onset of the infection did not respond to the disease and remained fairly constant relative to the control ones. However at the time of appearance of the secondary symptoms both total and inorganic phosphorus fell under the controls. This decrease in the total phosphorus as percentage of dry matter is in agreement with Ryzhkov and Vorobjeva (1942), Ryzhkov (1943) and Vayonis (1950). The last one was able to report a decrease in

total as well as inorganic phosphorus only when the tobacco plants grew in nutrient solutions. Holden and Tracey (1948) observed the opposite effect even though the total phosphorus per plant decreased. Apparently the accelerated decrease of matter in systemically infected tobacco plants relative to that of total phosphorus does not take place in the presence of optimum growth conditions as are maintained in the greenhouse-grown tobacco plants (Vayonis, 1950).

The phosphate esters' fraction includes the triose and hexosephosphate compounds closely related to the respiration of the plant and serves as stepping stone in the transfer of energy. This important phosphorus fraction exhibited a noteworthy increase in the inoculated plants during the early stages of the infection. These disturbances become more important when they are considered in connection with the increase in respiration observed on the same tobacco leaves during the same time intervals, reported in the first phase of the present work. What could have caused this simultaneous increase on both the phosphate esters' fraction and the respiration at that stage of the infection is only a matter of speculation. It is noteworthy, however, that both respiration and phosphate esters appear to be related to the mechanism of the virus formation. Both stages also are related to the normal pathway in the transfer of energy in living organisms.

IV. TRANSLOCATION OF RADIOACTIVE TOBACCO MOSAIC VIRUS

A. Introduction

In the first two phases of the present study evidence has been assembled suggesting an early establishment of abnormal metabolism in tobacco plants infected with mosaic virus. It was shown that within the first twelve hours after inoculation of a lower leaf, the respiratory rate and the phosphate esters' fraction of the upper, younger tobacco leaves were altered. It was suggested then that "these abnormalities are <u>not</u> brought about by virus <u>in situ</u>, but ...precede the appearance of virus particles." The above statement was made in light of the rates of virus translocation reported in the literature (Bawden, 1950; Steere, 1952) without any direct evidence substantiating it. Thus it was found necessary to attempt to trace the movement of the initial virus inoculum under the same experimental conditions as in the respiration and phosphorus studies in order to furnish information on the rate of such trnaslocation, a matter of paramount importance.

In general the studies on the translocation of viruses are hindered by the inherent difficulties present due to the minute quantities of the initial virus inoculum involved, in comparison to the amounts of experimental tissue under consideration. Thus most of the reported translocation studies have concentrated on the movements of infectious quantities of viruses, rather than on the actual translocation of the minute quantities of virus particles from the site of

inoculation to the newly infected tissues. The use of radioactive viruses, however, affords the best means available for these studies. The movement of tagged viruses in healthy plants can be followed by measuring the radioactivity of the different tissues, at frequent intervals after the inoculation of plants with radioactive virus. This approach assumes that the appearance of radioactivity indicates the presence of virulent agent. Stanley (1942), in the first reported attempt to use phosphorus-32 tagged mosaic virus in physiological studies pointed out that this assumption did not hold true. He found out that only 14.1 percent of the radioactivity introduced in tobacco plants with tobacco mosaic virus inoculum was isolated, after 12 days, in virus particles. Thus he suggested that the radioactive virus particles upon their introduction into the "host" cells undergo a breakdown and that the products of disintegration enter into the normal metabolic cycle of the plant. This observation is in agreement with the findings on bacteriophage (Putnam and Kozloff, 1950; Lesley, French and Graham, 1950).

The breakdown of virus inoculum complicates the picture when translocation studies are contemplated, since the appearance of radioactivity in tissues far distant from the site of virus entry does not necessarily imply the presence of a virulent agent. However, a scheme was followed in the present study whereby the relative rates of translocation of radioactive tobacco mosaic virus and phosphorus-32 in the form of phosphate radical were compared.

B. Experimental Material and Procedures

Radioactive tobacco mosaic virus was prepared according to the method described by Wynd and Vayonis (unpublished data),

by the incorporation of phosphorus-32 into the virus particles. In summary the following procedure was carried out: Nicotiana tabacum, var. Havana, No. 38, plants were cultured hydroponically in nutrient solutions low in phosphorus. When they attained heights of approximately 12 inches, 10 millicuries of radioactive phosphorus in the form of orthophosphoric acid, obtained from the National Laboratory, Oak Ridge, Tennessee, were added to the nutrient solutions. Simultaneously the tobacco plants were infected by rubbing with fresh tobacco mosaic virus suspension. The plants were harvested 14 days after the addition of radioactive phosphorus to the nutrient solutions. They were frozen for three days, thawed, their cell sap extracted and the virus purified by repeated differential ultracentrifugations (Stanley, 1940). The sediment after the seventh high speed centrifugation was suspended in 10 milliliters of distilled water. This suspension of virus was used in the study of translocation.

The experimental plants used were Nicotiana tabacum, var. Havana, No. 38. They were grown under the same conditions as those described in the respiration study. Eighteen tobacco plants with 10 leaves each were selected and their leaves were numbered as is described in the respiration study, starting from the lower, older leaf as number 1. The plants were separated into two groups. In the first one, composed of 15 plants, leaves 3 and 4 were inoculated with a water suspension of the radioactive tobacco mosaic virus. In the second group, composed of three plants, leaves 3 and 4 were treated similarly but instead of using radioactive virus suspension a water solution of phosphorus-32 in the phosphate form exhibiting approximately equal activity as the radioactive virus suspension, was employed. At intervals of 1/2, 1 1/2, 2 1/2 and 4 1/2 days the leaves of three virus infected tobacco plants were harvested, and their activity was measured by the following method: From each of the three equally-numbered leaves two discs, nine

millimeters in diameter were punctured taking care not to include any vein tissues. The total number of six discs were placed on a planchet and their activity measured by a Geiger-Müller tube. The radioactivity of the phosphorus-32 inoculated plants was followed in a similar manner, except that the plants were not harvested, but the discs were removed each time from the intact leaves. Thus in the case of the virus infected plants the appearance of radioactivity was followed at the different time intervals through successive plants, while in the case of the phosphorus-32 treated plants the same individual plants were used throughout the experiment.

C. Experimental Results

The tobacco plants infected with radioactive tobacco mosaic virus exhibited visible secondary symptoms of the disease on the growing tip within the fourth day after inoculation, while the phosphorus-32 treated plants did not exhibit any visible abnormality at any time.

The radioactivity, in counts per minute per six discs of leaf tissue, of the different leaves from the virus and phosphorus-32 treated tobacco plants is presented in Table XIV. Leaves 3 and 4, which received the radioactive inoculum or the phosphorus-32 water solution became radioactive immediately upon application and remained active throughout the period of observation. The phosphorus-32 treated plants exhibited radioactivity in the leaves of the growing tip within the second day after inoculation. Such was not the case with the tobacco mosaic-infected plants. The latter plants did not show any significant activity in any of their leaves, with the exception of leaves 3 and 4, (Standard Error Deviation of the different measurements amounted to $\pm$ 1.5 counts per minute). On the fourth day,

when the first mottling of the leaves appeared, while the phosphorus-32 treated plants measured 10 to 15 counts per minute above background in the growing tips, the corresponding virus-infected leaves did not exhibit any activity.

On the eigth day after inoculation the very young growing tips of all the 15 mosaic-infected tobacco plants were tested for radioactivity. In all cases the results were negative, while the respective tips of the three phosphorus-32 treated plants exhibited radioactivity of the order of six to eight counts per minute per six discs of leaf tissue above background.

D. Discussion

Viruses are assumed to move within the infected plants because of their striking property to cause systemic infection. This <u>apparent</u> movement, however, is not in general agreement with some of the known properties of viruses, such as their large proteineous molecule or their inability to diffuse through semipermeable membranes. However, we still consider that, "Something moves, and as this leads to the production of further virus, it is simple and most reasonable to assume that the something is virus," (Bawden, 1950). The absence, however, of any radioactivity in the systemically infected young tobacco leaves suggests that the initial virus inoculum did not move to any detectable degree from the site of its entry. It is thus implied that if the above phenomenon holds true tne "something" is not the virus proper. What could possibly have caused the appearance of the new viruses in far distant tissues at the moment remains only a matter of speculation.

The comparative ease with which radioactivity was detected in the tip of leaves of phosphorus-32 treated plants suggests that were the radioactive fraction of the tobacco mosaic virus particle as mobile as the phosphate phosphorus-32 radical then it could be detected as it was the case with the latter. Thus the absence of radioactivity in the tip leaves of the infected tobacco plants implies than not only the virus particles were not translocated to the younger leaves but also, that any phosphate phosphorus-32 of the breakdown products of the mosaic virus was not translocated in detectable quantities.

V. GENERAL DISCUSSION

The inoculation of tobacco plants with tobacco mosaic virus was shown to be accompanied by profound physiological disturbances. Most of these abnormalities are the after-effects of the infection. In the present study, however, evidence has been accumulated suggesting that some metabolic changes may precede the appearance of the virus particles rather than follow it. The implication of such observations leads us to speculate on the nature of virus particles more in accordance with the "metabolic" rather than the "organismic" theory of their origin. When the early establishment of respiratory and phosphorus disturbances, and the apparent early immobility of the virus inoculum are coupled with the subsequent establishment of systemic infection of young tobacco leaves, the "organismic" theory of the virus origin does not offer us much of an explanation as the mode of action of the initial inoculum. However, the findings of the present study are best intergrated when we consider as Cohen (1947) did, that viruses, upon their introduction into the "host" cells, alter the metabolism of the cells and deviate their normal cycle into the production of new virus particles. Thus it can be suggested that the inoculation of a lower leaf of tobacco plants brought about changes in the metabolism of the entire plant within a few hours. This altered metabolism in turn gave rise to the appearance of new virus particles. Such a mechanism of virus formation does not necessarily exclude the movement of virus particles, but it considers that this movement from the site of inoculation to newly infected tissues is not a necessary condition for the establishment of the disease.

VI. SUMMARY AND CONCLUSIONS

The data presented in this report point to an early establishment of disturbances in the metabolism of mosaic virus infected tobacco plants.

The respiratory rates of leaves from mosaic-infected tobacco plants, expressed in terms of dry weight, increased above the controls within 36 hours after the inoculation of a lower leaf. This increased respiration became first apparent among the younger leaves within 12 hours after inoculation. It preceded by approximately five days the appearance of visible systemic mosaic symptoms. Most leaves, at the time when the symptoms appeared, respired at lower rates than the corresponding control leaves.

The phosphorus metabolism of infected plants was disturbed by the disease. The total, inorganic and residual phosphorus fractions were not consistently affected at the onset of the disease, while at later stages the first two fractions fell below the control and the third one increased above the control with time. The responses of total, inorganic and residual phosphorus fractions to the mosaic infection suggest that these observed abnormalities follow rather than precede the appearance of newly formed virus particles. The phosphate esters' fraction, associated with the respiratory activities of the plant, exhibited an increased concentration in the younger leaves within 12 hours after inoculation of a lower leaf. There existed a positive correlation between both the respiratory and phosphate esters' fraction increase to the infection during the first two days after inoculation.

Radioactive tobacco mosaic virus was prepared by the incorporation of phosphate phosphorus-32 into the virus particles. The translocation of the virus inoculum was

studied. Plants infected with radioactive virus suspension exhibited systemic infection within four days after inoculation. No activity was detected in systemically infected tissues. When phosphorus-32 was used in the form of phosphate radical, of approximately double the activity of the virus inoculum, radioactivity was detected in the growing tip within 36 hours after inoculation of a lower leaf.

These data suggest that the observed abnormalities in the respiratory rates and the phosphate esters' fraction in leaves of mosaic-infected tobacco plants do not follow but RATHER PRECEDE the appearance of new virus particles.

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TABLE I

THE USE OF OXYGEN IN CUBIC MILLIMETERS BY TWELVE EXCISED DISCS FROM LEAVES OF HEALTHY AND INOCULATED TOBACCO PLANTS. H, LEAVES FROM HEALTHY PLANTS; In, LEAVES FROM INOCULATED PLANTS.

Time	Leaf 3		Leaf 4		Leaf 5		Leaf 6		Leaf 7		Leaf 8		Leaf 9		Leaf 10	
	H	In	H	In	H	In	H	In	H	In	H	In	H	In	H	In
							1/2 day									
min.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.
15	16.3	12.2	13.8	11.5	12.3	11.0	12.2	11.8	14.9	16.3	16.6	18.4	--	--	--	--
30	33.4	24.3	30.2	23.1	24.6	22.0	24.6	25.0	30.2	31.1	34.1	36.6	--	--	--	--
45	51.2	36.1	42.8	35.3	36.7	33.9	38.1	36.8	45.3	45.9	51.5	55.1	--	--	--	--
60	68.3	48.8	56.4	46.6	49.4	45.8	49.4	50.1	60.1	61.7	69.2	74.5	--	--	--	--
							1 1/2 days									
15	15.6	14.1	15.3	14.2	14.0	11.5	12.9	12.6	14.9	16.7	19.4	20.4	--	--	--	--
30	31.4	29.2	30.4	28.2	28.2	25.6	27.5	25.5	31.3	33.2	39.1	41.4	--	--	--	--
45	48.7	45.0	48.4	43.8	42.4	39.2	42.3	38.3	47.4	48.3	59.8	60.6	--	--	--	--
60	66.3	60.2	65.0	58.8	57.7	52.4	57.2	52.3	63.4	64.6	79.3	80.3	--	--	--	--
							2 1/2 days									
15	13.4	14.3	15.3	15.7	15.0	15.1	11.8	13.3	16.5	16.2	19.4	19.4	--	--	--	--
30	27.4	29.7	32.3	32.6	30.5	30.3	24.4	25.9	32.7	33.2	37.7	39.6	--	--	--	--
45	40.9	44.0	49.5	49.8	46.0	45.4	37.7	40.1	48.6	49.9	56.8	60.4	--	--	--	--
60	54.9	59.4	65.8	66.2	61.5	59.8	52.3	53.6	64.8	65.8	76.0	78.7	--	--	--	--
							3 1/2 days									
15	15.8	12.2	11.2	12.0	10.8	12.3	8.6	9.1	12.7	11.5	15.2	13.7	--	--	--	--
30	27.0	24.7	24.2	24.2	21.0	24.2	18.8	20.0	25.4	24.5	28.8	28.2	--	--	--	--
45	40.4	37.3	36.4	37.2	30.9	38.0	28.6	32.1	37.2	38.2	42.2	42.2	--	--	--	--
60	58.2	49.5	50.8	50.3	41.5	50.2	39.5	43.4	50.0	51.1	57.1	56.6	--	--	--	--

Continued next page

TABLE I (Continued)

Time	Leaf 3		Leaf 4		Leaf 5		Leaf 6		Leaf 7		Leaf 8		Leaf 9		Leaf 10	
	H	In	H	In	H	In	H	In	H	In	H	In	H	In	H	In
							5 1/2 days									
min.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.
15	9.4	12.1	12.0	10.4	11.0	9.5	9.5	10.6	13.4	--	16.1	13.2	--	--	--	--
30	19.0	24.2	23.1	20.2	22.9	19.5	20.4	21.5	28.8	--	31.8	27.2	--	--	--	--
45	28.5	35.9	35.4	31.6	33.9	29.9	31.3	32.4	41.5	--	48.5	42.1	--	--	--	--
60	38.1	48.8	47.3	40.0	45.1	40.5	42.5	43.0	54.5	--	65.1	55.6	--	--	--	--
							7 1/2 days									
15	--	--	--	--	11.7	10.0	11.1	10.4	14.4	12.5	15.4	15.1	19.3	17.8	24.8	23.4
30	--	--	--	--	23.1	21.2	22.6	21.7	29.2	26.1	31.5	27.5	38.3	31.5	49.6	47.3
45	--	--	--	--	36.0	32.0	35.0	33.5	43.4	39.6	47.4	41.7	56.0	51.7	73.7	70.8
60	--	--	--	--	47.3	41.8	46.3	45.6	57.1	51.9	64.1	57.1	74.3	68.1	97.1	94.2
							9 1/2 days									
15	--	--	--	--	11.2	12.7	9.8	12.0	11.8	13.7	12.3	13.4	14.4	17.7	19.3	21.5
30	--	--	--	--	22.0	24.8	21.3	24.1	25.0	26.9	25.0	26.7	29.1	35.6	39.6	42.5
45	--	--	--	--	32.7	36.3	32.2	37.0	38.2	39.6	38.1	39.9	44.4	52.8	59.6	63.3
60	--	--	--	--	43.2	48.8	43.7	47.4	50.0	52.6	51.2	53.7	59.8	70.2	79.2	84.1

TABLE II

THE USE OF OXYGEN IN CUBIC MILLIMETERS BY EXCISED DISCS FROM LEAVES OF HEALTHY AND INOCULATED TOBACCO PLANTS, CALCULATED PER 10 MILLIGRAMS OF DRY PLANT TISSUE. H, LEAVES FROM HEALTHY PLANTS; In, LEAVES FROM INOCULATED PLANTS.

Time	Leaf 3		Leaf 4		Leaf 5		Leaf 6		Leaf 7		Leaf 8		Leaf 9		Leaf 10	
	H	In	H	In	H	In	H	In	H	In	H	In	H	In	H	In
							1/2 day									
min.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.
15	11.9	12.3	12.5	12.8	13.4	12.5	12.6	13.6	15.2	17.5	16.0	18.4	--	--	--	--
30	24.4	24.5	27.5	25.7	26.7	25.0	25.4	28.7	30.8	33.4	32.8	36.6	--	--	--	--
45	37.4	36.5	38.9	39.2	39.9	38.5	39.3	42.3	46.2	49.3	49.5	55.1	--	--	--	--
60	49.9	49.3	51.3	51.8	53.7	52.0	50.9	57.6	61.3	66.3	66.6	74.5	--	--	--	--
							1 1/2 days									
15	10.1	10.8	11.8	12.6	12.5	11.2	12.4	14.8	15.2	17.0	18.0	18.7	--	--	--	--
30	20.4	22.5	23.4	25.0	25.2	24.9	26.5	30.0	31.9	33.9	36.2	38.0	--	--	--	--
45	31.6	34.6	37.2	38.8	37.9	38.1	40.7	45.0	48.3	49.3	55.4	55.6	--	--	--	--
60	43.0	46.3	50.0	52.0	51.5	50.9	55.0	61.6	64.7	65.9	73.4	73.6	--	--	--	--
							2 1/2 days									
15	11.8	10.9	12.3	12.2	13.9	15.7	12.6	14.0	16.5	16.9	18.7	19.2	--	--	--	--
30	24.0	22.7	26.1	25.3	28.2	31.6	26.0	27.3	32.7	34.6	36.3	39.2	--	--	--	--
45	35.9	33.6	39.9	38.6	42.6	47.3	40.1	42.2	48.6	52.0	54.6	59.9	--	--	--	--
60	48.1	45.3	53.1	51.3	56.9	62.3	55.6	56.4	64.8	68.6	73.1	78.0	--	--	--	--
							3 1/2 days									
15	12.5	10.9	10.2	10.6	12.6	12.6	10.2	10.3	14.4	13.1	17.1	14.9	--	--	--	--
30	21.4	22.1	22.0	21.4	24.4	24.8	22.4	22.7	28.9	27.8	32.4	30.7	--	--	--	--
45	32.1	33.3	33.1	32.9	35.9	38.9	34.1	36.5	42.3	43.4	47.4	45.9	--	--	--	--
60	46.2	44.2	46.2	44.5	48.3	51.4	47.0	49.3	56.8	58.0	64.2	61.5	--	--	--	--

Continued next page

TABLE II (Continued)

Time	Leaf 3		Leaf 4		Leaf 5		Leaf 6		Leaf 7		Leaf 8		Leaf 9		Leaf 10	
	H	In	H	In	H	In	H	In	H	In	H	In	H	In	H	In
							5 1/2 days									
min.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.	cu.mm.
15	10.4	10.7	12.8	11.4	11.0	10.8	10.0	11.3	15.6	--	15.5	13.9	--	--	--	--
30	21.1	21.4	24.6	22.2	22.9	22.2	21.5	22.9	33.5	--	30.6	28.6	--	--	--	--
45	31.7	31.8	37.7	34.7	33.9	34.0	33.0	34.5	48.3	--	46.7	44.3	--	--	--	--
60	42.3	43.2	50.3	44.0	45.1	46.0	44.8	45.8	63.4	--	62.6	58.5	--	--	--	--
							7 1/2 days									
15	--	--	--	--	11.7	10.9	11.1	11.2	13.9	13.3	13.9	15.1	17.5	17.3	20.3	19.2
30	--	--	--	--	23.1	23.0	22.6	23.3	28.1	27.8	28.4	27.5	34.8	30.6	40.7	38.8
45	--	--	--	--	36.0	34.8	35.0	36.0	41.8	42.1	42.7	41.7	50.9	50.2	60.4	58.1
60	--	--	--	--	47.3	45.4	46.3	49.0	54.9	55.2	57.8	57.1	67.5	66.1	79.6	77.2
							9 1/2 days									
15	--	--	--	--	12.3	13.5	11.4	12.2	13.7	14.0	14.5	13.4	14.8	16.2	18.4	17.2
30	--	--	--	--	25.3	26.4	24.8	24.6	29.1	27.4	29.4	26.7	30.0	32.6	37.7	34.0
45	--	--	--	--	37.6	38.6	37.4	37.7	44.4	40.4	44.8	39.9	45.8	48.4	56.7	50.6
60	--	--	--	--	49.6	51.9	50.8	48.3	58.2	53.7	60.2	53.7	61.7	64.4	75.4	67.2

TABLE III

THE USE OF OXYGEN BY TWELVE EXCISED DISCS FROM LEAVES OF INOCULATED TOBACCO PLANTS CALCULATED AS PERCENTAGE OF THE OXYGEN USE BY TWELVE CORRESPONDING EXCISED DISCS FROM LEAVES OF HEALTHY PLANT.

Days after inocula-tion	Leaf 3 %	Leaf 4 %	Leaf 5 %	Leaf 6 %	Leaf 7 %	Leaf 8 %	Leaf 10 %
	Oxygen used in 15 minutes						
1/2	74.8	83.3	89.4	96.7	109.3	110.8	-
1 1/2	90.4	92.8	82.1	97.7	112.1	105.2	-
2 1/2	106.7	102.6	100.7	112.7	98.2	100.0	-
3 1/2	77.2	107.1	113.9	105.8	90.6	90.1	-
5 1/2	128.1	86.7	86.4	111.6	-	82.0	-
7 1/2	-	-	85.5	93.7	86.8	98.1	94.4
9 1/2	-	-	113.4	122.4	116.1	108.9	111.4
	Oxygen used in 30 minutes						
1/2	72.8	76.5	89.4	101.6	103.0	107.3	-
1 1/2	93.0	92.8	90.8	92.7	106.1	105.0	-
2 1/2	108.4	100.9	99.3	106.1	101.5	105.0	-
3 1/2	91.5	100.0	115.2	106.4	96.5	97.9	-
5 1/2	127.4	87.4	85.2	105.4	-	85.5	-
7 1/2	-	-	91.8	96.0	89.4	87.3	95.4
9 1/2	-	-	112.7	113.1	107.6	106.8	107.3
	Oxygen used in 45 minutes						
1/2	70.5	82.5	92.4	96.6	101.3	107.0	-
1 1/2	92.4	90.5	92.5	90.5	101.9	101.3	-
2 1/2	107.6	100.6	98.7	106.4	102.7	106.3	-
3 1/2	92.3	102.2	123.0	112.2	102.7	100.0	-
5 1/2	126.0	89.3	88.2	103.5	-	86.8	-
7 1/2	-	-	88.9	95.7	91.2	88.0	96.1
9 1/2	-	-	111.0	114.9	103.7	104.7	106.2
	Oxygen used in 60 minutes						
1/2	71.4	82.6	92.7	101.4	102.7	107.7	-
1 1/2	90.8	90.5	90.8	91.4	101.9	101.3	-
2 1/2	108.2	100.6	97.2	102.5	101.5	103.6	-
3 1/2	85.1	99.0	121.0	109.9	102.2	99.1	-
5 1/2	128.1	84.6	89.8	101.2	-	85.4	-
7 1/2	-	-	88.4	98.5	90.9	89.1	97.0
9 1/2	-	-	113.0	108.5	105.5	104.9	106.2

TABLE IV

THE USE OF OXYGEN BY 10 MILLIGRAMS DRY WEIGHT OF EXCISED DISCS FROM LEAVES OF INOCULATED TOBACCO PLANTS CALCULATED AS PERCENTAGE OF THE OXYGEN USE BY THE CORRESPONDING EXCISED DISCS FROM LEAVES OF HEALTHY PLANTS

Days after inoculation	Leaf 3 %	Leaf 4 %	Leaf 5 %	Leaf 6 %	Leaf 7 %	Leaf 8 %	Leaf 10 %
	Oxygen used in 15 minutes						
1/2	103.4	102.4	93.3	107.9	115.1	115.0	-
1 1/2	106.9	106.8	89.6	119.4	111.8	103.9	-
2 1/2	92.4	99.2	112.9	111.1	102.4	102.7	-
3 1/2	87.2	103.9	100.0	101.0	91.0	87.1	-
5 1/2	102.3	89.1	98.2	112.0	-	89.6	-
7 1/2	-	-	93.2	100.9	95.6	108.6	94.5
9 1/2	-	-	104.7	107.0	102.2	92.4	93.4
	Oxygen used in 30 minutes						
1/2	100.4	93.5	93.6	113.0	108.4	111.6	-
1 1/2	110.3	106.8	98.8	113.2	106.3	105.0	-
2 1/2	94.6	96.9	112.1	105.0	105.8	108.0	-
3 1/2	103.3	97.3	101.6	101.3	96.2	94.8	-
5 1/2	101.4	90.2	96.9	106.5	-	93.5	-
7 1/2	-	-	99.6	103.1	95.6	96.8	95.3
9 1/2	-	-	104.3	99.2	102.2	90.8	90.2
	Oxygen used in 45 minutes						
1/2	97.6	100.8	96.5	107.6	106.7	111.3	-
1 1/2	109.5	104.3	100.5	110.6	102.1	100.4	-
2 1/2	93.6	96.7	111.0	105.2	107.0	109.7	-
3 1/2	103.7	99.4	108.4	107.0	102.6	96.8	-
5 1/2	100.3	92.0	100.3	104.5	-	94.9	-
7 1/2	-	-	96.7	102.9	100.7	97.7	96.2
9 1/2	-	-	102.7	100.8	91.0	89.1	89.2
	Oxygen used in 60 minutes						
1/2	98.8	101.0	96.8	113.2	108.2	111.9	-
1 1/2	107.7	104.0	98.8	112.0	101.9	100.3	-
2 1/2	92.4	96.6	109.5	101.4	105.9	106.7	-
3 1/2	95.7	96.3	106.4	104.9	102.1	95.8	-
5 1/2	102.1	87.5	102.0	102.2	-	93.5	-
7 1/2	-	-	96.0	105.8	100.5	98.8	97.0
9 1/2	-	-	104.6	95.1	92.3	89.2	89.1

TABLE V

TOTAL PHOSPHORUS OF HEALTHY TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3			Leaf 4			Leaf 5			Leaf 6		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.56	0.55	0.56	0.58	0.57	0.58	0.81	0.81	0.81	0.69	0.68	0.69
1 1/2	0.55	0.55	0.55	0.54	0.51	0.53	0.67	0.67	0.67	0.66	0.66	0.66
2 1/2	0.53	0.52	0.53	0.57	0.55	0.56	0.57	0.57	0.57	0.63	0.63	0.63
3 1/2	0.49	0.52	0.51	0.59	0.59	0.59	0.66	0.64	0.65	0.74	0.74	0.74
5 1/2	0.73	0.74	0.74	0.57	0.57	0.57	0.63	0.63	0.63	0.69	0.69	0.69
7 1/2	-	-	-	-	-	-	0.73	0.74	0.74	0.74	0.74	0.74
9 1/2	-	-	-	-	-	-	0.78	0.77	0.78	0.81	0.81	0.81

Days after inoculation	Leaf 7			Leaf 8			Leaf 9			Leaf 10		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.73	0.71	0.72	0.65	0.65	0.65	-	-	-	-	-	-
1 1/2	0.74	0.75	0.75	0.77	0.77	0.77	-	-	-	-	-	-
2 1/2	0.70	0.72	0.71	0.77	0.78	0.78	-	-	-	-	-	-
3 1/2	0.78	0.78	0.78	0.88	0.89	0.89	-	-	-	-	-	-
5 1/2	0.69	0.68	0.69	0.82	0.83	0.83	-	-	-	-	-	-
7 1/2	0.77	0.77	0.77	0.81	0.81	0.81	0.82	0.82	0.82	0.84	0.85	0.85
9 1/2	0.74	0.74	0.74	0.76	0.78	0.77	0.76	0.75	0.76	0.82	0.81	0.82

TABLE VI

TOTAL PHOSPHORUS OF MOSAIC-VIRUS INFECTED TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3			Leaf 4			Leaf 5			Leaf 6		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.58	0.56	0.57	0.58	0.56	0.57	0.66	0.63	0.65	0.72	-	0.72
1 1/2	0.53	0.53	0.53	0.55	0.55	0.55	0.59	0.60	0.60	0.68	0.68	0.68
2 1/2	0.54	0.55	0.55	0.56	0.57	0.57	0.67	0.67	0.67	0.72	0.72	0.72
3 1/2	0.61	0.59	0.60	0.56	0.55	0.56	0.68	0.69	0.69	0.75	0.75	0.75
5 1/2	0.73	0.69	0.71	0.66	0.65	0.66	0.77	0.77	0.77	0.76	0.76	0.76
7 1/2	-	-	-	-	-	-	0.58	0.58	0.58	0.63	0.64	0.64
9 1/2	-	-	-	-	-	-	0.61	0.63	0.62	0.77	0.77	0.77

Days after inoculation	Leaf 7			Leaf 8			Leaf 9			Leaf 10		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.75	0.76	0.76	0.80	0.81	0.81	-	-	-	-	-	-
1 1/2	0.71	0.71	0.71	0.77	0.78	0.78	-	-	-	-	-	-
2 1/2	0.72	0.73	0.73	0.79	0.78	0.79	-	-	-	-	-	-
3 1/2	0.73	0.76	0.75	0.87	0.87	0.87	-	-	-	-	-	-
5 1/2	0.73	0.75	0.74	0.89	0.92	0.91	-	-	-	-	-	-
7 1/2	0.68	0.67	0.68	0.67	0.69	0.68	0.74	0.74	0.74	0.77	0.78	0.78
9 1/2	0.75	0.74	0.75	0.73	0.73	0.73	0.79	0.81	0.80	0.77	0.77	0.77

TABLE VII

INORGANIC PHOSPHORUS FRACTION OF HEALTHY TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3			Leaf 4			Leaf 5			Leaf 6		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.41	0.41	0.41	0.43	0.43	0.43	0.46	0.47	0.47	0.42	0.44	0.43
1 1/2	0.37	0.37	0.37	0.36	0.36	0.36	0.43	0.42	0.43	0.41	0.41	0.41
2 1/2	0.33	0.32	0.33	0.30	0.31	0.31	0.37	0.37	0.37	0.40	0.40	0.40
3 1/2	0.33	0.33	0.33	0.36	0.35	0.36	0.40	0.38	0.39	0.49	0.48	0.49
5 1/2	0.51	0.51	0.51	0.38	0.38	0.38	0.40	0.40	0.40	0.43	-	0.43
7 1/2	-	-	-	-	-	-	0.51	0.49	0.50	0.47	0.48	0.48
9 1/2	-	-	-	-	-	-	0.58	0.59	0.59	0.58	0.57	0.58

Days after inoculation	Leaf 7			Leaf 8			Leaf 9			Leaf 10		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.44	0.45	0.45	0.44	0.45	0.45	-	-	-	-	-	-
1 1/2	0.47	0.46	0.47	0.45	0.46	0.46	-	-	-	-	-	-
2 1/2	0.43	0.43	0.43	0.46	0.47	0.47	-	-	-	-	-	-
3 1/2	0.52	0.52	0.52	0.49	0.48	0.49	-	-	-	-	-	-
5 1/2	0.43	0.43	0.43	0.49	0.49	0.49	-	-	-	-	-	-
7 1/2	0.50	0.51	0.51	0.54	0.53	0.54	0.51	0.52	0.52	0.47	0.46	0.47
9 1/2	0.53	0.51	0.52	0.53	0.55	0.54	0.53	0.50	0.52	0.51	0.51	0.51

TABLE VIII

INORGANIC PHOSPHORUS FRACTION OF MOSAIC-VIRUS INFECTED TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3			Leaf 4			Leaf 5			Leaf 6		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.40	0.40	0.40	0.35	0.36	0.36	0.40	0.41	0.41	0.43	0.43	0.43
1 1/2	0.35	0.36	0.36	0.35	0.35	0.35	0.35	0.37	0.36	0.42	0.42	0.42
2 1/2	0.35	0.35	0.35	0.33	0.33	0.33	0.43	0.42	0.43	0.45	0.45	0.45
3 1/2	0.41	0.41	0.41	0.35	0.36	0.36	0.46	0.46	0.46	0.47	0.47	0.47
5 1/2	0.51	0.50	0.51	0.45	0.48	0.47	0.52	0.53	0.53	0.49	0.48	0.49
7 1/2	-	-	-	-	-	-	0.36	0.37	0.37	0.40	0.40	0.40
9 1/2	-	-	-	-	-	-	0.39	0.40	0.40	0.53	0.53	0.53

Days after inoculation	Leaf 7			Leaf 8			Leaf 9			Leaf 10		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.43	0.44	0.44	0.43	0.43	0.43	-	-	-	-	-	-
1 1/2	0.40	0.40	0.40	0.40	0.40	0.40	-	-	-	-	-	-
2 1/2	0.44	0.44	0.44	0.49	0.46	0.48	-	-	-	-	-	-
3 1/2	0.51	0.49	0.50	0.56	0.55	0.56	-	-	-	-	-	-
5 1/2	0.47	0.46	0.47	0.60	0.58	0.59	-	-	-	-	-	-
7 1/2	0.43	0.44	0.44	0.42	0.43	0.43	0.45	0.44	0.45	0.40	0.41	0.41
9 1/2	0.50	0.50	0.50	0.46	0.46	0.46	0.51	0.50	0.51	0.43	0.41	0.42

TABLE IX

PHOSPHATE ESTERS FRACTION OF HEALTHY TOBACCO LEAVES,
EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	Leaf 8	Leaf 9	Leaf 10
1/2	0.08	0.08	0.09	0.09	0.08	0.08	-	-
1 1/2	0.12	0.11	0.08	0.08	0.07	0.09	-	-
2 1/2	0.11	0.11	0.11	0.12	0.09	0.10	-	-
3 1/2	0.07	0.06	0.07	0.06	Traces	Traces	-	-
5 1/2	0.04	0.05	0.05	0.04	0.04	0.04	-	-
7 1/2	-	-	0.12	0.08	0.09	0.07	0.10	0.08
9 1/2	-	-	0.03	0.04	0.07	0.10	0.04	0.07

TABLE X

PHOSPHATE ESTERS FRACTION OF MOSAIC-VIRUS INFECTED TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	Leaf 8	Leaf 9	Leaf 10
1/2	0.08	0.11	0.10	0.11	0.10	0.11	-	-
1 1/2	0.08	0.11	0.10	0.09	0.09	0.11	-	-
2 1/2	0.12	0.12	0.08	0.08	0.08	0.05	-	-
3 1/2	0.02	0.02	0.02	0.01	Traces	0.02	-	-
5 1/2	0.05	0.03	0.01	0.03	0.05	0.05	-	-
7 1/2	-	-	0.08	0.08	0.05	0.11	0.10	0.10
9 1/2	-	-	0.10	0.10	0.10	0.11	0.09	0.14

TABLE XI

RESIDUAL PHOSPHORUS FRACTION OF HEALTHY TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3			Leaf 4			Leaf 5			Leaf 6		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.07	0.07	0.07	0.07	-	0.07	0.18	-	0.18	0.13	-	0.13
1 1/2	0.10	0.07	0.09	0.04	0.04	0.04	0.06	0.09	0.08	0.07	0.07	0.07
2 1/2	0.10	0.10	0.10	0.05	0.06	0.06	0.11	0.12	0.12	0.13	0.15	0.14
3 1/2	0.12	0.08	0.10	0.09	0.11	0.10	0.14	0.15	0.15	0.14	0.13	0.14
5 1/2	0.14	-	0.14	0.12	0.13	0.13	0.16	0.15	0.16	0.16	0.16	0.16
7 1/2	-	-	-	-	-	-	0.13	0.13	0.13	0.16	0.15	0.16
9 1/2	-	-	-	-	-	-	-	-	-	-	-	-

Days after inoculation	Leaf 7			Leaf 8			Leaf 9			Leaf 10		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.15	-	0.15	0.07	-	0.07	-	-	-	-	-	-
1 1/2	0.06	0.08	0.07	0.16	-	0.16	-	-	-	-	-	-
2 1/2	0.17	-	0.17	0.19	0.15	0.17	-	-	-	-	-	-
3 1/2	0.19	0.18	0.19	0.24	0.21	0.23	-	-	-	-	-	-
5 1/2	0.17	0.17	0.17	0.20	0.17	0.19	-	-	-	-	-	-
7 1/2	0.14	0.16	0.15	0.16	0.17	0.17	0.25	0.21	0.23	0.26	0.26	0.26
9 1/2	0.12	-	0.12	0.12	-	0.12	0.15	-	0.15	0.12	-	0.12

TABLE XII

RESIDUAL PHOSPHORUS FRACTION OF MOSAIC-VIRUS INFECTED TOBACCO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Days after inoculation	Leaf 3			Leaf 4			Leaf 5			Leaf 6		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.10	-	0.10	0.09	0.08	0.09	0.12	0.12	0.12	0.17	-	0.17
1 1/2	0.03	0.03	0.03	0.06	0.05	0.06	0.07	0.07	0.07	0.08	0.06	0.07
2 1/2	0.08	0.06	0.07	0.11	0.09	0.10	0.15	0.14	0.15	0.17	0.18	0.18
3 1/2	0.11	0.12	0.12	0.12	0.12	0.12	0.13	0.16	0.15	0.17	0.16	0.17
5 1/2	0.12	0.12	0.12	0.13	0.12	0.13	0.14	0.14	0.14	0.16	0.16	0.16
7 1/2	-	-	-	-	-	-	0.15	0.14	0.15	0.16	0.15	0.16
9 1/2	-	-	-	-	-	-	0.13	0.13	0.13	0.16	0.15	0.16

Days after inoculation	Leaf 7			Leaf 8			Leaf 9			Leaf 10		
	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.	Det.1	Det.2	Ave.
1/2	0.20	-	0.20	0.15	0.13	0.14	-	-	-	-	-	-
1 1/2	0.18	-	0.18	0.20	0.24	0.22	-	-	-	-	-	-
2 1/2	0.18	0.19	0.19	0.21	0.22	0.22	-	-	-	-	-	-
3 1/2	0.16	0.18	0.17	0.20	0.19	0.20	-	-	-	-	-	-
5 1/2	0.17	0.17	0.17	0.19	0.20	0.20	-	-	-	-	-	-
7 1/2	0.13	-	0.13	0.15	0.15	0.15	0.20	0.19	0.20	0.26	0.25	0.26
9 1/2	0.15	0.15	0.15	0.18	0.17	0.18	0.23	0.23	0.23	0.26	0.24	0.25

TABLE XIII

PHOSPHORUS FRACTIONS OF LEAVES FROM MOSAIC-VIRUS INFECTED TOBACCO PLANTS, CALCULATED AS PERCENTAGES OF THE CORRESPONDING PHOSPHORUS FRACTIONS OF LEAVES FROM HEALTHY PLANTS

Days after inoculation	Leaf 3				Leaf 4			
	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus
1/2	98	100	143	102	119	138	129	97
1 1/2	97	67	33	96	97	100	150	104
2 1/2	106	109	70	104	106	109	167	102
3 1/2	124	29	120	118	100	33	120	95
5 1/2	100	125	86	96	124	60	100	116
7 1/2	-	-	-	-	-	-	-	-
9 1/2	-	-	-	-	-	-	-	-

Days after inoculation	Leaf 5				Leaf 6			
	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus
1/2	87	111	67	80	100	122	131	104
1 1/2	84	125	88	90	102	113	100	103
2 1/2	116	73	125	118	108	67	129	114
3 1/2	118	29	100	106	94	17	121	101
5 1/2	133	20	88	122	123	75	100	110
7 1/2	74	67	115	78	77	100	100	86
9 1/2	68	333	-	79	70	250	-	95

Continued next page

TABLE XIII (Continued)

Days after inocula-tion	Leaf 7				Leaf 8			
	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus
1/2	98	125	133	106	96	138	200	125
1 1/2	85	129	257	95	87	122	138	101
2 1/2	102	89	112	103	102	50	129	101
3 1/2	96	(traces)	89	96	114	(traces)	87	98
5 1/2	109	125	100	107	83	125	105	110
7 1/2	86	56	87	88	80	157	88	84
9 1/2	96	143	125	99	85	110	150	95

Days after inocula-tion	Leaf 9				Leaf 10			
	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus	Inorganic Phosphorus	Phosphate Esters	Residual Phosphorus	Total Phosphorus
1/2	-	-	-	-	-	-	-	-
1 1/2	-	-	-	-	-	-	-	-
2 1/2	-	-	-	-	-	-	-	-
3 1/2	-	-	-	-	-	-	-	-
5 1/2	-	-	-	-	-	-	-	-
7 1/2	86	100	87	90	87	125	100	92
9 1/2	98	225	153	105	82	200	208	94

TABLE XIV

RADIOACTIVITY IN COUNTS PER MINUTE PER SIX DISCS OF LEAF TISSUE, FROM TOBACCO PLANTS INOCULATED WITH RADIOACTIVE TOBACCO MOSAIC VIRUS (TMV) SUSPENSION AND PHOSPHORUS-32 SOLUTION

Leaf Number	Days after inoculation							
	1/2		1 1/2		2 1/2		4 1/2	
	P-32	TMV	P-32	TMV	P-32	TMV	P-32	TMV
1	4.4	-*	0.3	0.6	0	1.8	0	0.3
2	2.4	-	0	0	0.1	3.3	0	0.9
3	354	123	290	128	240	128	220	133
4	290	118	231	101	208	150	150	236
5	4.5	1.9	1.0	0	0	0.5	-	0
6	0	0	0	1.1	0	0	-	0
7	1.6	1.2	3.4	0	0.9	0	0	0.1
8	1.5	0.1	0.3	2.2	1.1	0	1.0	1.3
9	1.5	0	1.6	0	1.0	0	0	0.8
10	1.4	0	0	0.7	0	-	0	1.9
11	-	-	2.5	1.8	1.3	3.0	1.0	1.7
12	-	-	5.5	1.1	1.4	0	1.2	0.1
13	-	-	12.4	1.9	4.2	0	5.0	0.7
14	-	-	-	0	11.1	-	16.2	1.3
15	-	-	-	-	13.7	2.6	15.9	0
16	-	-	-	-	-	-	13.5	0
17	-	-	-	-	-	-	8.5	0

* - = Measurements were not taken during the indicated days.

Figure 1. The oxygen consumption of 12 excissed discs from leaves of mosaic-infected tobacco plants. The ordinates represent the percentage of the oxygen use in terms of the use by 12 discs from healthy leaves of comparable age. The abscissae represent the days elapsed after inoculation.

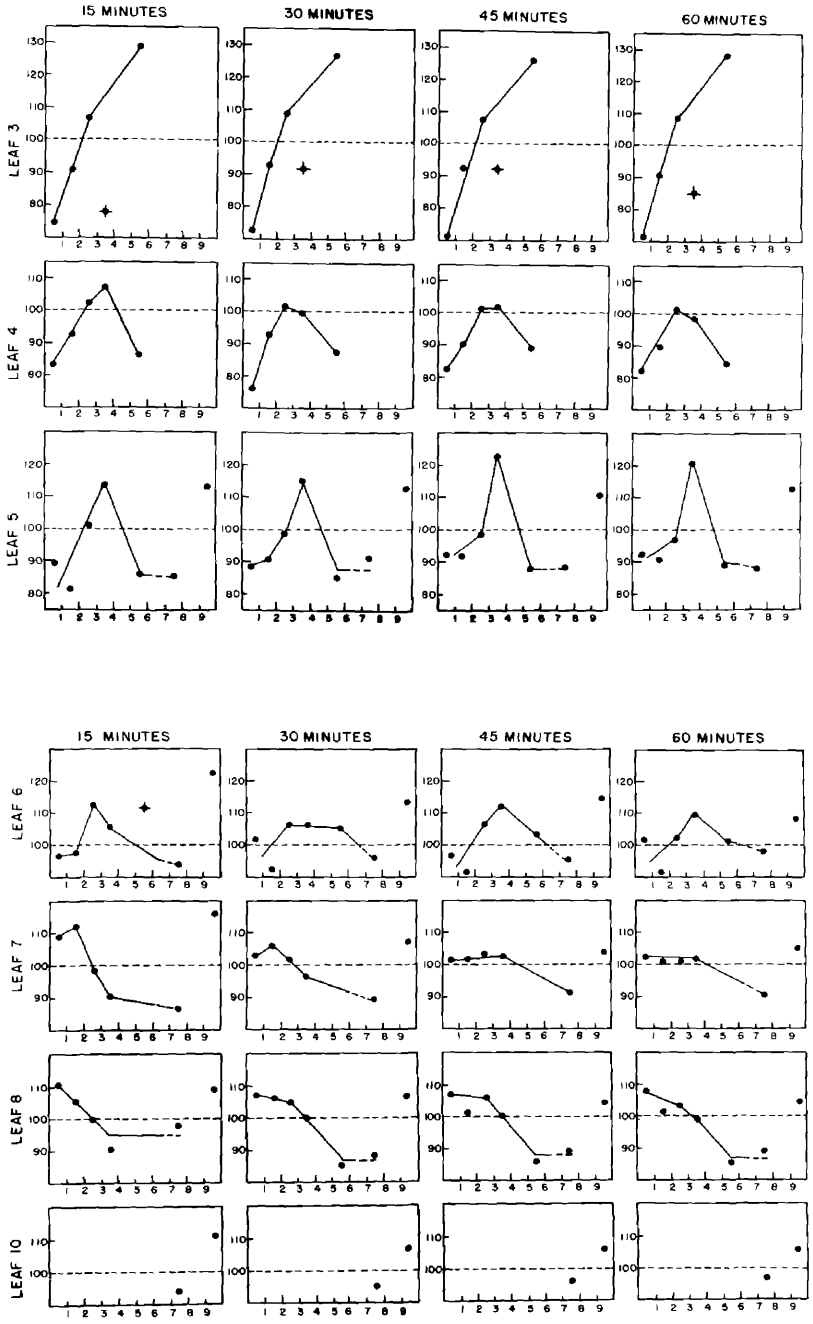
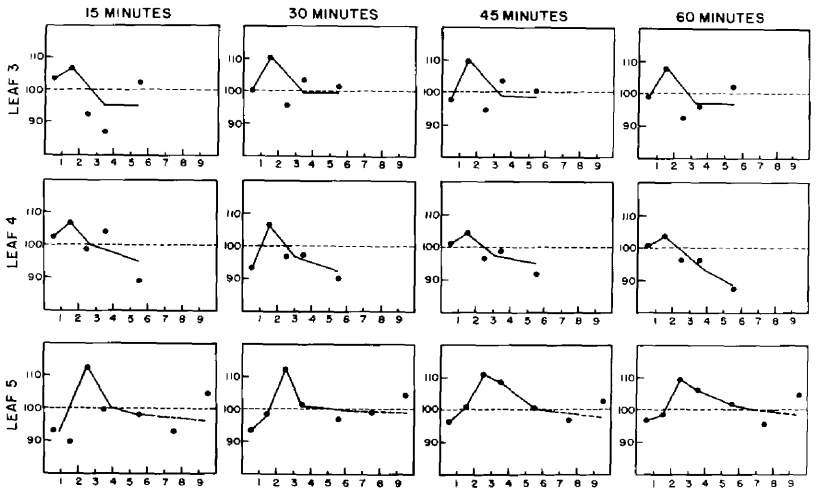
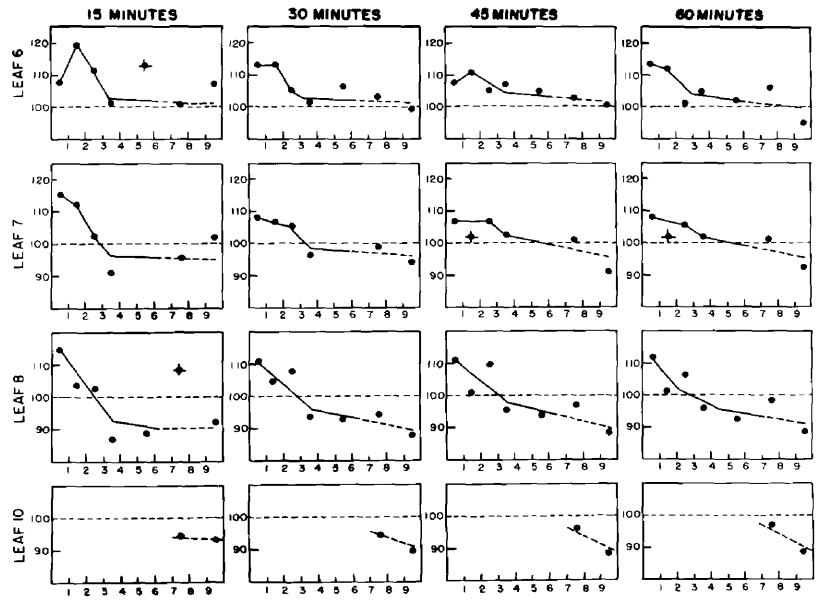

15 MINUTES
30 MINUTES
45 MINUTES
60 MINUTES
LEAF 3
LEAF 4
LEAF 5
LEAF 6
LEAF 7
LEAF 8
LEAF 10

Figure 2. The oxygen consumption of leaves from mosaic-infected tobacco plants. The ordinates represent the percentage of the oxygen use by 10 milligrams of dry tissue in terms of the use by tissue from healthy leaves of comparable age. The abscissae represent the days elapsed after inoculation.


15 MINUTES
30 MINUTES
45 MINUTES
60 MINUTES
LEAF 3
LEAF 4
LEAF 5


15 MINUTES
30 MINUTES
45 MINUTES
60 MINUTES
LEAF 6
LEAF 7
LEAF 8
LEAF 10

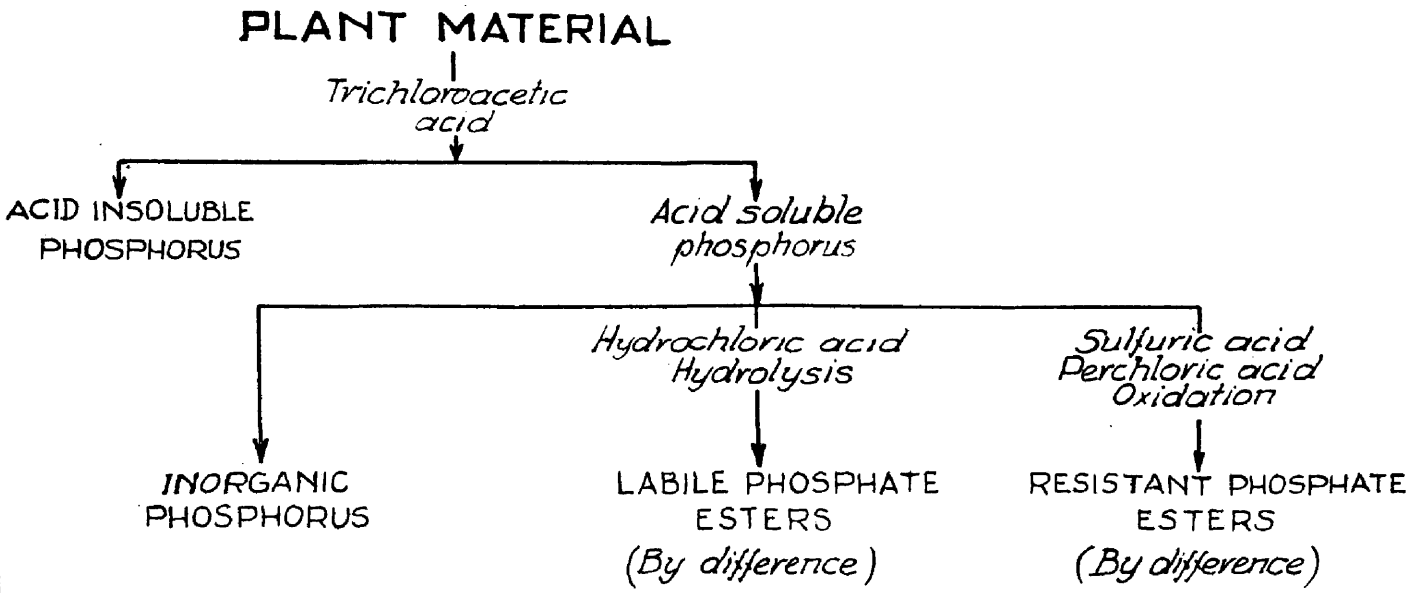


FIGURE 3.- *Phosphorus fractionation of plant material.*

Figure 4. Total phosphorus of leaves from mosaic-infected tobacco plants. The ordinates represent the percentage of total phosphorus calculated on the basis of dry weight in terms of the total phosphorus content from healthy leaves of comparable age. The abscissae represent the days elapsed after inoculation.

FIGURE 4

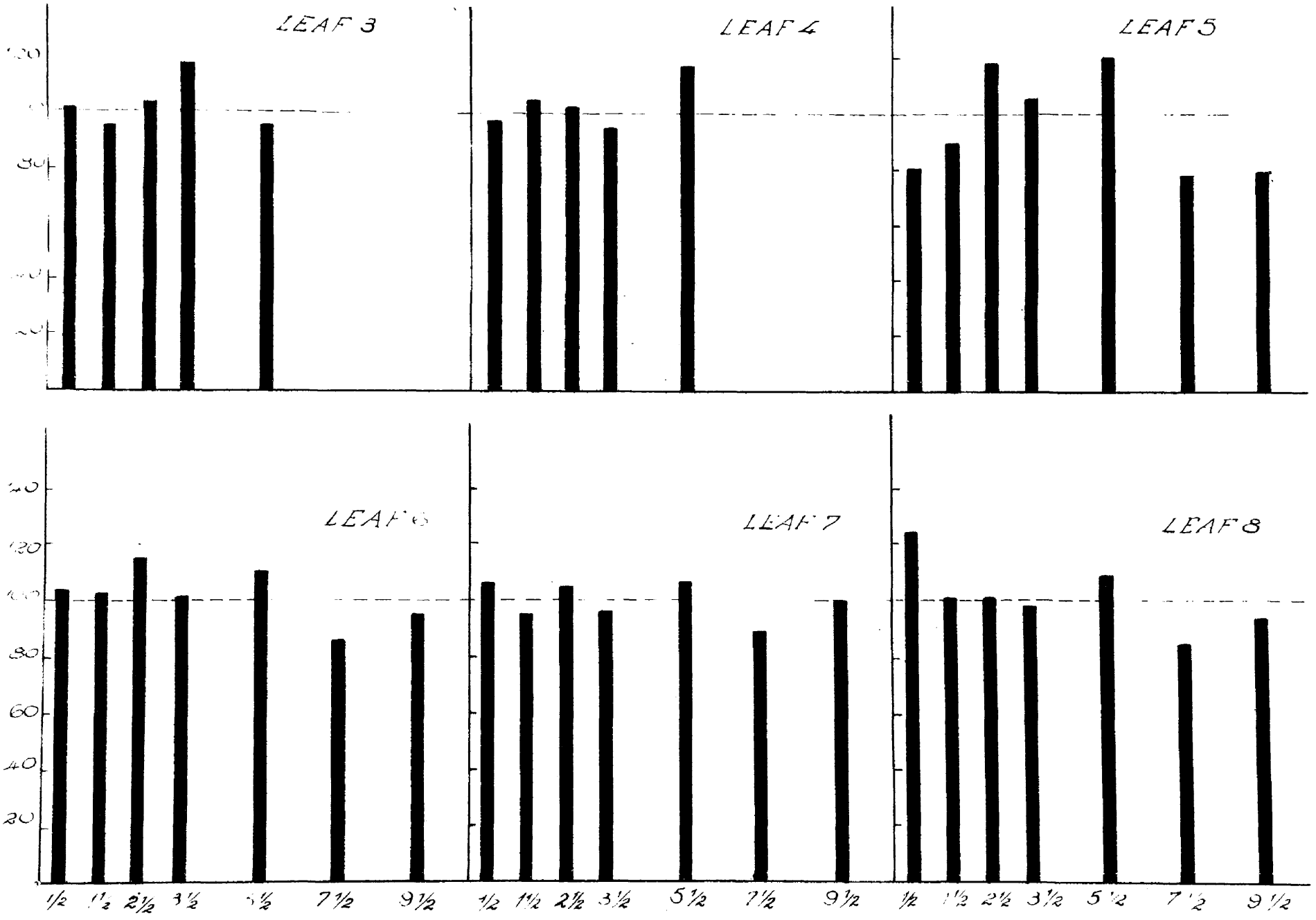

LEAF 3
LEAF 4
LEAF 5
LEAF 6
LEAF 7
LEAF 8
80
60
40
20
1/2
1 1/2
2 1/2
3 1/2
5 1/2
7 1/2
9 1/2

Figure 5. Inorganic phosphorus fraction of leaves from mosaic-infected tobacco plants. The ordinates represent the percentage inorganic phosphorus calculated on the basis of dry weight in terms of the inorganic phosphorus content from healthy leaves of comparable age. The abscissae represent the days elapsed after inoculation.

FIGURE 5

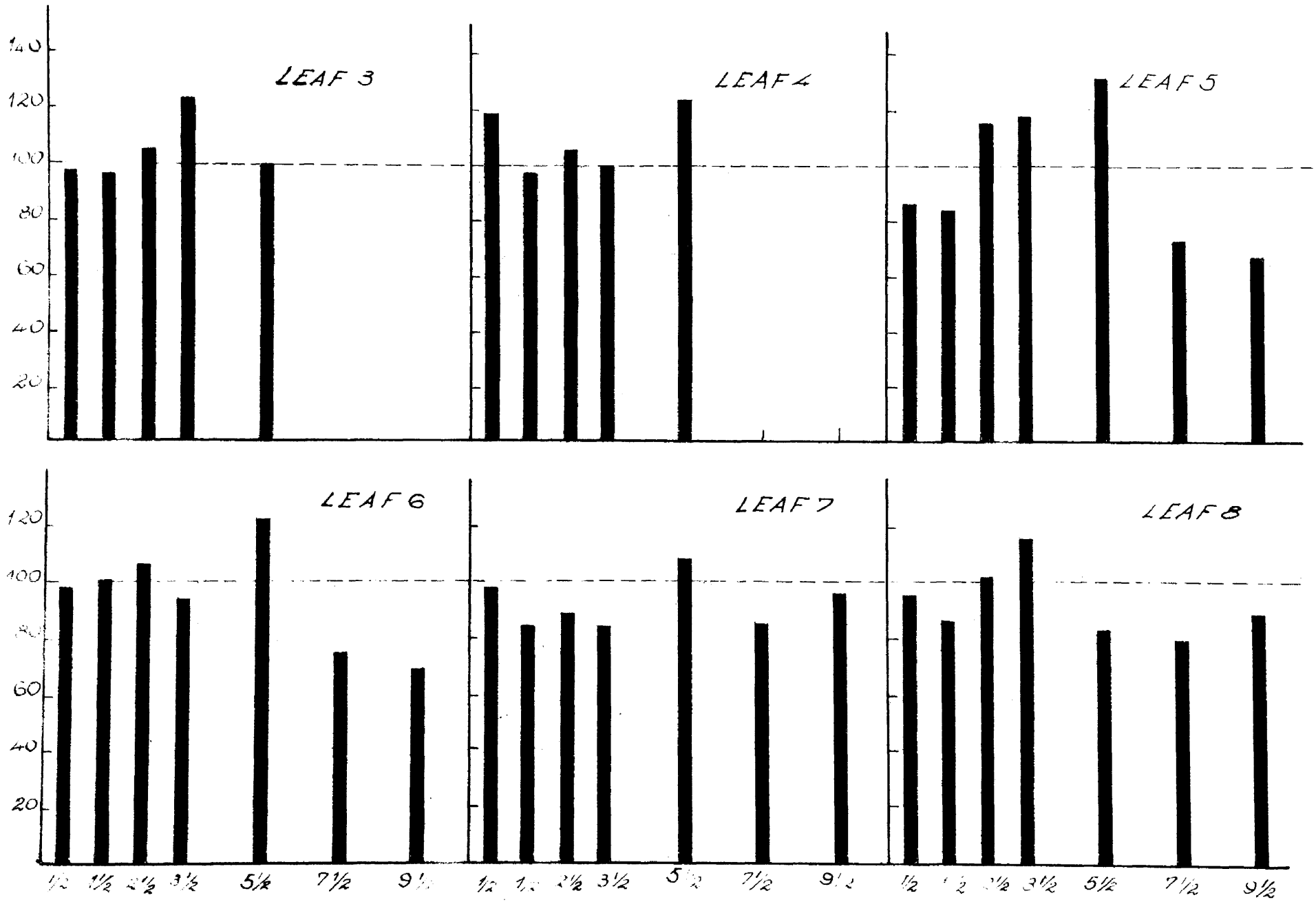

LEAF 3
LEAF 4
LEAF 5
LEAF 6
LEAF 7
LEAF 8
140
120
100
80
60
40
20
1/2
1 1/2
2 1/2
3 1/2
5 1/2
7 1/2
9 1/2

Figure 6. Phosphate esters' fraction of leaves from mosaic-infected tobacco plants. The ordinates represent the percentage phosphorus in phosphate esters calculated on the basis of dry weight in terms of the phosphorus content of phosphate esters from healthy leaves of comparable age. The abscissae represent the days elapsed after inoculation.

FIGURE 6

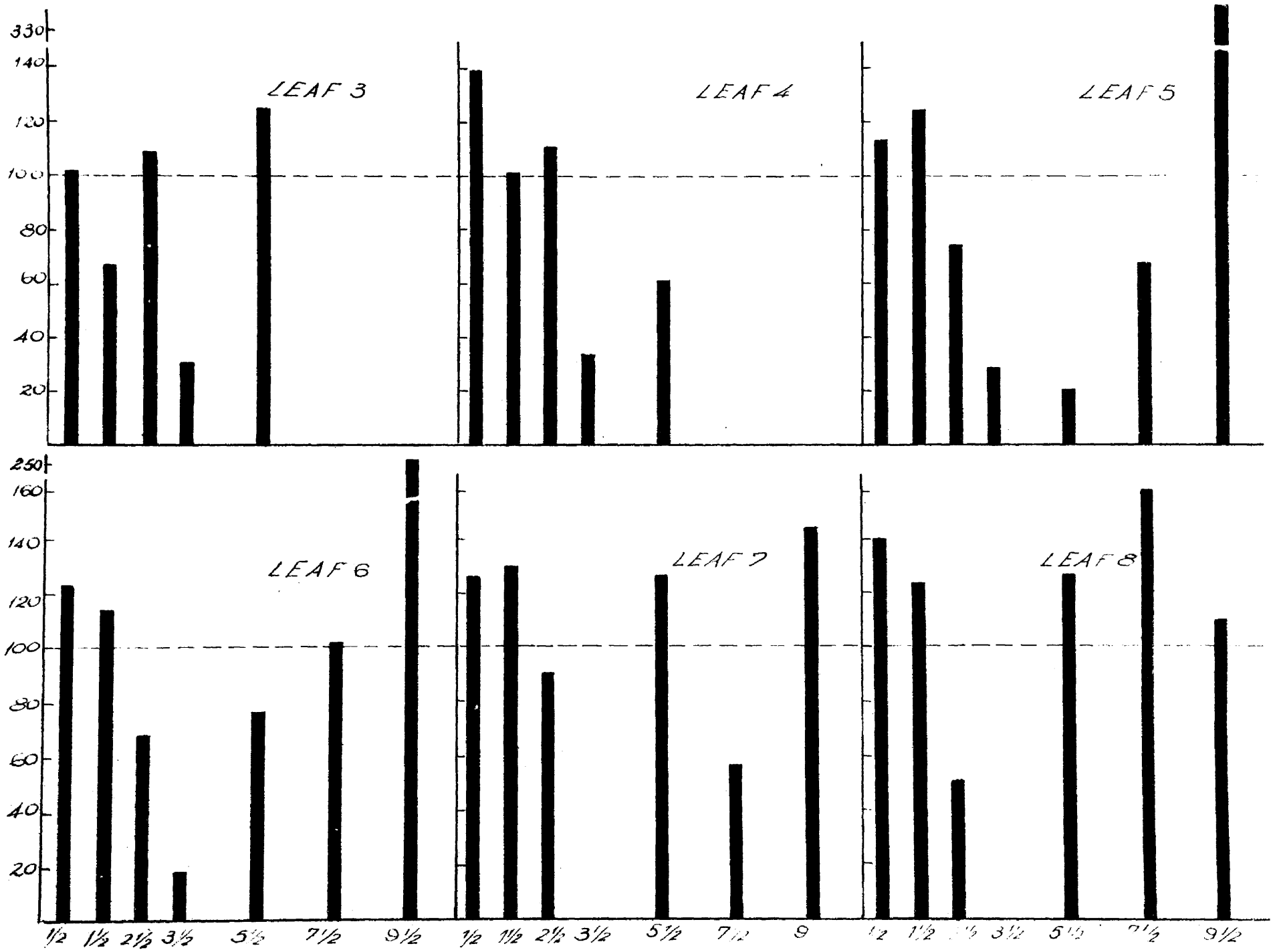
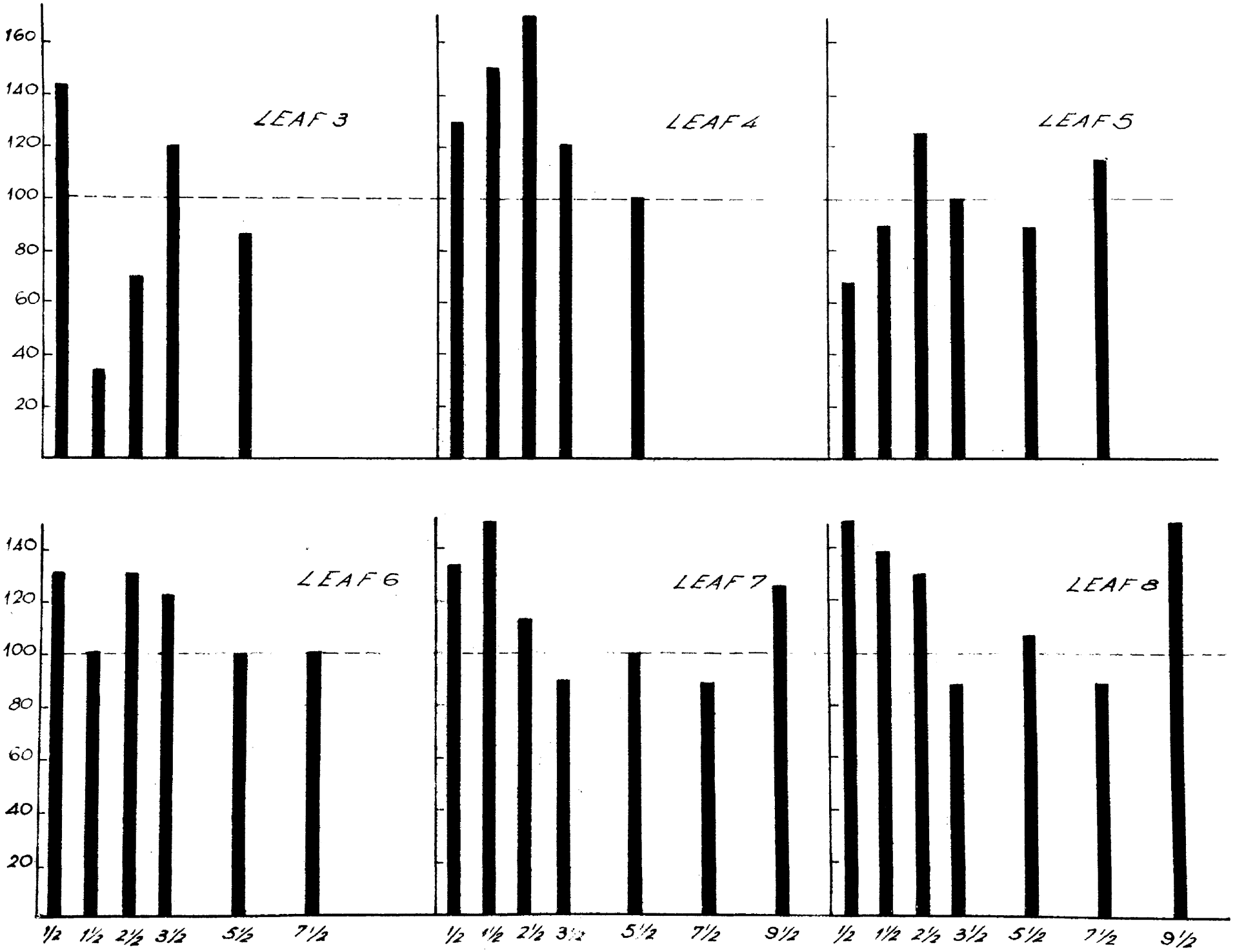

LEAF 3
LEAF 4
LEAF 5
LEAF 6
LEAF 7
LEAF 8
330
140
120
100
80
60
40
20
250
160
140
120
100
80
60
40
20
1/2 1 1/2 2 1/2 3 1/2 5 1/2 7 1/2 9 1/2
1/2 1 1/2 2 1/2 3 1/2 5 1/2 7 1/2 9
1/2 1 1/2 3 1/2 5 1/2 7 1/2 9 1/2

Figure 7. Residual phosphorus fraction of leaves from mosaic-infected tobacco plants. The ordinates represent the percentage residual phosphorus calculated on the basis of dry weight in terms of the residual phosphorus from healthy leaves of comparable age. The abscissae represent the days elapsed after inoculation.

FIGURE 7


160
140
120
100
80
60
40
20
LEAF 3
LEAF 4
LEAF 5
LEAF 6
LEAF 7
LEAF 8
1/2
1 1/2
2 1/2
3 1/2
5 1/2
7 1/2
9 1/2

A P P E N D I X

PHOSPHORUS FRACTIONS IN PLANT MATERIAL

Table of Contents

A. PREPARATION OF THE EXTRACT

Reagents

Trichloroacetic acid. 7.5 percent solution
Norite. Activated carbon

Procedure

Place 250 milligrams of dried, finely ground plant material in 100 milliliters beaker. Add 60 milliliters of ice-cooled 7.5 percent trichloroacetic acid.

Stir gently with a mechanical stirrer for 30 minutes in an ice bath. If the plant material adheres to the sides of the beaker wash it down with a minimum of water.

Filter into a 100 milliliters beaker. Add 10 milliliters of 7.5 percent trichloroacetic acid to the residue and filter through the same filter

paper. Wash the beaker with the plant material twice with 5 milliliters of water, and add the washings to the filter paper.

Save the residue for the determination of residual phosphorus fraction.

Add about 0.2 gram of norite to the combined filtrate and washings, shake well and let it stand for about 15 minutes.

Filter into 100 milliliters volumetric flask and bring up to volume with distilled water. This solution is 5.25 percent in respect to trichloroacetic acid.

Designate the solution as "Extract 1".

B. DEVELOPMENT OF COLOR WITH AMMONIUM MOLYBDATE

Reagents

Ammonium molybdate-sulphuric acid solution.

Dissolve five grams of ammonium molybdade C.P. in approximately 80 milliliters of warm, 50° C., water. Add 28 milliliters of concentrated sulphuric acid to the cooled solution and dilute to 100 milliliters with distilled water. This solution should not be used if white residue is formed.

Hydroquinone solution.

Dissolve 0.5 gram of hydroquinone in 100 milliliters of distilled water made slightly acid with one drop of concentrated sulphuric acid per 100 milliliters of solution.

Keep in refrigerator.

Sodium succinate solution.

Dissolve 20 grams of anhydrous sodium succinate in 100 milliliters of distilled water.

Standard phosphate.

Dissolve 0.4394 gram of potassium dihydrogen phosphate in one liter of distilled water. One milliliter contains 0.1 milligram of phosphorus.

Ammonium hydroxide. 1:1 Dilution

Boric acid, Saturated.

Fifty grams of boric acid crystals are dissolved in one liter distilled water.

Bromo-phenol blue indicator.

Dissolve 0.05 gram of bromo-phenol blue indicator in 2.4 milliliters of 0.01 normal sodium hydroxide and dilute to 175 milliliters with distilled water.

Procedure

Pipette an aliquot of or up to 10 milliliters containing not more than 0.3 milligram phosphorus into 25 milliliters volumetric flask. Add 5 milliliters saturated boric acid solution. If the solution is acid, add two drops of bromo-phenol blue indicator and titrate by adding ammonium hydroxide drop by drop, until the solution turns blue. Then add the following reagents in order, mixing well after each addition. Maximum elapse of time between the addition of each reagent without an effect on the development of blue color is five minutes.

2 milliliters ammonium molybdate solution
2 milliliters hydroquinone solution
2.5 milliliters sodium succinate solution

Make up to volume with distilled water. Allow to stand 30 minutes and measure the color intensity, in a Coleman spectrophotometer employing 775 mμ wave length and PC-5 filter. The color is stable from half to four hours.

Effective range is 0.002 to 0.3 milligram of phosphorus.

C. PHOSPHORUS FRACTIONS

1. Inorganic Phosphorus

Procedure

Pipette five milliliters aliquot of "Extract 1" into 25 milliliters volumetric flask. Add five milliliters of distilled water.

Develop the color and read optical density as it is indicated in Section B.

Obtain a standard phosphorus curve with the following concentrations of phosphorus per flask; 0.01, 0.05 and 0.10 milligrams.

2. Labile Esters' Phosphorus

Reagent

Hydrochloric acid. 1:1 Dilution

Procedure

Pipette five milliliters aliquot of "Extract 1" into 25 milliliters volumetric flask. Add five milliliters of distilled water and two milliliters of 1:1 dilution hydrochloric acid. This solution is about 1 normal with respect to hydrochloric acid.

Place the flask into boiling water bath, stir mechanically for seven minutes. Cool the hydrolysate in an ice-cold water bath.

Obtain a standard phosphorus curve as in C-1 but with the addition of two milliliters 1:1 dilution hydrochloric acid per flask.

Calculations

Percent Labile Esters' Phosphorus = Percent Phosphorus found - Percent Inorganic Phosphorus.

3. Resistant Esters' Phosphorus

Reagents

Sulphuric acid, Concentrated

Perchloric acid, Concentrated

Nitric acid, Concentrated

Nitric acid. Approximately 0.1 normal

Procedure

Pipette five milliliters aliquot of "Extract 1" into 30 milliliters beaker. Add two milliliters of concentrated nitric acid, three drops of concentrated sulphuric acid and one milliliter of concentrated perchloric acid. Place on hot plate, and digest to approximate dryness. To the cooled beaker add 5 milliliters of 0.1 normal nitric acid, warm and transfer quantitatively into 25 milliliters volumetric flask. Wash the beaker three times with two milliliters of 0.1 normal nitric acid. Add washings to the volumetric flask.

Develop the color and read optical density as indicated in Section B.

Obtain a standard phosphorus curve using the same concentrations as in Section C-1, but in 0.1 normal nitric acid.

Calculations

Percent Resistant Esters' Phosphorus = Percent Phosphorus found - (Percent Inorganic Phosphorus + Percent Labile Esters' Phosphorus).

4. Residual Phosphorus

Reagents

Digestion mixture. Sulphuric acid, perchloric acid mixture. Add 100 milliliters of concentrated sulphuric acid to 200 milliliters of concentrated perchloric acid.

Nitric acid, Concentrated

Nitric acid. 1:4 Dilution

Procedure

Transfer the residue of the plant material obtained in the "Preparation of the Extract" into 250 milliliters beaker. Add 10 milliliters of concentrated nitric acid and 10 milliliters of the digestion mixture. Place the beaker on a hot plate and heat at moderate temperature until all the organic matter has been oxidized and white fumes are evolved. Use more digestion mixture if necessary.

Add 10 milliliters of 1:4 nitric acid, 60 milliliters of hot distilled water and pour into 100 milliliters volumetric flask. Make up to volume.

Pipette five milliliters aliquot into 25 milliliters volumetric flask and add five milliliters of distilled water.

Develop the color and measure optical density as indicated in Section B.

Obtain a standard phosphorus curve as in Section C-3.

5. Total Phosphorus

Reagents

Digestion mixture. As in Section C-4

Nitric acid, Concentrated

Nitric acid. 1:4 Dilution

Kerosene

Procedure

Place one-gram sample of plant material in a micro-Kjeldahl flask. Add 10 milliliters of concentrated nitric acid, three milliliters of the digestion mixture and two drops of kerosene. Let it stand overnight. Heat on micro-Kjeldahl digestion unit. When white fumes are evolved, the digestion is complete and a colorless liquid of about one to two milliliters remains in the flask.

Add to the digestion flask 10 milliliters of 1: 4 nitric acid and 20 milliliters of distilled water. Warm, pour into 100 milliliters volumetric flask, make up to volume with distilled water.

Develop the color and measure the optical density as in Section B.

Obtain a standard phosphorus curve as in Section C-4.