EFFECTS OF THE MOSAIC DISEASE ON THE PHYSIOLOGY OF TOBACCO PLANTS

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EFFECTS OF THE MOSAIC DISEASE ON THE PHYSIOLOGY OF TOBACCO PLANTS

by

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EFFECTS OF THE MOSAIC DISEASE ON THE PHYSIOLOGY OF TOBACCO PLANTS[1]

I. INTRODUCTION

There is considerable evidence showing that virus diseases of plants induce profound physiological disturbances. The visual symptoms appear as irregular light green areas on the leaf blade, and they become manifest within about three weeks after the plant has been inoculated. The time of their appearance somewhat depends on the physiological state of the host plant. These symptoms appear first in the growing tip, and later appear spread on all of the actively metabolizing leaves of the plant.

Long before the appearance of the visible symptoms, physiological disturbances associated with the disease are in progress. For example, Wildman, Cheo and Bonner (1949) report their detection of virus protein in the leaves by electrophoretic methods three days after inoculation. This observation is in essential agreement with the results reported by Wynd (1943). This author showed an increase in the respiratory

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[1] The expenses of this study were borne jointly by the Agricultural Experimental Station, Michigan State College, Project 39, and by a grant-in-aid provided by the American Cancer Society, Committee on Growth.

rate of the plant on the fourth day after inoculation and suggested that this phenomenon was accompanied by the dissemination of the virus particles throughout the plant.

The nature and character of various types of disturbances induced by the mosaic disease have been the subject of numerous publications and review articles. Bawden (1943) and Wynd (1943) reviewed the subject thoroughly, and based their general concepts of the problem on the published literature to the date of their publications. Wynd (1943) in his review on the metabolic phenomena associated with the virus infection emphasized that the permeability of cytoplasmic membranes, involving the differential absorption of the mineral constituents as well as the translocation of the products of photosynthesis, is altered by the virus disease since many conflicting data could be explained by means of this physiological mechanism.

The present study was undertaken as a phase of the investigation of the general problem of the physiological nature of the virus diseases in plants now in progress in this laboratory. The study specifically was concerned with the further investigation of the conclusion of Wynd (1943) that "The permeability of cytoplasm or its membranes is greatly altered in respect to soluble substances. This accounts for the accumulation of carbohydrates in the leaves, and the accumulation of nutrient ions in the roots at the expense of tops." The study was based on the detailed analysis of tobacco plants grown

under field conditions and also of plants grown in nutrient solutions under controlled experimental conditions in the greenhouse.

II. EXPERIMENT 1

A. Purpose

Most of the literature on the disturbances accompanying mosaic diseases has been based on material grown under field conditions. Experiment 1, therefore, represents a determination of the chemical composition of normal and mosaic diseased tobacco plants grown in the field, and a detailed comparison of the data with those previously described by other workers. Special emphasis was placed on the application of the data to the hypothesis of Wynd that the disease induces an altered permeability of the protoplasmic membranes and the consequent changes in absorption and translocation.

B. Experimental material

1. The soil

The healthy and mosaic infected tobacco plants were grown on a Fox sandy loam, located in Essex County, Ontario, Canada. They were supplied through the courtesy of M. F. Murwin, superintendent, Experimental Station, Harrow, Ontario, Canada. Fox sandy loam is described by Richards, Caldwell and Norwick (1949) as a brown silt loam underlain by a dark brown loam which may verge into a clay loam. The Fox soil series exhibits the characteristics of the gray-brown podesolic soils. The topography of this soil type typically is undulating to rolling, and the profile possesses good natural drainage. The soil reaction is moderately acid, varying from pH 6.0 in the upper layer or A horizon to 6.6 in the B horizon. The crops

generally grown on this soil type are comprised of a few orchards and such cash crops as corn, tobacco and canning vegetables. The main fertility deficiencies consist of organic matter and phosphate, and to a minor degree of potash.

The cultural and fertilizer practice on the field producing the tobacco plants examined in the present study during the previous five-year period may be summarized as follows:

1945 Alfalfa; sod manured and ploughed during the following spring, and then planted to tobacco.

1946 Burley tobacco; fertilized with a 4-8-10 mixture at the rate of 1000 pounds per acre.

1947 Corn.

1948 Oats; seeded with clover.

1949 Burley tobacco; with a heavy application of barnyard manure. Additional nitrogen fertilizer was added at the time the ground was prepared for tobacco and the 4-8-10 fertilizer was applied in two applications, half at the time of transplanting the tobacco and half added later as a side-dressing.

2. The tobacco leaves

Nicotiana tobacum, var. burley was grown under commercial conditions. Leaves of the secondary growth exhibiting tobacco mosaic symptoms and also those of healthy plants were collected during the first week of October, 1949, and were dried in the greenhouse. The

dry leaves were ground in a micro Wiley mill to pass a 60-mesh screen and the powdered material was then stored in air-tight containers until the chemical analyses were carried out.

C. Experimental results

1. Inorganic constituents

a. Total ash

The percentage of total ash was determined as carbonates by dry ashing 1 gram of the sample in a platinum crucible at a temperature of 650° C. (See appendix 1.)

The percentages of total ash are presented in table 1. The ash content of healthy tobacco leaves is seen to be greater than that of the virus infected leaves. The normal leaves contained 23.60 percent while the diseased leaves contained only 16.86 percent. When this difference in the ash content is calculated as a percentage of the ash content of healthy leaves it is seen that the virus disease lessened the percentage of ash 28.55 percent.

It was suggested in the introduction that the mosaic disease had an effect on the permeability of cells and the translocation of soluble nutrients within the host plant. If this be true, it would be expected that the percentages of ash in the leaves would be changed by the disease. The above data obtained in the present experiment are consistent with this hypothesis. The above finding is supported by the previous work by True, Black and Kelly (1918). These authors, working with normal and blighted spinach leaves,

observed that the total ash of healthy leaves was 21.41 percent of the dry matter, while in virus infected plants, the percentage was only 16.68. This represents a decrease of 22.08 percent with respect to the value for normal plants. Bailey (1924) also reported a lower percentage of ash in the leaves of mosaic infected tobacco plants. Freiberg's report (1917) on the composition of mosaic infected tobacco plants is too incomplete to furnish data concerning the percentage of total ash.

b. Silica

The silica content of the leaves was determined by the difference in the weight of the total ash before and after treatment with hydrofluoric acid. The one-gram samples used for the total ash determination were treated as described by Piper (1950). (See appendix 2.)

The percentages of silica in healthy and infected leaves are presented in table 1. Mosaic infected leaves contained conspicuously less silica than did the normal leaves. The normal leaves contained 4.94 percent and the diseased leaves contained only 1.00 percent. These data represent a diminution of 79.75 percent when the value for the normal plants is taken as 100. This tremendous decrease in the concentration of silica contradicts the previous reports by True, Black and Kelly (1918) and Iyengar (1928). These authors, working with blighted spinach and spiked sandal, respectively, reported an increase in the silica concentration in the leaves. True, Black and Kelly observed a 1.87 percent increase of the silica in infected

spinach calculated on the basis of the dry matter.

The silica contents of the leaves are expressed in table 2 as percentages of the total ash contents. The value was 37.53 percent in the normal leaves and only 12.70 percent in the diseased leaves. This decrease in the percentage of silica in the total ash indicates that silica accumulation in the diseased plants was inhibited to a greater degree than was the total ash.

c. Individual components

(1) Preparation of sample

A 0.5 gram sample of the powdered dry material was wet ashed with nitric and sulphuric acid and the residue treated with 10 milliliters of 1:4 nitric acid and then diluted to 100 milliliters with distilled water. Appropriate aliquots were taken for the determination of the different ions.

(2) Calcium

Calcium was determined by the ammonium hexanitrate cerate method according to Reitemeier (1943). An aliquot of 5 milliliters, equivalent to 25 milligrams of dry plant material, was used which required about 4 to 6 milliliters of the 0.01 N cerate reagent. (See appendix 3.)

The calcium concentrations of the samples appear in table 1 and are expressed as percentages of the dry matter. The percentage of calcium in the mosaic leaves is smaller than that in the normal leaves. The normal leaves contained 3.70 percent while the diseased leaves

contained 2.94 percent. These values represent a 20.54 percent decrease in the calcium concentration of virus leaves when calculated on the basis of the concentration in the healthy leaves.

The data in table 2 express the percentages of calcium in the total ash. It is evident that the diminution of the calcium concentration in the diseased plants is of lesser magnitude than the decrease in the total ash, since the percentage of calcium in the ash increased 9.41 percent, even though the percentage in the dry material decreased 0.76 percent. This increase of the ratio of calcium to the total ash agrees with the data reported by True, Black and Kelly (1918) who noted that calcium, calculated as CaO, increased from 6.48 percent of the total ash in healthy plants to 11.88 percent in blighted spinach.

Iyengar (1928) observed the opposite result since his data show that the ratio of calcium to the total ash in spiked sandal decreased.

Freiberg (1917) detected no significant changes in the calcium content of mosaic tobacco leaves.

The decrease in the calcium concentration in the dry matter of the diseased tobacco plants may not be as significant physiologically as the changes in the percentage of calcium in respect to the total ash. This may produce a far more reaching physiological disturbance than the mere change in its absolute amount would suggest.

(3) Magnesium

Magnesium was determined in the aliquot after the removal of calcium as the oxalate. The general procedure of Reitemeier (1943)

was followed. The precipitate of magnesium phosphate, $Mg(NH_4)PO_4$, obtained by this procedure, was dissolved in 1 N sulphuric acid. The phosphate ion in the solution was determined colorimetrically by the molybdenum blue reaction as described by Ritson and Mellon (1944). This procedure gave very satisfactory results. An aliquot of 5 milliliters of the calcium-free solution, equivalent to 5 milligrams of dried plant material, was used for the determination. (See appendix 4.)

The percentages of magnesium in the dry plant tissues are presented in tables 1 and 2. The average percentage of magnesium in the healthy tissue was 0.71 percent, while only 0.43 percent was found in the diseased leaves. When calculated as a percentage of the value for normal leaves, it is seen that the magnesium in the diseased leaves was lessened by 39.44 percent. When the magnesium content is calculated as a percentage of the total ash, table 2 shows that the ash of normal leaves contained 5.39 percent, while the value for the diseased leaves was 5.48 percent. These data indicate that the percentage of magnesium in the ash increased 1.67 percent in the virus infected leaves. This increase indicates that the amount of total ash in the diseased plants decreased a little more than did the magnesium component.

In view of the significant differences in the magnesium content of normal and virus infected tobacco observed in the present study, it is interesting to note that Freiberg (1917) reported no effect of the mosaic disease on the magnesium content of the tobacco plant.

The percentages of magnesium and calcium in the dry matter exhibited the same quantitative and qualitative responses to the mosaic disease. The virus infection decreased the percentages of both of these ions. This relationship is not observed when the magnesium and calcium concentrations are expressed as percentages of the total ash since the values in this instance differ considerably. The percent increase of the magnesium ion is almost zero while that of calcium ions is of the order of 33.43 percent of the total ash. The reciprocal relationship between calcium and magnesium presented in Wadd's (1940) review is not supported by the data in tables 1 and 2. This uniformity of responses of the divalent ions should be borne in mind, since it has a direct bearing on the theory of virus induced physiological changes to be discussed in a later section.

(4) Potassium

Potassium was determined by the procedure described by Peech (1941) by precipitation with sodium cobaltinitrite. The precipitate was brought into solution with sulfuric acid and the potassium was determined colorimetrically with nitroso-R-salt. An aliquot of 3 milliliters, equivalent to 15 milligrams of dried plant material, was used for the determination. (See appendix 5.)

Tables 1 and 2 indicate the percentages of potassium in the dry matter and in the total ash. The percentage of potassium in the mosaic infected leaves is decreased. In the normal leaves the percentage was 3.63, while in the diseased leaves 3.33 percent was found. This decrease appears to be small, but when it is calculated on the basis of the amount in normal tissue being 100, then the decrease is seen to be 8.3 percent. This effect of virus infection on the potassium content agrees with the general trend described by Wynd (1943).

Freiberg (1917) reported that the effect of mosaic disease on the potassium content of tobacco leaves was small.

The absorption of potassium was less disturbed by the disease than was that of most of the other major metallic ions. This fact is in agreement with the general opinion that potassium is more readily translocated within the plant body than is calcium or magnesium. On the other hand, the absorption of potassium by the diseased plants was actually less, and this should not be overlooked in view of the discussion by Wynd and Vavonis (in press) of the occasional masking of the early symptoms of the Little peach disease by the symptoms of potassium deficiency.

(5) Sodium

The sodium content of the leaves was determined by the use of the Perkin-Elmer flame photometer according to the method described by Toth, Prince, Wallace and Mikkelsen (1948). A 0.5 gram was ashed as described above, and the residue dissolved in 100 milliliters of

0.1 N nitric acid.

The percentages of sodium in healthy and mosaic infected leaves are presented in tables 1 and 2. There is only a small and probably insignificant increase in the concentrations of sodium in the diseased leaves, but when the sodium is expressed as a percentage of the total ash, the value is 0.43 percent in the healthy leaves and 0.74 in the infected leaves. These data show that the absorption of sodium was essentially unaffected by the disease although the effect on the total ash was very great.

The similarity in the percentages of sodium in the healthy and mosaic leaves indicates that the virus disease has little or no effect on the accumulation of this nutrient in tobacco plants. The increase observed when the percentage of sodium to total ash is considered indicates that sodium, like potassium, is more mobile and is more readily translocated within the plan than are most of the other cations. It is to be noted that potassium and sodium follow the same trend of increase when they are expressed as percentages of total ash. This observation conflicts with the data summarized from the published literature by Wynd (1943).

(6) Iron

Iron was determined by the procedure described by Hummell and Willard (1938), by using hydroquinone as a reducing agent and O-phenathroline as an indicator. An aliquot of 10 milliliters, equivalent to 50 milligrams of dry plant material, was used. (See appendix 6.)

The iron content of the healthy and infected leaves is given in tables 1 and 2. Mosaic diseased leaves contained less than half the amount of iron in the healthy leaves. The normal leaves contained 0.106 percent while the diseased leaves contained only 0.051 percent. This decrease corresponds to 52 percent of the iron in the normal plants. When the iron content is calculated as percentages of the total ash, the value of 0.81 was obtained for normal plants and 0.65 was obtained for the diseased plants. These values indicate that the absorption of iron was lessened by the disease to a significantly greater degree than was the total ash.

The lower percentage of iron in the mosaic infected tobacco leaves is in agreement with the data of Coleman (1917) who reported that the spike disease of sandal caused a lower iron content in the mature leaves.

(7) Manganese

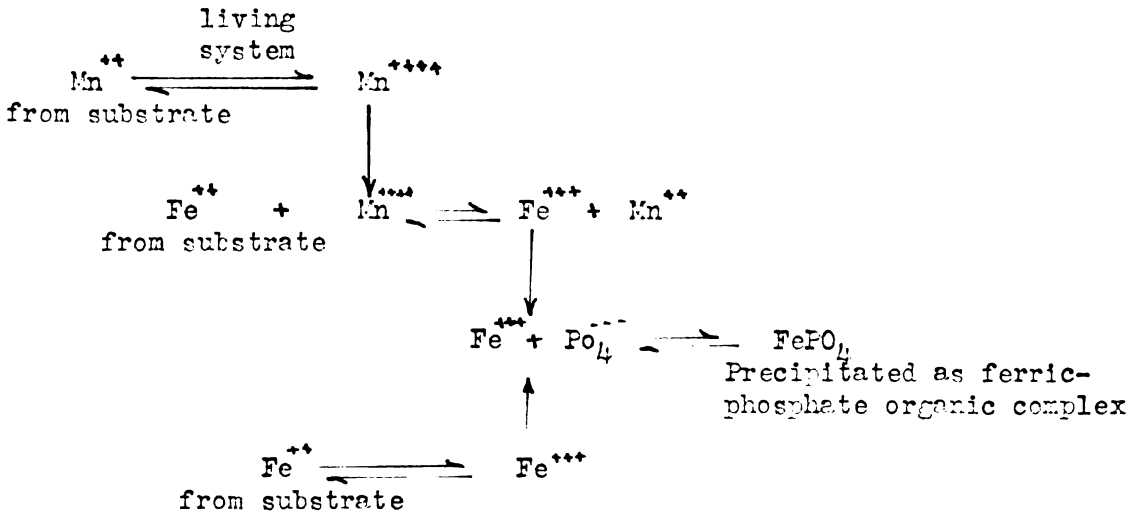
Manganese was determined according to the method described by Peech (1947). The manganese in the aliquot was oxidized by potassium metaperiodate and the color of the permanganate solution was determined in the Coleman spectrophotometer. An aliquot of 20 milliliters, representing 0.5 gram of dry plant material was used. (See appendix 7.)

The manganese content of the healthy and virus infected leaves is given in tables 1 and 2. The manganese concentration in the healthy leaves was 0.019 percent, but it increased to 0.026 percent in the diseased leaves. The increase by 0.007 percent on the basis

of dry matter represents a 36.84 percent increase when it is calculated on the basis of the value for normal leaves taken as 100. When the data are calculated in terms of percentages of the total ash, 0.14 is obtained for normal leaves and 0.33 for diseased leaves. This increase represents a 135.7 percent increase in terms of the percentage of manganese in the total ash. The data agree with those reported by True, Black and Kelly (1918) based on blighted spinach plants. These authors observed an increase of manganese, calculated as Mn_3O_4, in the tops of blighted spinach from 0.021 to 0.045 percentage of dry weight. This represents a 114.2 percent increase over the normal content of healthy leaves which is in remarkably close agreement with the value obtained in the present study.

The observed increase of percentage of manganese in the mosaic infected tobacco leaves indicates how profoundly the physiology of the plants was altered by the disease. This increase becomes even more important when the iron content also is considered.

The importance of the reciprocal relationship between manganese and iron was described by Somers and Shive (1942), who suggested that the relative abundance of these two ions is far more important than is their total concentrations in the tissue. Manganese, since it is the stronger oxidizing agent, reacts with the ferrous, physiologically active, iron, transforming it into the ferric form and rendering it inactive as it precipitates in the ferric-phosphate organic form. The suggested mechanism for this interaction between manganese and iron may be summarized as follows:

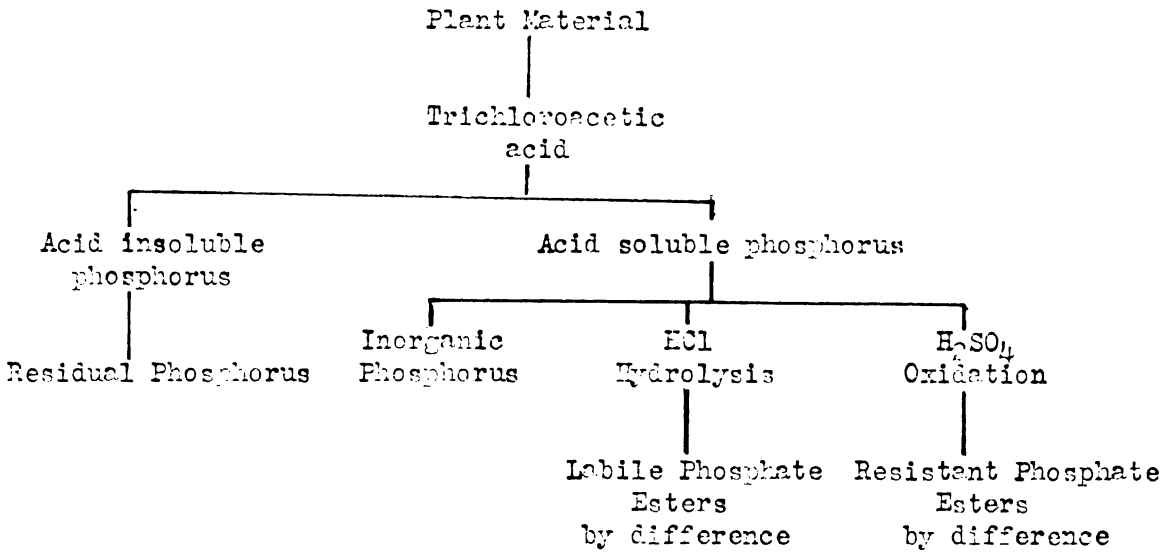


Very often, an iron deficiency in plants is not caused by the lack of iron, but rather by manganese toxicity resulting from the inactivation of the iron in the plant.

The reciprocal relationship between the absorption of iron and manganese was reported by True, Black and Kelly (1918), who observed that an increase in the manganese content of the tops was accompanied by a decrease in the iron content.

(8) Phosphorus fractions

The separation of phosphorus fractions was carried out by a modification of Arney's procedure (1939). (See appendix 7.) The scheme of separation was as follows:



In all cases the phosphorus content of the different extracts and solutions was determined as recommended by Ritson and Mellon (1944), utilizing the ammonium molybdate-hydroquinone procedure. (See appendix 4.)

A one-gram sample was used for the original extraction and appropriate aliquots ranging from 2 to 5 milliliters and corresponding to 10 to 25 milligrams of dry tissue were employed for the determinations. (See appendix 8.)

(a) Total phosphorus

The virus infected leaves contain more total phosphorus than the healthy leaves, table 5. The increase calculated as percentage of the normal content of healthy leaves is 5.64, table 4. This finding is in agreement with the data presented by Holden and Tracey (1948) when the total phosphorus found is expressed as percentage of the total dry matter, in the systemic infected mosaic tobacco plants.

Freiberg (1917) did not observe any appreciable difference in the total phosphorus content of mosaic and healthy tobacco leaves. Wynd (1943) in his review described an inconsistent effect of virus diseases on the percentage of total phosphorus in the plants. Ryzhkov and Vorob'eva (1942) report a decrease in the total phosphorus of mosaic infected tobacco.

(b) Inorganic phosphorus

Table 4 gives the percentages of inorganic phosphorus in the healthy and mosaic leaves and the percentage difference when calculated on the basis of normal content of healthy leaves. The healthy leaves contained 0.185 and the diseased leaves contained 0.206 percent inorganic phosphorus in the dry plant tissue. The data show that the virus disease lessened the inorganic phosphorus in the leaves 11.35 percent when the decrease is calculated as a percentage of the amount present in the normal plants.

(c) Labile phosphate esters

The labile esters fraction represents the phosphorus fraction most intimately related to the respiratory mechanism of the protoplasm. The normal leaves contained 0.026 percent of phosphorus present as labile esters, while the infected leaves contained 0.035 percent. This difference represents an increase of 34.62 percent over the amount in the normal leaves.

(d) Resistant phosphate esters

The resistant esters fraction contains such phosphate compounds as the hexosephosphates, triosephosphate, phosphopyruvic acid, and phosphoglyceric acid. Table 4 indicates that the amount in normal leaves was 0.046 percent and the amount in the diseased leaves was 0.038. This comparatively small decrease, calculated on the dry weight basis, amounts to 6.5 percent when calculated on the basis of the amount in the normal leaves.

(e) Residual phosphorus

The fraction indicated as residual phosphorus contains all of the phosphoproteins and phospholipids which are precipitated by trichloroacetic acid.

The virus infection increased the residual phosphorus fraction by 57.69 percent over the content of healthy leaves, table 4.

(f) Discussion of phosphorus fractions

The data in table 4 show that the total phosphorus recovered in the various fractions varies from 91.3 to 108.3 percent of the total. There is a greater consistency in the recovery of the higher amounts, although the absolute recovery is satisfactory in view of the limited accuracy of the fractionation procedure.

The data show that the virus disease increases not only the absorption of total phosphorus but also the different fractions, with the exception of the resistant ester fraction. The most pronounced effect is exerted on the amount of residual phosphorus, in which case the increase was 57.69 percent. Perhaps this might be expected from

the increase in the phosphoproteins in the virus particles themselves, although this phenomenon would not account for the greater total phosphorus. The labile esters fraction exhibited the next highest increase which was found to be 34.62 percent when calculated on the basis of the amount of this fraction in healthy leaves.

Table 5 presents the ratios of the various phosphorus fractions to each other. There is a decrease in the ratio of labile esters to residual phosphorus. These data suggest two mechanisms, one following the other, which may account for the increase in the labile esters fraction. Bawden (1943) and Wynd (1943) in their reviews indicate that the respiratory rate of virus infected plants is related to the stage to which the disease has progressed. In the early stages of the disease, namely on the fourth day after inoculation, Wynd (1943) reported a maximum in the rate of respiration of mosaic infected tobacco plants. This increase was followed by a rate of respiration smaller than the normal. It may be surmized that an increased amount of labile esters would accompany an increased rate of respiration. When the respiratory rate decreased in the later stages of the disease, and when the virus particles accumulated in the tissue, the labile esters might remain intact while the other phosphate compounds were utilized for the formation of virus particles. This mechanism would explain the increased labile ester fraction of mosaic infected tobacco leaves. The magnitude of the decrease in the percentage of resistant esters is not significant when the duplicate determinations are considered.

The changes observed in the amounts of the different phosphorus fractions are of interest in considering a possible mode of virus formation and multiplication. Although the mechanism of virus formation and the translocation of the particles within the host plant do not fall within the scope of this study, it is worthy of mention that the very labile phosphate group of the virus particle might serve as one of the possible avenues of approach to these perplexing problems.

f. Discussion of inorganic constituents

The data described above show that the virus infected tobacco leaves contained less total ash than did normal leaves when calculated by the sum of the cations determined. This decrease in the amounts of the total cations was accompanied by reciprocal differences between such physiologically related cations as manganese and iron. The amounts of magnesium and calcium did not exhibited a similar reciprocal relationship.

There was no appreciable difference in the sodium concentration in the normal and diseased leaves when their percentages were calculated on the basis of the dry matter. The same situation was not evident when sodium was expressed as percentages of the total ash.

A study of the data in table 1, assembled in the column 'Percentage difference', discloses that the valence of the element concerned is related to the degree of the disturbance of its concentration in the tissue caused by the virus disease. With the exception of silica, the magnitudes of the percentage difference induced by the

disease are directly proportional to the valence of the ions. Iron and manganese which are present mostly in their oxidized state in normal soils and which undergo the greatest changes in valence are subject to the greatest changes in absorption as a result of the virus disease of the plant. Calcium and magnesium which have a valence of two are disturbed to a lesser degree, while sodium and potassium which possess but one valence are affected to a still lesser extent by the disease.

These observations suggest that the nature of the physiological disturbances brought about by the mosaic disease may be related to the electrical status of the colloidal structure of the protoplasm. That such alterations in the electrical condition of the colloidal complex of living protoplasm may produce profound physiological disturbances cannot be overemphasized.

The importance of the electrical forces of the colloidal surfaces of the living cells to the mechanism of absorption of ions from the colloidal particles in the soil have been described in the works of Jenny and co-workers. A change in the electrical state of the protoplasm in the root hair will markedly affect the uptake of nutrients when the electrical forces play an important role in nutrient absorption.

A comparable study of the physiological disturbances induced by virus disease carried out with plants grown in nutrient solution, where the part of the electrical forces in the absorption of nutrients is minimized, would throw light on the problem.

This disturbance in the electrical state of the protoplasmic colloids is augmented as the disease proceeds since the differential uptake of nutrients, resulting in their altered ratios in the cells, causes further disturbances.

2. Organic constituents

a. Carbohydrates

The total carbohydrates were determined according to the method described in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1945). One-gram samples of the dry powdered plant material were used for the initial hydrolysis. After clearing with neutral lead acetate, the filtered solution was made up to 500 milliliters. An aliquot of 3 milliliters of this extract, equivalent to 6 milligrams of dry material, was used for the determination of reducing sugar. (See appendix 9.)

Table 8 presents the concentrations of carbohydrates found in healthy and virus infected leaves expressed as percentages of dried matter. The normal leaves contained 11.38 and the diseased leaves contained 10.35 percent. This decrease of the carbohydrate content of the diseased leaves represents a 9.05 percent decrease over the content of healthy leaves.

The observed decrease in carbohydrate content is in agreement with numerous data in the literature. Bailey (1924) and Dunlap (1930) also observed a decrease in the percentage of carbohydrates in mosaic infected tobacco leaves. Such an effect on the tobacco

leaves seems to be a consistent response to the disease, although similar symptoms are not observed in the case of many other virus diseases.

b. Fats

The determination of crude fats was carried out by an ether extraction of two-gram samples of the dry plant material in a Goldfish extractor. (See appendix 10.)

Mosaic infected leaves contained more crude fats than did healthy leaves, as is evident from the data in table 8. The normal leaves contained 1.71 percent while the diseased leaves contained 2.31 percent. These results indicate an increase of 35.08 percent over the amount in the normal leaves.

c. Total nitrogen

The total nitrogen was determined by the Micro Kjeldahl procedure as described by Ma and Cussava (1942). Samples of the dry material ranging from 20 to 35 milligrams were used, which required about 4 to 5 milliliters of 0.02 N hydrochloric acid for the titration of the ammonia released. (See appendix 11.)

Table 9 presents the percentages of total nitrogen in the leaves. The normal leaves contained 4.66 percent nitrogen as compared to the 5.26 in the diseased leaves. The increase of 0.60 percent on the basis of the dry matter represents an increase of 12.8 percent over the amount in the healthy leaves taken as 100.

The observed increase in the percentage of total nitrogen in the mosaic diseased tobacco leaves is in agreement with data of previous investigators. Cordingley, Grainger, Pearsall and Wright (1934) reported an increase of approximately 10 percent in the total nitrogen content of their mosaic infected tobacco plants. The same results were obtained by Dunlap (1930). True and Hawkins (1918), however, reported a decrease in the percentage of total nitrogen in the leaves of blighted spinach.

According to Bawden (1943), the effect of the mosaic disease on the nitrogen content of the host plant is less clearly defined than the effect on the percentage of carbohydrates.

d. Discussion of organic constituents

The effects of the mosaic disease on the percentage of organic constituents in tobacco leaves can be separated into two classes: those causing an increase as exemplified by the total nitrogen and crude fats, and those bringing about a decrease as in the instance of carbohydrates.

Tables 10 and 11 present comparisons between the carbohydrate and total nitrogen content of healthy and mosaic infected leaves, together with the resultant changes in the carbon:nitrogen ratios.

Dunlap (1930) determined the carbohydrate:nitrogen ratios for a number of plants suffering from different diseases. A tabulation of his data showing the effects of mosaic disease on different plants follows:

Host	Ratios: Diseased plants/Healthy plants		
	Nitrogen	Carbohydrate	C/N Ratios
Tobacco	1.10	0.77	0.71
Tomato	1.09	0.81	0.76
Squash	1.15	0.86	0.76
Pokeweed	1.14	0.78	0.67
Pepper	1.03	0.93	0.88
Cucumber	1.07	0.75	0.70

A comparison of Dunlap's data with those in table 11 indicates a substantial agreement.

The observed accumulation of the nitrogenous compounds and the diminution of the carbohydrates in the virus infected tobacco leaves cannot be explained on the basis of changed rates of translocation and alterations in the permeability of the cell membranes to soluble constituents. The dilution factor introduced by the increase in the amount of total nitrogen would itself augment the difference in the carbon:nitrogen ratio.

III. EXPERIMENT 2

A. Purpose

The mosaic infected tobacco plants grown under field conditions exhibited marked physiological disturbances. The data obtained in experiment 1 showed that the disease caused a decrease in the total ash content and altered the ratios of individual constituents. The carbohydrate, fat and total nitrogen accumulation in the leaves was altered also. A hypothesis which suggested that the observed disturbances were brought about by an alteration in the electrical state of the protoplasmic colloids was proposed. This change of protoplasmic state is associated with a differential absorption of nutrient ions, causing alterations in their ratios in the tissues. The electrical nature of this disturbance at the site of absorption was suspected from the effects which the disease exerted on the accumulation of nutrients possessing different valence numbers.

The present experiment was arranged to present a more complete picture of the distribution of the different constituents in healthy and mosaic infected tobacco plants and especially to obtain further data pertinent to the hypothesis of electrical disturbances of the protoplasm induced by the disease. Hydroponic cultures were used since this procedure offered a better control over the conditions of growth of the experimental plants, and especially because the absorption of nutrients from solutions minimizes the participation of electrical forces involving absorption. When the absorbing root hair

is surrounded by nutrients in solution, the primary forces acting on the mineral uptake by the plant are those of permeability of the protoplasm itself. The necessity for the root hair to remove the ions from the electrically charged surfaces of the colloidal soil particles does not exist in the water cultures. The absorption of the nutrients is then a function of permeability of the absorbing cells rather than an electrical tug-of-war with the soil colloids.

B. Experimental methods and materials

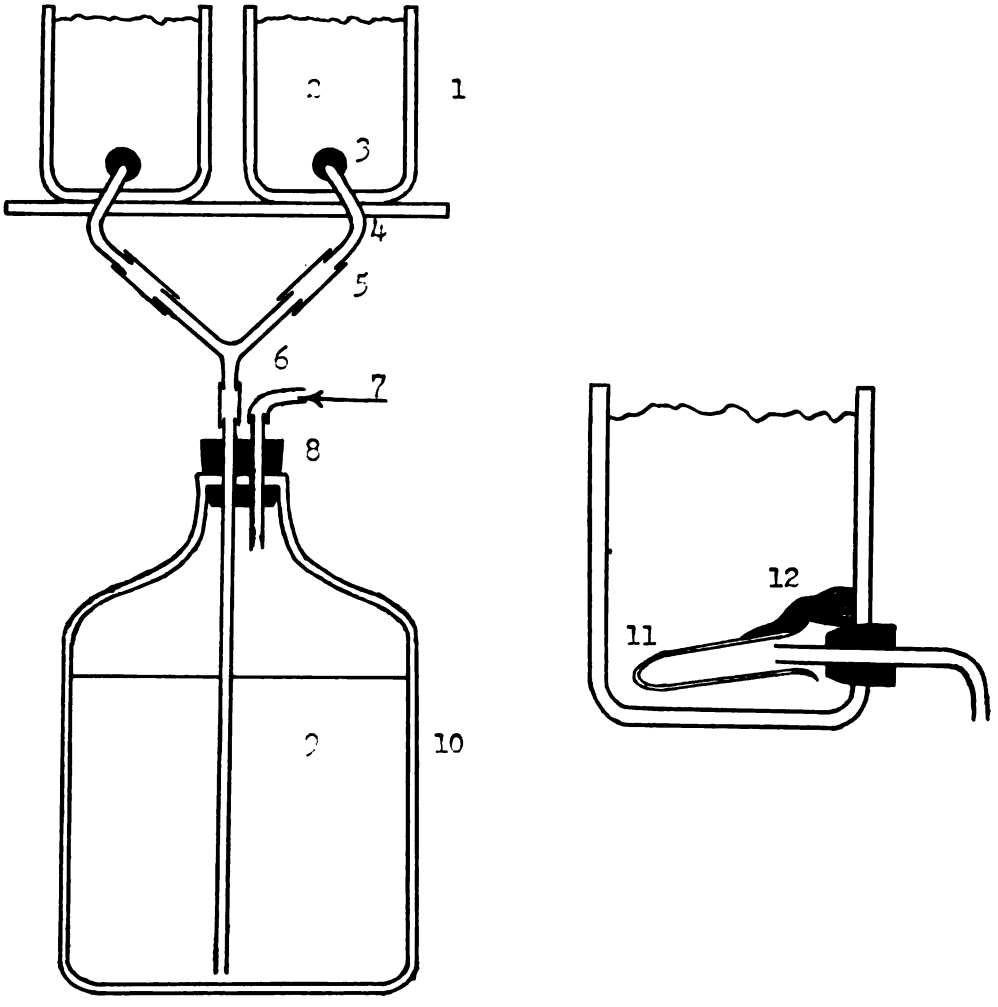
The plants studied in experiment 2 were grown in the greenhouse room No. 53 of the Plant Science Greenhouses at Michigan State College. The space available included a bench, twenty feet long and two feet wide. The room was equipped with an automatic ventilating system and with a temperature control set for 17-18° C.

The mechanical arrangement for the flooding of the culture pots periodically with nutrient solution is indicated in figure 1.

An air pump, activated every two hours by an electric time clock, flooded the culture pots every two hours. The level of the nutrient solution in the pots was controlled by a hydrostatic column at the end of the air pressure line. The culture pots were placed far enough apart to permit a sufficient illumination of the plants.

The nutrient solution recommended by Spencer (1941) was used. The final composition of the solution was as follows:

Figure 1. Arrangement of the culture pots



1, One-gallon glazed culture pot; 2, Quartz gravel, number 8 mesh; 3, Stopper, size No. 3; 4, L-shaped glass tubing; 5, Rubber tubing connection; 6, Y-shaped glass tube; 7, Air pressure line; 8, Stopper, size No. 12; 9, Nutrient solution, 16 liters; 10, Carboy, 20 liters capacity; 11, Test tube, six inches long; 12, Glass wool.

Salt	Grams per liter
$MgSO_4.7H_2O$	7.9
$Ca(NO_3)_2.4H_2O$	20.8
KH_2PO_4	14.9
$(NH_4)_2SO_4$	3.5

Concentrated stock solutions of each of the nutrient salts were prepared to have the following concentrations:

Salt	Milliliters of stock solution added per carboy
$MgSO_4.7H_2O$	64
$Ca(NO_3)_2.4H_2O$	176
KH_2PO_4	206
$(NH_4)_2SO_4$	53

The final volume in each carboy was made up to 16 liters with distilled water. Microelements were supplied by adding 10 milliliters per carboy of a stock solution having the following composition.

Salt	Grams per liter
H_3BO_3	4.56
$MnSO_4.H_2O$	3.762
$CuSO_4.5H_2O$	0.176

Iron was omitted from the solutions since previous experiments indicated that sufficient iron was obtained as contaminants. The final concentrations of the micro elements in the nutrient solution were as follows:

Element	Parts per million
Boron	0.5
Manganese	0.5
Copper	0.02

Nicotiana tobacum var. Havana No. 38 seeds were planted on April 1, 1950. The seeds were supplied through the courtesy of Dr. James Johnson of the University of Wisconsin. When the fifth leaf appeared, the plants were transplanted into the gravel pots. The average height at the time of transplanting was from 7 to 8 inches.

When the appearance of the plants showed that they had fully recovered from the transplanting, they were infected with tobacco mosaic virus by rubbing the two lower leaves of each plant with cheese cloth soaked in a virus preparation. The inoculations were performed on May 4, 1950. The inoculum was prepared by soaking the dry powdered diseased leaves in water, and then pressing the mass in a hydrolic press. The liquid obtained was preserved in the refrigerator until used to infect the experimental plants. The first visual symptoms appeared the fifth day after inoculation on the leaves of the growing tip. The water level of the different carboys was

kept constant by the addition of water every three days. The nutrient solutions were replaced the third week after transplanting.

The tobacco plants were harvested on June 14, 1950. By that date, they had attained an average height of 3 feet and at no time did they show nitrogen deficiency symptoms. Each plant was separated into leaves, stems, and roots. The roots were washed with tap water to remove adhering gravel particles. Only the fibrous roots were retained. The different samples were dried in the oven at 100° C. for 24 hours, and then ground in a micro-Wiley mill to pass a 60-mesh sieve. The powdered material was stored in air-tight containers until the analyses were carried out.

The methods of analyses employed in this experiment were identical to those used in experiment 1.

C. Experimental results

1. Inorganic constituents

a. Total ash

The percentages of total ash, expressed as carbonates, appear in table 12. The diseased plants contained more total ash than did the normal plants. This was true for the leaves, stems, and roots. This increase, calculated on the basis of the amount in the healthy plants, was 9.6 percent for the leaves, 1.4 percent for the stems, and 1.6 percent for the roots.

Table 13 presents the data obtained by calculating the percentages of the individual ash components in the total ash when the

total ash is obtained by summation of the individual components obtained by analysis. When calculated in this manner, the total ash in leaves of the diseased plants increased 26.11 percent, while it increased 63.85 percent in the stems, and 4.11 percent in the roots.

b. Silica

The determinations of silica yielded traces too small for accurate estimation. This situation might be expected since the only source of silica to the plants was the contact of the roots with the quartz gravel used to support the plants in the nutrient solution.

c. Calcium

The calcium content of mosaic leaves is slightly higher than that in the normal leaves when it is calculated as a percentage of the dry matter. The data in table 12 show that the normal leaves contained 2.34 percent while the infected leaves contained 2.40 percent. When the concentration of calcium is calculated as a percentage of the amount in the normal plants, this increase amounts to 2.6 percent over the normal value. If the calcium contents are calculated as percentages of the total ash, table 13 shows that in normal leaves the value is 63.89 percent, and in the diseased leaves, the percentage is 51.97 percent. This shows that the increase in the calcium content of the diseased leaves was not as great as the increase in total ash.

The data in table 12 show that the calcium content in the stems of healthy plants was 0.80 percent, when calculated on the basis of the dry plant material. The percentage in the diseased stems was 0.75. These values represent a decrease of 6.3 percent when calculated on the basis of the amount in normal stems. The data in table 13 show that in normal stem tissue, the percentage of calcium in the total ash was 28.16, while in the ash of the stems of diseased plants, the percentage was 16.11. This decrease, relative to the total ash, represents 42.79 percent of the concentration of calcium in the ash of normal stems. These results show that not only was the percentage of calcium in the dry tissue of the stems lessened, but also that this decrease occurred even though the total ash increased.

The data concerning the percentage of calcium in the dry root tissue are presented in table 12. The percentage in normal roots was 1.12 percent and in the roots of the diseased plants the percentage was 1.02. This decrease represents 8.9 percent of the calcium content of the roots of normal plants. Table 13 presents the data calculated as percentages of the total ash. In normal roots this value was 54.13 and in the roots of the diseased plants the value was 47.35 percent. This indicates that the ash of diseased roots contained 12.53 percent less calcium, which shows that the diminution of calcium in the roots occurred even though the total ash increased.

d. Magnesium

The magnesium content in the mosaic diseased leaves is higher than that in the the healthy leaves. The data presented in table 12 show that the healthy leaves contained 0.59 percent magnesium, calculated on the basis of the dry matter, while the concentration in the diseased leaves was found to be 0.73 percent. This increase represents a 23.7 percent increase over the magnesium content of the healthy leaves. Table 13 shows that when the magnesium was calculated as a percentage of the total ash, the value was 16.11 percent, while in the diseased leaves it was only 15.81 percent. This percent decrease of the amount of magnesium in the ash of the diseased leaves represents a 1.81 percent decrease in comparison to the value for normal leaves. These data show that, although the magnesium content of the plants was increased, the amount of this increase was less than that of the total ash.

The stems of diseased plants contained less magnesium than did the healthy stems. The data in table 12 indicate that the percentages in healthy and diseased stems were 0.61 and 0.48 respectively, calculated on the basis of the dry matter. This decrease in the diseased stems amounts to 21.3 percent of the amount in the stems of healthy plants. When magnesium is calculated as a percentage of the total ash, table 13 shows that the healthy stems contained 21.47 percent, while in the virus diseased material there was only 10.31 percent. This represents a 51.97 percent decrease, calculated on the

basis of the percentage in the ash.

More magnesium was found in diseased than in healthy roots. Table 12 shows that the magnesium content of the healthy roots was 0.42 percent of the dry matter. The diseased roots contained 0.48 percent. This increase amounts to 14.3 percent of the amount of magnesium in the healthy roots. When the magnesium in the roots was calculated on the basis of the total ash, the value for healthy roots was 20.30 percent, while that for the diseased roots was 22.28 percent. This increase represents only 5.91 percent over the content in the ash of healthy roots. The differences in the amount of increase of magnesium calculated on the basis of the dry matter and on the basis of the total ash indicate that, although the magnesium content of diseased roots was increased by the disease, the amount of this increase was less than that of the total ash.

e. Potassium

The percentages of potassium in the healthy and diseased plants are presented in table 12. The leaves, stems, and roots of the diseased plants contained more potassium than did the healthy corresponding material. Healthy leaves contained 0.59 percent potassium in the dry matter, while the diseased leaves contained 1.34 percent. This increase represents a 127.1 percent over the amount in the healthy leaves. When the potassium content is calculated as a percentage of the total ash, the healthy and diseased leaves contained

16.11 and 29.01 percent, respectively. This increase of the potassium in the ash of the diseased leaves, as shown in table 13, represents a 80.07 percent increase over that in the healthy leaves. The data presented indicate that the absorption of potassium was increased by the disease, but the increase in the total ash was of smaller magnitude. This shows that the effect of the disease on the absorption of potassium was greater than on the total ash.

Table 12 shows that the stems of the healthy plants contained 1.31 percent potassium in the dry matter and the diseased stems contained 3.33 percent! This conspicuous increase amounts to 154.2 percent over the potassium content of the healthy stems. When the amount of potassium was calculated on the basis of the total ash, the healthy stems contained 46.11 percent, as is indicated in table 13, and the diseased material contained 71.53 percent. This increase over the content in healthy stems amounts to 55.13 percent. The data show that the disease induced a greater absorption of potassium, and that the magnitude of this increase was relatively greater than the increase in the total ash.

The percentage of potassium in the roots is presented in table 12. The healthy roots contained 0.36 percent in the dry matter while the diseased roots contained 0.48 percent. The observed increase in the potassium content in the roots of diseased plants was 33.3 percent over the corresponding value for the healthy roots. When the data are calculated on the basis of the total ash, the

healthy roots contained 17.40 percent and the diseased roots contained 22.14 percent. This represent an increase of 4.05 percent over the value obtained for the healthy roots. The data show that the virus infection caused a greater accumulation of potassium in the roots, but that this increase was not as relatively great as the increase in the total ash.

f. Sodium

The data concerning the sodium contents of healthy and diseased plants are presented in tables 12 and 13. Table 12 shows that the sodium in healthy leaves was present in the concentration of 0.09? percent of the dry matter, while in diseased leaves there was 0.0?4 percent. This small increase in the amount of sodium in the diseased leaves amounts to 1.1 percent of the amount in healthy leaves. The sodium is expressed as a percentage of the total ash in table 13. The healthy leaves contained 2.54 percent, while the diseased leaves contained 2.04 percent of the ash. This decrease in the sodium content of the ash of diseased leaves is 19.6 percent of that in healthy leaves. The data reported show that the percentage of sodium in the dry matter of the leaves was not significantly affected by the virus disease, although its ratio to the total ash was greatly lessened.

The sodium content of the healthy stems, indicated in table 12, was 0.10? percent, and in the diseased stems the sodium represented 0.0?? percent of the dry matter. The decrease of the sodium content

induced by the disease was 21.9 percent in respect to the amount in stems of healthy plants. When the concentrations of sodium found in the healthy and in the infected stems are expressed as percentages of the total ash, the value for healthy stems was 3.70 percent and that for diseased stems was 1.76 percent, as is shown by the data assembled in table 13. The decrease of sodium in the ash of the diseased stems is equivalent to 52.43 percent of the value obtained for the healthy stems. The data indicate, therefore, that the virus disease inhibited the accumulation of sodium in the stems, even though the total ash in the stems increased.

Table 12 shows that the healthy roots contained 0.106 percent of sodium and the diseased roots 0.094 percent when calculated on the basis of the dry matter. This decrease represents 11.3 percent of the content in the dry matter of the healthy roots. Table 13 presents the concentrations of sodium expressed in terms of the total ash. When calculated on this basis, the healthy roots contained 5.12 percent and the diseased roots contained 4.36 percent. This decrease in the sodium content in the ash of the roots is equal to 14.84 percent of the value obtained for healthy roots. These data show that the disease inhibited the accumulation of sodium in the roots, although the total ash was slightly augmented.

g. Iron

The iron content of the healthy leaves, presented in table 12, was 0.035 percent in the dry matter and the diseased leaves contained

0.041 percent. This percentage increase is equal to 17.1 over the content of healthy leaves. In table 13 the percentages of iron are expressed on the basis of the total ash. Calculated on this basis, the healthy leaves contained 0.96 percent while the diseased leaves contained 0.89 percent. This decrease is equal to 7.29 percent of the value obtained for healthy leaves. These results indicate that the increased iron content of the diseased leaves was of smaller magnitude than the percentage increase of the total ash.

In normal stems, the iron was present in the concentration of 0.013 percent of the dry matter, as shown in table 12, while the diseased stems contained 0.010 percent. This decrease amounts to 23.1 percent of the normal value. The content of iron in the stems, calculated as percentages of the total ash, are presented in table 13. The healthy stems contained 0.46 percent and diseased stems 0.21 percent. This decrease is equal to 54.34 percent of the value obtained for the ash of normal stems. The data indicate that the amount of iron in the stems was decreased by the disease, even though the total ash increased.

The iron content of normal roots was equal to 0.030 percent of the dry matter, as is indicated in table 12. The diseased roots contained 0.041 percent. This increase of iron content in the dry matter of the diseased roots is equal to 36.7 percent of the amount found in the roots of normal plants. When the iron content in the total ash was calculated, the ash of healthy roots contained 1.45

percent and that of the diseased roots contained 1.90 percent,as is shown by the data in table 13. This increase is equal to 31.03 percent of the value obtained for the ash of healthy roots. The percentages increase calculated on the basis of dry matter and total ash indicate that the rate of iron increase in the diseased roots is of the same order as that of the total ash,although it is true that the percentage increase of the iron itself was a little greater than was the percentage increase of the total iron.

h. Manganese

The manganese content of the healthy and virus diseased plants are given by the tables 12 and 13. The percentage in healthy leaves calculated on the basis of dry matter,was 0.014,while that in the diseased leaves was0.013 percent. This decrease amounts to 7.1 percent of the value for healthy leaves. When the manganese is calculated as a percentage of the total ash,as indicated in table 13, the value for the manganese of healthy leaves was 0.3 percent,and that for the diseased leaves 0.24 percent.This decrease was equivalent to 26.32 percent of the value obtained from the healthy material. It is apparent from the results reported,that the accumulation of manganese in the leaves is only slightly, or perhaps insignificantly, lessened by the disease, while the percentage of total ash was significantly increased.

The manganese content of healthy and diseased stems,expressed as percentages of the dry matter, was 0.013 in both cases, but when

the manganese is calculated on the basis of total ash, the value for healthy stems was 0.11 percent and that for diseased stems was 0.06 percent. This decrease in the percentage of manganese in the ash of the diseased stems corresponds to 45.45 percent of the manganese in the ash of healthy stems and shows that the amount of manganese in the stems was not affected by the disease, even though the total ash augmented.

The roots of healthy plants contained 0.033 percent manganese on the basis of the dry matter. The diseased roots, as indicated in table 12, contained 0.039 percent. These results represent a 18.2 percent increase in the roots of the diseased plants. The manganese in healthy roots, calculated as a percentage of the total ash, was 1.59 while that in the ash of diseased roots was 1.81. This increase is equivalent to 13.84 percent of the amount in the ash of healthy roots. These results show that both manganese and total ash were increased in the roots by the virus disease, but that the increase in the ash was relatively a little greater.

i. Phosphorus fractions

(1) Total phosphorus

The virus infection decreased the total phosphorus concentrations in the leaves, stems, and roots of the tobacco plants. Table 14 shows that the leaves, stems, and roots of healthy plants contained 0.862, 0.432, and 1.355 percent phosphorus in the dry matter, respectively, while the values for the diseased plants were 0.690, 0.381, and 1.177

percent. The percentage decrease in the leaves was 19.95, in the stems 11.81, and in the roots 13.14, when the data are calculated in terms of the corresponding values for normal plants.

(2) Inorganic phosphorus

The virus infection exerted the same qualitative effect on the amounts of inorganic phosphorus as on the total phosphorus. In the healthy plants, as indicated in table 14, there was 0.483, 0.207, and 0.905 percent inorganic phosphorus in the dry matter of the leaves, stems, and roots respectively. The corresponding contents of inorganic phosphorus in the diseased plants were 0.357, 0.158, and 0.820 respectively. The percentage decreases amounted to 26.08, 23.67, and 9.39 percent when calculated on the basis of the amounts in the corresponding parts of healthy plants.

Table 15 presents the ratios of the amount of inorganic phosphorus to the total phosphorus. The data show that the virus disease lessened the amounts of total phosphorus and of inorganic phosphorus in the leaves, stems and roots. The relative decrease of the inorganic phosphorus in the leaves and stems is greater than that of the total phosphorus, while the opposite is true in the roots.

(3) Labile phosphate esters

The labile phosphate ester fraction in the leaves, stems, and roots of healthy and diseased plants contained only traces of phosphorus, as is indicated in table 14.

(4) Resistant phosphate esters

The virus disease decreased the resistant phosphate esters in the leaves, stems, and roots, as is shown by the data arranged in table 14. The percentages of phosphorus present in this form in the healthy leaves, stems, and roots were 0.263, 0.179, and 0.327 percent of dry matter, respectively. The corresponding concentrations in the diseased plants were 0.165, 0.157, and 0.260 percent. The decreases of the resistant phosphate esters fraction appear in table 14, and they were found to be 36.50, 12.29, and 20.49 percent for leaves, stems, and roots, respectively.

The ratios among the different fractions of phosphorus appear in table 15. The magnitude of the decrease of the resistant esters fraction was greater than that of the inorganic phosphorus fraction for the leaves and roots of the mosaic infected plants, while the opposite was true for the stems. The ratios of resistant esters phosphorus to the total phosphorus are smaller in all the three parts of the diseased plants than in the corresponding parts of healthy plants.

(5) Residual phosphate esters

The virus infected tobacco plants contained more phosphorus in the residual esters form than did the healthy plants. Table 14 shows that the healthy leaves, stems, and roots contained, respectively, 0.125, 0.031, and 0.111 percent phosphorus in the residual

esters form in the dry matter. The diseased plants contained in their leaves, stems, and roots 0.138, 0.040, and 0.139 percent in the dry matter. These increases amount to 10.40, 29.03, and 25.23 percent over the corresponding values obtained from healthy leaves, stems, and roots.

The ratios between the different phosphorus fractions, as indicated in table 15, show that the ratios of inorganic to residual phosphorus, and the ratios of resistant esters to residual phosphorus are smaller in the diseased than in the healthy plants. There is an increase, however, in the ratio of the residual to the total phosphorus in the diseased tissue.

(6) Discussion of phosphorus fractions

The data for the different phosphorus fractions, presented in tables 14 and 15, show that the percentage recovery of the total phosphorus in the fractions varies from 93.4 to 103.4 percent of the amount determined as "total phosphorus." This degree of recovery is satisfactory in view of the analytical problems which the fractionation procedure imposed.

The data presented show that the virus disease decreased not only the total absorption of phosphorus, but also the amount in the inorganic and resistant phosphate esters forms. The residual phosphorus fraction, on the other hand, markedly increased. There was an insignificant amount of phosphorus in the labile esters fraction in all tissues of both the healthy and the mosaic diseased plants. The

actual increase of the residual phosphate fraction, while the concentrations of the total, inorganic, and resistant esters phosphorus were decreasing, is consistent with the phosphoproteinous nature of the virus particles themselves. It may be assumed that the virus particles were formed at the expense of the other phosphorus fractions. The actual decrease of the total phosphorus should not be overlooked, in view of the altered absorption and permeability of the protoplasm, induced by the virus disease.

The maximum absolute increase of the phosphorus in the residual fraction, induced by the disease, occurred in the roots. The actual increase was 0.028 percent on the dry matter basis. This value represents 30.4 percent of the decrease of the inorganic fraction, and 39.3 percent of the resistant esters fraction. The theory that the increase of the residual fraction is attributable to the formation of virus particles is especially reasonable in view of these relationships.

j. Discussion of inorganic constituents

The data assembled in table 12 indicate that the virus disease stimulated to a small degree the accumulation of total ash. The greatest concentration of total ash, as well as the greatest increase, occurred in the leaves. The roots also exhibited these effects, but their magnitudes were smaller. The stem tissue exhibited the same qualitative effects, but to the least degree.

The potassium content of the diseased leaves, stems, and roots was markedly effected. All of these diseased tissues contained more

potassium than the corresponding healthy tissues. The accumulation of potassium is especially evident since the ratio of potassium to the total ash also increased, indicating that the magnitude of the increase of this element was greater than that of the other mineral constituents. The greatest absolute increase in the percentage of potassium occurred in the stems. A lesser increase occurred in the leaves, and a still smaller increase was detected in the roots. The marked accumulation of potassium in the stems may be related to the moisture content of the tissue. Potassium is mostly found in the plant tissue in a water soluble state and it can be leached almost quantitatively from the tissue. The stems contained the highest percentage of moisture which may account for the great accumulation of potassium observed. A positive correlation between moisture content and the concentration of potassium would presume that the percentage of potassium in the tissues studied would follow the order stems, leaves, and roots. The data in table 12 substantiate this presumption.

The effect of the virus disease on the sodium accumulation was less in the leaves than in the other tissues. The stems and roots of the diseased plants contained less sodium than did similar healthy tissues. There appears to be a reciprocal response of potassium and sodium to the virus disease. A consideration of the magnitude of these responses indicates, however, that the reciprocal relationship is qualitative only, rather than strictly quantitative.

The concentrations of calcium and magnesium were both higher in the diseased leaves and stems, but a reciprocal response was detected in the roots. The concentrations of calcium were more markedly affected than were those of magnesium.

Iron and manganese were accumulated to a greater degree in the diseased than in the healthy roots. This increase is indicated by their percentages in the dry matter and also by their percentages in the total ash. The iron in the stems was decreased by the disease, while it was increased in the leaves. The response of manganese was completely different. No effect was detected in the accumulation of manganese in the stems, while the leaves contained less manganese on the dry matter basis and in the total ash. The actual accumulation of iron and manganese in the roots at the expense of the tops suggests an inhibition of the translocation of these elements caused by the disease.

The relationship of the valence of the ions to the degree to which their accumulation in the leaves was affected by the disease was not observed in this experiment.

2. Organic components

a. Carbohydrates

The carbohydrate contents of the healthy and diseased plants appear in table 19. The healthy leaves, stems, and roots contained 19.01, 19.37, and 11.8% percent of the total, acid hydrolyzable carbohydrates in the dry matter. The percentage of carbohydrates in

the diseased plants were 17.15, 13.16, and 12.60 percent in the leaves, stems, and roots respectively. These values indicate that the infected roots contained more carbohydrates than did the healthy roots. The opposite effect was produced by the disease on the accumulation of carbohydrates in the leaves and stems of the tobacco plants. The same effects are indicated in table 22 where the ratios of the carbohydrates in the diseased plants to the amounts in healthy plants are reported.

b. Fats

The virus infection produced the same qualitative effect on the accumulation of total fats in the different parts of the tobacco plants as on the carbohydrate content. Table 19 shows that the healthy leaves, stems, and roots contained 7.81, 3.38, and 2.34 percent crude fat in the dry matter, respectively. The corresponding fat contents of the diseased plants were 5.93, 2.95, and 2.47 percent.

c. Nitrogen

The diseased leaves and roots contained more total nitrogen than did the healthy corresponding tissues, while the diseased stems contained less. The data are presented in table 20. The percent nitrogen in the leaves, stems, and roots of healthy plants were 4.27, 2.80, and 3.67 respectively. The corresponding percentages of nitrogen in the diseased leaves, stems, and roots were 4.77, 2.56, and 3.88 percent of the dry matter.

The ratios of carbohydrates to total nitrogen, so often reported in the literature as an indication of the nutritional state of the plants, are reported in table 21. The ratios for healthy leaves and stems were 4.217 and 6.561, while that of roots was 3.226. In the diseased leaves, stems, and roots the ratios were 3.595, 7.904, and 3.247. The C:N ratios in the healthy and diseased plants are compared in table 22.

d. Discussion of organic components

The concentrations of carbohydrate and fat in the leaves and stems were lessened as a result of the virus disease, while their concentrations in the roots were increased. The total nitrogen increased in the roots and leaves of the infected plants, while it decreased in the stems.

The ratios of carbohydrate to nitrogen, presented in table 21, follow the general trends described in the literature. The mosaic infection decreases the ratio in tobacco leaves, according to Dunlap (1930). The absence of similar data for the stems and roots does not permit us to compare tables 21 and 22 with published data. The ratios of nitrogen, carbohydrate and carbohydrate:nitrogen ratio of the diseased and healthy plants are given in table 22.

IV. GENERAL DISCUSSION

The development of the tobacco mosaic disease is accompanied by pronounced physiological disturbances in the plant. The nature of these induced abnormalities is related to the virus particle itself, to the physiology of the host, and to the nutritional environment in which the plant is growing.

The first attempt to unify the published data on the nature of the physiological disturbances caused by virus diseases and to explain some of the discrepancies in the results obtained by various investigators was the review of Wynd (1943). This critical review emphasized the importance of the alteration of the normal permeability of the cytoplasm or of its membranes as a result of the disease.

The data obtained in the present study show that the nutritional conditions under which the plants were grown were of primary importance in verifying the effects of the disease on the absorption of nutrients, and they also suggest a possible mechanism by which these disturbances were brought about.

The nature of the disturbances in the accumulation of phosphorus in the different fractions differed under different nutritional conditions. The total phosphorus, expressed as a percentage of the dry tissue, increased when the plants were grown in soil, but decreased when the plants were grown in nutrient solutions. This shows that the effect of the disease on the accumulation of total phosphorus was not an intrinsic feature of the disease itself, but that it depended

on the nature of the source of the available phosphorus.

The inorganic and labile esters increased in respect to the dry weight in the plants grown in soil, but decreased in the plants grown in nutrient solutions. There were significant amounts of phosphorus in the form of labile esters in the plants grown in soil, but only traces were found in those grown in solutions. Consequently, a comparison of the effects of the disease under the different conditions was not possible.

Although the analytic data for the resistant esters obtained in experiment 1 did not check as closely as might be desired, the data do show that the percentage of phosphorus in this form was less in the dry matter of the plants grown under both nutritional conditions. The percentage of phosphorus in the residual esters increased under both nutritional conditions. Since the resistant esters and residual esters were the only fractions of phosphorus which were affected by the disease in the same manner under the different conditions of culture, it may be assumed that the disturbances of these fractions are intrinsic features of the disease.

The increase of the residual phosphorus fraction, representing the phosphoproteins and phospholipids, at the expense of the resistant esters fraction, chiefly represented by the hexosephosphates and triosephosphates, shows that the disease facilitated the formation of the residual phosphorus compounds at the expense of the resistant esters fraction. Since it is known that the tobacco mosaic particle

consists of phosphoprotein, represented in the residual phosphorus fraction, it seems probable that the phosphorus incorporated in the virus particle is supplied to it by the monosaccharide phosphate esters.

The concept that the monosaccharide esters furnish the phosphorus utilized in the formation of the virus particle implies that those tissues of the diseased plants exhibiting similar and prominent changes in the ratio of the residual to the total phosphorus are the tissues involved in the reproduction of the virus. Examination of the data reported shows such changes to have occurred in the leaves and roots of the diseased plants, but only a minor change in this ratio was detected in the stem. This situation suggests that the virus particles are formed in the roots as well as in the leaves. The fact that virus reproduction occurs only in actively metabolizing cells is not inconsistent with this possibility, since the total number of cells in the meristematic areas of all the roots probably exceed in number those found in the tip of the growing stem.

The positive correlation observed between the increase of the residual phosphorus fraction and total phosphorus does not exist between residual phosphorus and the carbohydrates, fats, and total nitrogen! This situation indicates the especial importance of an adequate supply of appropriate phosphorus groups in the process of virus formation.

The data concerning the amounts of inorganic constituents in the tissues of healthy and diseased plants exhibit striking differences

in the plants grown under the two nutritional conditions. Certain data have been regrouped in table 23 in order to emphasize these differences.

Thedata in table 23 show that only two mineral constituents exhibited an increased concentration in the dry matter of the diseased leaves of plants growing in soil. These components were sodium and manganese. The concentrations of the remaining nutrients were decreased. The data based on the plants grown in solution cultures show that all constituents,calculated as percentages of the dry matter, increased with the exception of manganese.

The data for the soil-grown plants illustrate a positive relationship between the degree of the disturbance and the valence of the ion concerned. This relationship between valence and degree of disturbance by the disease is not evident in the data based on the plants grown in nutrient solutions.

The above mentioned qualitative and quantitative differences in the disturbances of the absorption and accumulation of the nutrients in the tobacco plants growing under field conditions and in nutrient cultures suggest that the physiological problem of obtaining nutrients is different under these two conditions.

The roots obtain metallic ions from the surfaces of the electrically charged soil colloids, and must therefore remove the ions from an electric field, while in solution cultures this aspect of absorption is absent. In other words, absorption of ions by plants growing in soil is affected by protoplasmic factors of permeability and accumulation,

and also by the electric problem imposed externally by the soil colloids. In solution cultures, on the other hand, the external electric problem is minimized. It is reasonable to assume, therefore, that the observed differences in the effects of the disease on the absorption of nutrients by plants grown in soil and in solutions could be attributed to the difference in the physiological problem involved. Since the effect of the disease on plants grown in soil can be correlated with the valence of the ions, but not so with plants grown in solution, it seems logical that the virus disease has altered the ability of the absorbing cells to compete with the electrical forces of the soil colloids.

One may only wonder what are the changes that the virus disease induced in the protoplasm of the absorbing cells which alter their relationship to the electrical tug-of-war involved in removing ions from the soil colloids. But since the problem appears to be electric in nature, we may only assume that the electric property of the cell colloids has been affected by the disease. The unavoidable result of any change in the state of the protoplasmic colloids on the over-all phenomena of permeability, translocation, and accumulation represents one of the most fundamental effects of virus disease on plants.

V. SUMMARY AND CONCLUSIONS

1. Healthy and mosaic infected plants were grown under field conditions and in nutrient solutions. The dry, powdered plant material was analyzed for total ash, silica, calcium, manganese, magnesium, potassium, sodium, iron, total phosphorus, inorganic phosphorus, labile phosphate esters, resistant phosphate esters, residual phosphorus, total hydrolyzable carbohydrate, crude fat, and total nitrogen.

2. The relationships between the different phosphorus fractions indicated that the phosphate component necessary for the formation of the virus particles was derived from the resistant phosphate esters represented by such compounds as hexosephosphate, triosephosphate, pyruvic acid phosphate, and glyceric acid phosphate. The amount of residual phospho-compounds, represented by phosphoproteins and phospholipids, was positively related to the levels of the total phosphorus concentration. The above mentioned relationships strongly suggested that the multiplication of virus protein took place in the actively metabolising cells of the root as well as in those of the growing shoot.

3. The absorption, translocation and accumulation of the cations were affected differently by the mosaic infection of plants growing in the soil and in nutrient solutions. The degree of the disturbance in the absorption of cations by plants growing in the soil was related to the valence of the cations concerned. This influence of the valence was not observed in the plants grown in nutrient solutions.

Since the absorption of nutrients from the soil involves the competition of the absorbing cells with the electrical forces residing on the surfaces of the soil colloids, it is suggested that the disturbed absorption of cations by tobacco plants grown in soil resulted from an altered electrical state of the protoplasmic colloids.

4. The organic components represented by carbohydrate, fat, and total nitrogen responded differently to the mosaic disease in the plants grown in soil and in nutrient solutions.

5. The theory proposed by Wynd that virus diseases profoundly alter the permeability of the cytoplasm, or of its membranes, was verified.

VI. LITERATURE CITED

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Table 1. Chemical constituents in healthy and mosaic infected tobacco leaves grown under field conditions, expressed as percentages of dry matter.

Constituent	Healthy Leaves			Mosaic Infected Leaves			Difference relative to healthy	Percentage difference relative to healthy
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.		
Total ash[1]	23.67	23.52	23.60	16.80	16.86	16.86	-6.74	-28.55
Silica (SiO_2)	4.89	5.03	4.94	1.00	0.99	1.00	-3.94	-79.75
Calcium	3.74	3.66	3.70	2.85	3.02	2.94	-0.76	-20.54
Magnesium	0.70	0.72	0.71	0.43	0.42	0.43	-0.28	-39.44
Potassium	3.66	3.60	3.63	3.33	3.33	3.33	-0.30	- 8.26
Sodium	0.055	0.059	0.057	0.055	0.060	0.058	+0.001	+ 1.75
Iron	0.110	0.102	0.106	0.052	0.049	0.051	-0.055	-51.88
Manganese	0.018	0.020	0.019	0.026	0.026	0.026	+0.007	+36.84

[1] Total ash as carbonates.

Table 2. Chemical constituents in healthy and mosaic infected tobacco leaves grown under field conditions, expressed as percentages of total ash. The total ash obtained by addition of individual components.

Constituent	Healthy Leaves	Mosaic Infected Leaves	Difference relative to healthy	Percentage Difference relative to healthy
Total ash	13.162	7.833	- 5.329	-40.48
Silica	37.53	12.70	-24.83	-66.16
Calcium	28.11	37.52	+ 9.41	+33.48
Magnesium	5.39	5.48	+ 0.09	+ 1.67
Potassium	27.59	42.50	+ 14.92	+54.09
Sodium	0.43	0.74	+ 0.31	+72.09
Iron	0.81	0.65	- 0.16	-19.75
Manganese	0.14	0.33	+ 0.19	+135.7

Table 3. Chemical constituents in healthy and mosaic infected tobacco leaves, grown under field conditions, expressed as milliequivalents per 100 grams dry matter.

Constituent	Healthy Leaves			Mosaic Infected Leaves		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Calcium	184.6	192.6	184.6	142.2	150.7	146.5
Magnesium	57.6	59.2	58.4	34.5	35.4	35.0
Potassium	93.6	92.1	92.9	85.2	85.2	85.2
Sodium	2.39	2.57	2.48	2.39	2.61	2.50
Iron[1]	5.91	5.48	5.70	2.79	2.63	2.71
Manganese[2]	0.66	0.73	0.70	0.95	0.95	0.95
Phosphorus	30.78	30.88	30.83	31.66	33.59	32.63
Nitrogen[3]	-----	-----	338.9	-----	-----	375.7

1 As ferric.
2 As manganous.
3 Average based on five determinations.

Table 4. Phosphorus fractions in healthy and mosaic infected leaves, grown under field conditions, expressed as percentages of dry matter.

Fraction	Healthy Leaves			Mosaic Infected Leaves			Difference relative to healthy	Percentage Difference relative to healthy
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.		
Total phosphorus	0.318	0.319	0.319	0.327	0.347	0.337	+0.018	+5.64
Total phosphorus by addition of fractions	0.293	0.323	0.308	0.365	0.365	0.365	-----	-----
Percent recovery of total phosphorus in fractions	91.8	101.2	96.5	108.3	108.3	108.3	-----	-----
Inorganic phosphorus	0.187	0.182	0.185	0.204	0.208	0.206	+ 0.021	+11.35
Labile esters	0.025	0.026	0.026	0.035	0.034	0.035	+ 0.009	+34.62
Resistant esters	0.046	-----	0.046	0.048	0.038	0.043	-0.003	- 6.52
Residual phosphorus	0.035	0.069	0.052	0.078	0.085	0.082	+ 0.030	+57.69

Table 5. Ratio between phosphorus fractions, based on percentages in dry matter, of healthy and mosaic infected tobacco leaves, grown under field conditions.

Ratio	Healthy Leaves	Mosaic Infected Leaves
Inorganic/Labile Esters	7.12	5.88
Inorganic/Resistant Esters	4.02	4.79
Inorganic/Residual	3.55	2.51
Inorganic/Total	0.58	0.61
Labile Esters/Resistant Esters	0.565	0.814
Labile Esters/Residual	0.500	0.426
Labile Esters/Total	0.082	0.104
Resistant Esters/Residual	0.884	0.524
Resistant Esters/Total	0.144	0.128
Residual/Total	0.163	0.243

Table 6. Ratio of milliequivalents of chemical constituents per 100 grams dry matter in healthy and mosaic infected tobacco leaves grown under field conditions.

Ratio	Healthy Leaves	Mosaic Infected Leaves
K:Na	37.46	34.08
K:Ca	0.503	0.582
K:Mg	1.59	2.43
K:Mn	132.7	89.7
K:Fe	16.30	31.44
K:Total Cations	0.269	0.312
Na:Ca	0.0134	0.0171
Na:Mg	0.0425	0.071
Na:Mn	3.54	2.63
Na:Fe	0.435	0.922
Na:Total Cations	0.0072	0.0092
Ca:Mg	3.16	4.19
Ca:Mn	263.7	154.2
Ca:Fe	32.39	54.06
Ca:Total Cations	0.535	0.537
Mg:Mn	83.43	36.84
Mg:Fe	10.25	12.92
Mg:Total Cations	0.169	0.128
Mn:Fe	0.123	0.351
Mn:Total Cations	0.00203	0.00348
Fe:Total Cations	0.0165	0.0099
Monovalent:Divalent	0.483	0.604
Monovalent:Total Cations	0.277	0.321
Divalent:Total Cations	0.664	0.618

Table 7. Ratio of percentages of constituents in dry matter of healthy and mosaic infected tobacco leaves, grown under field conditions. Total ash obtained by addition of individual constituents.

Ratio	Healthy Leaves	Mosaic Infected Leaves
K:Na	63.68	57.41
K:Ca	0.981	1.133
K:Mg	5.113	7.744
K:Mn	191.1	128.1
K:Fe	34.25	65.29
K:SiO_2	0.735	3.33
K:Total ash	0.276	0.425
Na:Ca	0.015	0.020
Na:Mg	0.080	0.135
Na:Mn	3.000	2.231
Na:Fe	0.538	1.137
Na:SiO_2	0.012	0.058
Na:Total ash	0.0043	0.0074
Ca:Mg	5.211	6.837
Ca:Mn	194.7	113.1
Ca:Fe	34.91	57.60
Ca:SiO_2	0.748	2.94
Ca:Total ash	0.281	0.375
Mg:Mn	37.37	16.54
Mg:Fe	6.698	8.431
Mg:SiO_2	0.144	0.430
Mg:Total ash	0.054	0.055

Table 7. (Concluded)

Ratio	Healthy Leaves	Mosaic Infected Leaves
Mn:Fe	0.179	0.509
Mn:SiO_2	0.004	0.026
Mn:Total ash	0.0014	0.0033
Fe:SiO_2	0.021	0.051
Fe:Total ash	0.0081	0.0065
SiO_2:Total ash	0.375	0.128
Monovalent:Divalent	0.832	0.969
Monovalent:Total ash	0.280	0.432
Divalent:Total ash	0.336	0.446

Table 8. Crude fat and total carbohydrate in healthy and mosaic infected tobacco leaves grown under field conditions, expressed as percentages of dry matter.

Constituents	Healthy Leaves			Mosaic Infected Leaves		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Fats	1.84	1.57	1.71	2.32	2.30	2.31
Carbohydrates	11.52	11.25	11.38	10.54	10.15	10.35

Table 9. Total nitrogen in healthy and mosaic infected tobacco leaves grown under field conditions, expressed as percentages of dry matter.

Determination	Healthy Leaves	Mosaic Infected Leaves
1	4.91	5.21
2	4.63	5.31
3	4.61	5.07
4	4.64	5.11
5	4.63	5.61
Ave.	4.66	5.26

Table 10. Carbohydrate, nitrogen and C/N ratio in healthy and mosaic infected tobacco leaves grown under field conditions, based on percentages in dry matter.

Constituent	Healthy Leaves	Mosaic Infected Leaves
Carbohydrates	11.38	10.35
Total Nitrogen	4.66	5.26
Ratio: C/N	2.44	1.97

Table 11. Ratios of carbohydrate, nitrogen, and C/N ratio, based on percentage values, in diseased to that in healthy leaves, grown under field conditions.

Ratios: Diseased Leaves/Healthy Leaves		
Nitrogen	Carbohydrate	C/N Ratio
1.129	0.909	0.807

Table 12. Chemical constituents in healthy and mosaic infected tobacco plants grown in nutrient solutions, expressed as percentages of dry matter.

Constituents	Healthy Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Total ash[1]	17.19	16.72	16.96	13.77	14.08	13.93	13.73	13.86	13.80
Silica	Trace	Trace	-----	Trace	Trace	-----	Trace	Trace	-----
Calcium	2.35	2.33	2.34	0.81	0.79	0.80	1.07	1.17	1.12
Magnesium	0.57	0.61	0.59	0.61	0.60	0.61	0.42	----	0.42
Potassium	0.63	0.55	0.59	1.18	1.43	1.31	0.29	0.43	0.36
Sodium	0.094	0.092	0.093	0.110	0.100	0.105	0.106	0.106	0.106
Iron	0.034	0.036	0.035	0.013	0.013	0.013	0.029	0.031	0.030
Manganese	0.014	0.014	0.014	0.003	0.003	0.003	0.034	0.031	0.033

1 Total ash as carbonates.

Table 12. (Continued)

Constituents	Mosaic Infected Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Total ash[1]	18.60	18.58	18.59	14.22	14.02	14.12	14.00	14.04	14.02
Silica	Trace	Trace	-----	Trace	Trace	-----	Trace	Trace	-----
Calcium	2.35	2.45	2.40	0.76	0.74	0.75	1.06	0.97	1.02
Magnesium	0.74	0.72	0.73	0.43	0.52	0.48	0.49	0.47	0.48
Potassium	1.38	1.30	1.34	3.33	----	3.33	0.54	0.41	0.48
Sodium	0.094	0.094	0.094	0.080	0.084	0.082	0.094	0.094	0.094
Iron	0.037	0.044	0.041	0.009	0.011	0.010	0.043	0.040	0.041
Manganese	0.012	0.014	0.013	0.002	0.003	0.003	0.038	0.039	0.039

[1] Total ash as carbonates.

Table 12. (Concluded)

Constituents	Difference relative to healthy			Percentage difference relative to healthy		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Total ash[1]	+1.63	+0.19	+0.22	+9.6	+1.4	+1.6
Silica	----	----	----	---	---	---
Calcium	+0.06	-0.05	-0.10	+2.6	-6.3	-8.9
Magnesium	+0.14	-0.13	+0.06	+23.7	-21.3	+14.3
Potassium	+0.75	+2.02	+0.12	+127.1	+154.2	+33.3
Sodium	+0.001	-0.003	-0.012	+1.1	-21.9	-11.3
Iron	+0.006	-0.003	+0.011	+17.1	-23.1	+36.7
Manganese	-0.001	0.000	+0.006	-7.1	----	+18.2

[1] Total ash as carbonates.

Table 13. Chemical constituents in healthy and mosaic infected tobacco plants grown in nutrient solutions, expressed as percentages of total ash. The total ash obtained by addition of individual components.

Constituents	Healthy Plants			Mosaic Infected Plants			Difference relative to healthy			Percentage difference relative to healthy		
	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
Total ash	3.662	2.841	2.069	4.613	4.655	2.154	+ 0.956	+1.814	+0.085	+26.11	+63.85	+4.11
Calcium	63.89	28.16	54.13	51.97	16.11	47.35	-11.92	-12.05	-6.78	-18.65	-42.79	-12.53
Magnesium	16.11	21.47	20.30	15.81	10.31	22.23	- 0.30	-11.16	+1.18	- 1.86	-51.97	+5.81
Potassium	16.11	46.11	17.40	29.01	71.53	22.28	+12.90	+25.42	+4.88	+80.07	+55.13	+28.05
Sodium	2.54	3.70	5.12	2.04	1.76	4.36	- 0.50	- 1.94	-0.76	-19.68	-52.43	-14.84
Iron	0.96	0.46	1.45	0.89	0.21	1.90	- 0.07	- 0.25	+0.45	- 7.29	-54.34	+31.03
Manganese	0.38	0.11	1.59	0.28	0.06	1.81	- 0.10	- 0.05	+0.22	-26.32	-45.45	+13.84

Table 14. Phosphorus fractions of healthy and mosaic infected tobacco plants, grown in nutrient solutions, expressed as percentages of dry matter.

Fractions	Healthy Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Inorganic phosphorus	0.466	0.500	0.483	0.206	0.208	0.207	0.902	-----	0.905
Labile esters	Trace	Trace	-----	Trace	Trace	-----	Trace	Trace	-----
Resistant esters	0.289	0.246	0.263	0.184	0.174	0.179	0.327	-----	0.327
Residual phosphorus	0.134	0.115	0.125	0.033	0.028	0.031	0.111	-----	0.111
Total phosphorus	0.836	0.888	0.862	0.441	0.423	0.430	1.383	1.327	1.355
Total phosphorus by addition of fractions	0.889	0.861	0.871	0.423	0.410	0.417	1.343	-----	-----
Percent recovery of total phorphorus in fractions	102.1	99.9	101.0	97.9	94.9	96.4	99.1	-----	-----

Table 1. (Continued)

Fractions	Mosaic Infected Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Inorganic phosphorus	0.337	0.377	0.357	0.157	0.159	0.158	0.820	-----	0.820
Labile esters	Trace	Trace	-----	Trace	Trace	-----	Trace	Trace	-----
Resistant esters	0.099[1]	0.165	0.165	0.115[1]	0.157	0.157	0.260	-----	0.260
Residual phosphorus	0.141	0.135	0.138	0.040	0.039	0.040	0.139	-----	0.139
Total phosphorus	0.691	0.688	0.690	0.395	0.376	0.391	1.202	1.151	1.177
Total phosphorus by addition of fractions	0.577	0.677	0.627	0.312	0.355	0.334	1.219	-----	-----
Percent recovery of total phosphorus in fractions	83.7	98.1	-----[2]	82.1	93.4	-----[2]	103.4	-----	-----

[1] This value is probably erroneously low.
[2] Average omitted because of probable error in determination of resistant esters.

Table 14. (Concluded)

Fractions	Difference relative to healthy			Percentage difference relative to healthy		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Inorganic phosphorus	-0.126	-0.049	-0.085	-26.08	-23.67	- 9.39
Labile esters	-----	-----	-----	-----	-----	----
Resistant esters	-0.096	-0.022	-0.067	-36.50	-12.29	-20.49
Residual phosphorus	+0.013	+0.009	+0.028	+10.40	+29.03	+25.23
Total phosphorus	-0.172	-0.051	-0.178	-19.95	-11.81	-13.14
Total phosphorus by addition of fractions	-----	-----	-----	-----	-----	-----
Percent recovery of total phosphorus in fractions	-----	-----	-----	-----	-----	-----

Table 15. Ratio between phosphorus fractions based on percentage of dry matter of healthy and mosaic infected tobacco plants grown in nutrient solutions.

Ratio	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Inorganic/Resistant esters	1.837	1.156	2.767	2.164	1.006	3.154
Inorganic/Residual	3.884	6.677	8.153	2.586	3.959	5.899
Inorganic/Total	0.560	0.479	0.668	0.517	0.415	0.697
Resistant esters/Residual	2.104	5.774	2.946	1.196	3.925	1.871
Resistant esters/Total	0.305	0.414	0.241	0.239	0.412	0.221
Residual/Total	0.145	0.072	0.082	0.200	0.105	0.118

Table 16. Chemical constituents in healthy and mosaic infected tobacco plants grown in nutrient solutions, expressed as milliequivalents per 100 grams of dry matter.

Constituents	Healthy Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Calcium	117.3	116.3	116.8	40.4	39.4	39.9	53.4	58.4	55.9
Magnesium	46.9	50.2	48.6	50.2	49.3	49.8	34.5	----	34.5
Potassium	16.11	14.07	15.09	30.17	36.57	33.37	7.42	11.00	9.21
Sodium	4.09	4.00	4.05	4.78	4.35	4.57	4.61	4.61	4.61
Iron[1]	1.83	1.93	1.88	0.70	0.70	0.70	1.56	1.66	1.61
Manganese[2]	0.51	0.51	0.51	0.11	0.11	0.11	1.24	1.13	1.19
Phosphorus	90.9	85.9	83.4	42.7	40.9	41.8	133.9	128.5	131.2
Nitrogen[3]	-----	-----	305.0	-----	-----	200.0	------	------	262.1

1 As ferric.
2 As manganous.
3 Average based on five determinations.

Table 16. (Concluded)

Constituents	Mosaic Infected Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Calcium	117.3	122.3	119.8	37.9	36.9	37.4	52.9	48.4	50.7
Magnesium	60.9	59.2	60.1	35.3	39.5	37.4	40.3	39.7	39.5
Potassium	35.29	33.24	34.27	85.17	----	85.17	13.81	10.48	12.15
Sodium	4.09	4.09	4.09	3.48	3.65	3.57	4.09	4.09	4.09
Iron[1]	1.98	2.36	2.17	0.48	0.59	0.54	2.31	2.15	2.23
Manganese[2]	0.44	0.51	0.48	0.07	0.11	0.09	1.38	1.41	1.40
Phosphorus	66.9	66.6	66.8	37.3	36.4	36.9	116.4	111.4	113.9
Nitrogen[3]	-----	-----	340.7	-----	-----	192.7	------	------	277.1

1 As ferric.
2 As manganous.
3 Average based on five determinations.

Table 17. Ratio of percentages of constituents in dry matter of healthy and mosaic infected tobacco plants grown in nutrient solutions. Total ash obtained by addition of individual constituents.

Ratio	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
K:Na	6.34	12.48	3.40	14.25	40.61	5.11
K:Ca	0.25	1.64	0.32	0.56	4.44	0.47
K:Mg	1.00	2.15	0.86	1.34	6.93	1.00
K:Mn	42.1	436.6	10.9	103.1	1,110.0	12.3
K:Fe	168.6	100.8	12.0	32.7	333.0	11.7
K:Total ash	0.161	0.461	0.174	0.290	0.715	0.223
Na:Ca	0.040	0.131	0.095	0.039	0.109	0.922
Na:Mg	0.158	0.172	0.025	0.139	0.179	0.196
Na:Mn	6.64	35.00	3.21	7.23	27.33	2.41
Na:Fe	2.66	8.08	3.55	2.29	8.20	2.29
Na:Total ash	0.025	0.037	0.051	0.020	0.018	0.044
Ca:Mg	3.96	1.31	2.66	3.29	1.56	2.13
Ca:Mn	167.1	266.1	33.9	184.6	250.0	26.2
Ca:Fe	66.86	61.54	37.33	58.53	75.0	24.9
Ca:Total ash	0.639	0.281	0.541	0.519	0.161	0.474
Mg:Mn	42.14	203.33	12.73	56.15	160.0	12.3

Table 17. (Concluded)

Ratio	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Mg:Fe	16.86	46.92	14.00	17.9	48.0	11.7
Mg:Total ash	0.141	0.215	0.203	0.158	0.103	0.223
Mn:Fe	0.400	0.231	1.10	0.317	0.300	0.951
Mn:Total ash	0.003,8	0.001,1	0.015,9	0.002,8	0.000,6	0.018,1
Fe:Total ash	0.009,6	0.004,6	0.014,5	0.008,9	0.002,1	0.019,0
Monovalent:Divalent	0.290	1.001	0.296	0.456	2.767	0.373
Monovalent:Total ash	0.187	0.498	0.205	0.311	0.733	0.266
Divalent:Total ash	0.643	0.497	0.760	0.681	0.265	0.714

Table 12. Ratio of milliequivalents of chemical constituents per 100 grams dry matter in healthy and mosaic infected tobacco plants grown in nutrient solutions.

Ratio	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
K:Na	3.73	7.30	1.20	8.38	23.86	2.97
K:Ca	0.129	0.836	0.165	0.236	2.277	0.240
K:Mg	0.310	0.670	0.267	0.570	2.277	0.308
K:Mn	29.6	303.00	7.7	71.4	946.0	8.7
K:Fe	8.03	476.70	5.72	15.79	157.70	5.45
K:Total cations	0.081	0.260	0.086	0.155	0.519	0.110
Na:Ca	0.035	0.115	0.033	0.034	0.095	0.081
Na:Mg	0.083	0.092	0.134	0.681	0.095	0.104
Na:Mn	7.9	41.50	3.9	85.2	39.7	2.9
Na:Fe	2.15	6.53	2.86	1.88	6.61	1.83
Na:Total cations	0.017	0.036	0.043	0.019	0.022	0.03
Ca:Mg	2.40	0.80	1.62	1.99	1.00	1.28
Ca:Mn	229.0	362.7	47.0	249.6	415.6	36.2
Ca:Fe	62.1	57.0	34.7	55.2	69.3	22.7
Ca:Total cations	0.625	0.310	0.522	0.542	0.228	0.458
Mg:Mn	95.3	452.7	29.0	185.2	415.6	28.2

Table 1°. (Concluded)

Ratio	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Mg:Fe	25.9	71.1	21.4	27.7	69.3	17.7
Mg:Total cations	0.269	0.388	0.322	0.272	0.228	0.357
Mn:Fe	0.271	0.157	0.739	0.221	0.167	0.628
Mn:Total cations	0.002,73	0.000,86	0.011,19	0.002,17	0.000,06	0.012,65
Fe:Total cations	0.010,10	0.005,45	0.015,04	0.009,82	0.003,29	0.020,14
Monovalent:Divalent	0.115	0.422	0.151	0.213	1.185	0.177
Monovalent:Total cations	0.102	0.295	0.132	0.174	0.541	0.147
Divalent:Total cations	0.838	0.699	0.856	0.817	0.456	0.830

Table 19. Crude fat and total carbohydrate in healthy and mosaic infected tobacco plants grown in nutrient solutions, expressed as percentages of dry matter.

Constituent	Healthy Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Fat	7.75	7.87	7.81	3.24	3.53	3.39	2.28	2.40	2.34
Carbohydrate	17.80	18.23	18.01	18.98	17.97	18.37	11.84	----	11.84

Table 19. (Concluded)

Constituent	Mosaic Infected Plants								
	Leaves			Stems			Roots		
	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.	Det. 1	Det. 2	Ave.
Fat	5.94	5.92	5.93	2.75	3.15	2.95	2.40	2.53	2.47
Carbohydrate	17.34	16.93	17.15	17.97	18.29	18.16	12.60	12.60	12.60

Table 20. Total nitrogen in healthy and mosaic infected tobacco plants grown in nutrient solutions, expressed as percentages of dry matter.

Determination	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
1	4.28	2.35	3.71	4.71	2.57	3.91
2	4.31	2.20	3.61	4.85	2.62	3.96
3	4.25	2.28	3.74	4.74	2.53	3.88
4	4.21	2.32	3.69	4.77	2.62	3.86
5	4.32	2.25	3.60	----	2.48	3.78
Average	4.27	2.30	3.67	4.77	2.56	3.88

Table 21. Carbohydrate, nitrogen, and C/N ratios in healthy and mosaic infected tobacco plants, grown in nutrient solutions, based on percentages of dry matter.

Constituent	Healthy Plants			Mosaic Infected Plants		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Carbohydrates	18.01	18.37	11.84	17.15	18.16	12.60
Total Nitrogen	4.27	2.80	3.67	4.77	2.56	3.88
Ratio: C/N	4.217	6.561	3.226	3.595	7.904	3.247

Table 22. Ratios of carbohydrate, nitrogen, and C/N ratio, based on percentage values in diseased to that in healthy tobacco plants, grown in nutrient solutions.

Ratios: Diseased plants/Healthy plants								
Nitrogen			Carbohydrates			C/N		
Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
1.117	0.914	1.057	0.953	0.988	1.064	0.853	1.081	1.007

Table 23. Percentage disturbance of the concentrations of cations in the leaves of mosaic infected tobacco plants, listed in increasing order of magnitude.

Plants grown in soil				Plants grown in nutrient solution			
In dry matter		In total ash		In dry matter		In total ash	
Ion	Disturbance	Ion	Disturbance	Ion	Disturbance	Ion	Disturbance
Na	+ 1.75	Mg	+ 1.67	Na	+ 1.1	Mg	- 1.86
K	- 8.26	Fe	-19.75	Ca	+ 2.6	Fe	- 7.29
Ca	-20.54	Ca	+33.48	Mn	- 7.1	Ca	-13.65
Mn	+36.84	K	+54.09	Fe	+ 17.1	Na	-19.68
Mg	-39.44	Na	+ 72.09	Mg	+ 23.7	Mn	-26.32
Fe	-51.33	Mn	+135.7	K	+127.1	K	+90.07
Ash	-29.55	Ash	-40.48	Ash	+ 9.6	Ash	+26.11

VIII. APPENDIXES: ANALYTICAL METHODS

Appendix 1

DETERMINATION OF TOTAL ASH AS CARBONATES

REAGENTS

Ammonium carbonate. Saturated solution.

PROCEDURE

Add one-gram sample into pre-weighed platinum crucibles.

Place into a muffle oven and increase gradually the temperature until the samples start smoking. Ash at 650° C. for four hours. Cool and add about 1 milliliter of ammonium carbonate solution. Hold for half an hour at 100° C. and for two hours at 200° C. Cool and weigh. The increase in weight in grams times 100 gives the percentage total ash as carbonate.

Appendix 2

DETERMINATION OF SILICA BY THE [illegible] METHOD

REAGENTS

Hydrofluoric acid. Concentrated.

Sulfuric acid. Concentrated.

Nitric acid. 0.1 N.

PROCEDURE

To the ash obtained in the 'Total Ash Determination' add carefully 10 milliliters of 0.1 N nitric acid. Evaporate to dryness at intermediate heat on the hot plate. Cool, bring to constant weight.

To the ash add 5 milliliters of 0.1 N nitric acid, 1 milliliter of concentrated sulfuric acid and 5 milliliters of hydrofluoric acid. Warm on the hot plate at intermediate heat to decompose the silica and bring to dryness. Cool, bring to constant weight.

The decrease in weight expressed in milligrams times 100 represents the percentage of silica.

Appendix 3

SEMIMICRO DETERMINATION OF CALCIUM

REAGENTS

Ammonium oxalate. 4 percent solution.

Sodium hydroxide. 30 percent solution.

Hydrochloric acid. 1:15.

Ammonium hydroxide. 1:50.

Perchloric acid. 4 N. Dilute 340 milliliters of 70 percent perchloric acid to 1 liter with distilled water.

Sodium oxalate. 0.01 N standard solution.

Ammonium hexanitrato cerate mixture. 0.01 N ammonium hexanitrato cerate in 1 N $HClO_4$.

Dissolve 5.76 grams of "standard or reference purity" ammonium hexanitrato cerate in 250 milliliters of 4 N $HClO_4$ and dilute to 1 liter. The reagent should be standardized in the following manner: Pipet 5 or 10 milliliters of fresh standard 0.01 N sodium oxalate into a small beaker containing 5 milliliters of 4 N $HClO_4$, add 0.2 milliliter of nitro-ferroin indicator and titrate with the cerate solution to the colorless end point. Determine a blank titration correction on a similar sample minus the oxalate solution. The milliliters of oxalate used divided by the corrected milliliters of cerate times 0.01, provide the normality of the cerate. Do not

attempt to adjust the solution to exactly 0.01 M. Restandardize whenever the reagent is used several days or more apart. Keep in a dark bottle away from light.

Nitro-ferroin indicator. (nitro-orthophenanthroline ferrous sulphate, or nitro-orthophenanthroline ferrous perchlorate). When the sulphate is used, 0.1 milliliter in analysis and 0.2 milliliter in standardization is employed, with the perchlorate 0.5 milliliter is used.

Methyl red. 0.1 percent solution in 95 percent ethanol.

PROCEDURE:

An aliquot (5 milliliters) of the digested ash solution is pipetted into a conical, 15 milliliters centrifuge tube. One drop of methyl red solution is added, and 30 percent NaOH added drop by drop until the indicator turns yellow, (pH = 6.3). (It is best to stir with a thin glass rod during this and any other operation involving the addition of reagents to this type of centrifuge tube, since diffusion is very slow. The rod can be washed with a thin stream of water from a hypodermic needle. This will keep the volume down.) 1:15 HCl is added until the indicator turns barely pink. Two milliliters of ammonium oxalate are added, the solution stirred, and the precipitate of calcium oxalate is allowed to stand overnight. It is not essential to allow the precipitate to stand so long, but this procedure is convenient when one is working with a large number of samples.

Centrifuge at 3000 r.p.m. for 10 minutes. Carefully decant the supernatant liquid into a 25 milliliters volumetric flask (save for Mg determination). Stir the precipitate, then rinse the sides of the tube with a stream of 5 milliliters of 1:50 NH_4OH blown from a pipet.

Centrifuge at 3000 r.p.m. for 10 minutes. Decant the washings into the same flask. Drain the tube by inversion on filter paper for 10 minutes. Wipe the mouth of the tube with a clean towel or lintless filter paper.

Blow into the tube 3 milliliters of 4 N $HClO_4$ from a pipet. Use a thin stirring rod to break the precipitate. Wash the stirrer with a stream of 2 milliliters of 4 N $HClO_4$. Transfer quantitatively the solution into a 50 milliliters beaker. Wash the centrifuge tube with 5 milliliters of water and add it to the beaker. Add 0.1 milliliter of the nitro-ferroin indicator. Titrate with the cerate solution from a 10 milliliters burette to a colorless end point. Determine the blank correction in the same manner. It is usually about 0.03 milliliter.

Dilute the supernatant liquid in the volumetric flask to volume and save the magnesium determination.

Appendix 4

SEMIMICRO DETERMINATION OF MAGNESIUM

REAGENTS

Ammonium chloride. 30 percent. Dissolve 30 grams of recrystallized NH_4Cl in water and dilute to 100 milliliters. Filter before use. Place in pyrex bottle.

Ammonium dihydrogen phosphate. 5 percent. Dissolve 25 grams of ammonium dihydrogen phosphate in water and dilute to 500 milliliters. Filter before use. Store in pyrex bottle.

Phenolphthalein. 1 percent in 60 percent ethanol.

Ammonium hydroxide. Concentrated.

Ammoniacal wash liquid. Mix 20 milliliters of concentrated NH_4OH with 80 milliliters of distilled water, 100 milliliters of ethanol, and 100 milliliters of ether.

Sulphuric acid. 1 N.

Magnesium. Standard.

Weigh 101.4 milligrams of $MgSO_4.7H_2O$ and dilute to a liter. One milliliter contains 10 micrograms of magnesium.

Ammonium molybdate-sulfuric acid solution.

Dissolve 5.0 grams of c.p. ammonium molybdate in approximately 80 milliliters of warm, 50° C., water. Add 2.8 milliliters of concentrated sulfuric acid to the cooled solution and dilute to 100 milliliters with distilled water. This solution should not be used if a white

residue has settled. Do not prepare big quantities. Use fresh reagent.

Hydroquinone solution.

Dissolve 0.5 gram of hydroquinone in 100 milliliters of distilled water, made slightly acid with a drop of concentrated sulfuric 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. Keep in refrigerator.

Ammonium hydroxide. 1:1.

Boric acid.

50 grams of c.p. boric acid crystals are made up to 1000 milliliters with distilled water. (About 0.8 molar).

Brom-phenol blue indicator.

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

PROCEDURE

From the volumetric flask containing the Ca-free sample, pipet an aliquot (about 5 milliliters) into a 15 milliliters conical centrifuge tube and dilute or evaporate to 5 milliliters. Add 1 milliliter of 30 percent NH_4Cl, 1 milliliter of 5 percent $NH_4H_2PO_4$ and 1 drop of phenolphthalein. Heat to

90° C. in a water bath and while twirling the tube add concentrated NH_4OH dropwise until pink. Cool, add 2 milliliters of concentrated NH_4OH and stir with a thin glass rod. Withdraw the rod, stopper the tube, and let stand overnight. Centrifuge at 3000 r.p.m. for 10 minutes, decant carefully, drain on filter paper for 10 minutes, and wipe the mouth of the tube with a clean towel. Wash the precipitate and sides of the tube with a stream of 5 milliliters of the ammoniacal wash liquid from a pipet equipped with a rubber aspirator bulb. Centrifuge at 3000 r.p.m. for 5 minutes, decant and drain for 5 minutes, then wipe the mouth of the tube. Repeat this washing procedure once more.

Pipet 2 milliliters of 1 N H_2SO_4 into the tube and dilute to about 5 milliliters. After 5 minutes, transfer the contents quantitatively in a 25 milliliters volumetric flask. Wash the centrifuge with 3 to 4 milliliters of distilled water and transfer it in the volumetric flask.

If fluorides are present at this point, add 5 milliliters of 0.8 M boric acid. If the solution is acid, add 5 drops of the brom-phenol blue and neutralize 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 blue color development is 5 minutes,

provided blanks are used.

1. 2 milliliters of ammonium molybdate

2. 2 milliliters of hydroquinone solution

3. 2.5 milliliters of sodium succinate solution

Make up to volume with distilled water. Allow to stand 30 minutes and measure the color intensity. The color intensity is constant from 0.5 to 4 hours. Determine the phosphorus concentration in a colorimeter using a 775 mμ wave length and P3-6 (Coleman). A blank is run containing all the reagents used in the samples and make up to volume.

Make up a standard curve, carrying 0.01 to 0.10 milligrams of magnesium through the above procedure.

PROCEDURE

Pipet an aliquot, 3 milliliters, of digested ash solution into a 15 milliliters centrifuge tube. If the aliquot is less than 3 milliliters, dilute up to 3 milliliters with 0.1 N HNO_3. Add 1 milliliter of sodium cobaltinitrite reagent and mix the contents thoroughly by twirling the tube. Let it stand in the refrigerator for 1 hour, then add 4 milliliters of 70 percent ethanol, stir thoroughly with a thin glass rod, wash the rod with ethanol, and centrifuge for 15 minutes at 1700 r.p.m. Decant the supernatant liquid, drain the tube for several minutes on paper, add 5 milliliters of 70 percent ethanol down the walls of the tube, break up the precipitate with a stirring rod, wash the rod with 1 milliliter of alcohol, and centrifuge for 10 minutes at 1700 r.p.m. Decant the clear solution, allowing the tube to drain for several minutes, and repeat the washing with 5 milliliters of 70 percent alcohol. Dissolve the precipitate in 5 milliliters of 2 N H_2SO_4 by placing the tube in a bath at about 70° C. After the precipitate in the bottom of the tube has been dissolved, add 5 to 7 milliliters of distilled water and heat for another 5 to 10 minutes to dissolve any adhering precipitate on the sides of the tube. Cool, dilute to 10 milliliters, stopper, and mix thoroughly.

Introduce a 1 milliliter aliquot of the solution into a 25 milliliters volumetric flask. Add 1 milliliter of 5 percent sodium

pyrophosphate solution, dilute to a volume of about 20 milliliters with distilled water, add 1 milliliter of 2.5 N sodium acetate solution, mix, and then add 2 milliliters of 0.5 percent solution of nitroso R-salt, mix well again, make to volume, and read in the colorimeter after 15 minutes. Use a 570 mμ band, FG-4 filter and a water blank. Make up a standard curve with 0.1 to 2.0 milligrams of potassium in each centrifuge tube.

Appendix 6

DETERMINATION OF IRON

REAGENTS

Acetic acid. 2 N.

Dilute 114 milliliters of glacial acetic acid (Sp. Gr. 1.04) to one liter with distilled water.

Hydrochloric acid. 1:1.

Ammonium citrate. $(NH_4)_2HC_6H_5O_7$ 1 percent.

Dissolve 1 gram ammonium citrate in distilled water and dilute to 100 milliliters.

Bromophenol blue indicator solution. 0.4 percent.

Grind 1 gram of solid bromophenol blue in a morter with 3 milliliters of 0.05 N NaOH, transfer to a volumetric flask and dilute to 250 milliliters with distilled water.

Buffer solutions.

1. Solution of pH 3.5 Mix 6.4 milliliters of 2 M sodium acetate solution with 93.6 milliliters of 2 M acetic acid solution and dilute to a liter with distilled water.
2. Solution of pH 4.5 Mix 43 milliliters of 2 M sodium acetate solution with 57 milliliters of 2 M acetic acid solution and dilute to 1 liter.

Hydroquinone solution.

Dissolve 1 gram of hydroquinone in 100 milliliters of a buffer solution of pH 4.5, store in a refrigerator.

Discard as soon as color develops.

O-phenanthroline solution.

Dissolve 1 gram of o-phenanthroline monohydrate in distilled water, warming if necessary to effect solution and dilute to 200 milliliters.

Sodium acetate. 2 M.

Dissolve 272 grams of sodium acetate trihydrated in distilled water and dilute to 1 liter.

Iron. Standard solution.

Dissolve 1 gram of electrolytic iron in 50 milliliters of 10 percent H_2SO_4, warming if necessary to hasten the reaction. Cool, and dilute to 1 liter with distilled water. One milliliter contains one milligram of iron.

PROCEDURE

Pipet an aliquot of 10 milliliters of digested ash solution into both a 25 milliliters volumetric flask and a 25 milliliters Erlenmeyer flask. An aliquot is chosen which will fall in the range of the spectrophotometer, (.01-.10 milligrams of iron). To the solution in the Erlenmeyer flask is added 5 drops of bromophenol blue indicator and this section then is titrated with 2 M sodium acetate until the color matches that of an equal volume of buffer solution of pH 3.5 containing the same quantity of indicator. Add 1 milliliter of the hydroquinone solution and 2 milliliters of o- phenanthroline

reagent to the solution in the volumetric flask and adjust the pH of the contents to 3.5 by adding the same volume of sodium acetate. If a turbidity develops upon adjustment of the pH of the aliquot in the Erlenmeyer flask, add 1 milliliter of ammonium citrate solution to the volumetric flask before adding the sodium acetate solution. Make to volume, mix and let stand for 1 hour to assure complete color development.

Compare the color in the Coleman spectophotometer using a PC-4 filter and wave length 510 mμ against a water blank. Make a standard curve containing .01 to .10 milligram of iron and develop color as above.

Appendix 7

DETERMINATION OF MANGANESE

(Micro colorimetric, as permanganate)

REAGENTS

Sodium metaperiodate. Fine powder.

Phosphoric acid. 85 percent.

Sulphuric acid. Concentrated.

Sodium sulphite. Fine powder.

Potassium permanganate. Standard solution containing 0.0250 milligram manganese per milliliter.

Prepare a 0.10 N standard potassium permanganate solution. Add 22.0 milliliters of the standard solution to a 250 milliliters Erlenmeyer flask, then add about 50 milliliters of distilled water and 1 milliliter of concentrated H_2SO_4. Heat to boiling and reduce the permanganate by adding sodium sulphite powder. Avoid a large excess of the sulphite. Boil off the excess sulphur dioxide and dilute to a liter. Each milliliter contains 0.0250 milligram of manganese.

PROCEDURE

Pipette an aliquot of 1 to 20 milliliters, depending on the amount of manganese present, into a 25 milliliters volumetric flask. Add 1 milliliter of 85 percent phosphoric acid, dilute to 25 milliliters with distilled water, add 0.3 milliliter of

distilled water to allow for the evaporation. Mix with a thin stirring rod.

Place in a water bath at 95° C., add about 50 milligrams of the sodium periodate, mix and let stand in the water bath for one hour or more.

Cool, make to volume if necessary, mix, and measure optical density using a wave length of 520 mμ and P-4 filter with the Coleman spectrophotometer.

CALIBRATION CURVE

Prepare a series of aliquots of the standard permanganate in 25 milliliters volumetric flasks corresponding to 0.005 to 0.050 milligrams of manganese. Add 1 milliliter of 85 percent phosphoric acid, and proceed as described above.

$$2Mn(NO_3)_2 + 5KIO_4 + 3H_2O \rightarrow 2HMnO_4 + 5HIO_3 + 4HNO_3$$

$$2MnSO_3 + 4KIO_4 + 3H_2O \rightarrow 2HMnO_4 + 4KIO_3 + 2H_2SO_4$$

$$KMnO_4 + H_2SO_4 \rightarrow K^+ + MnO_4^- + 2H^+ + SO_4^=$$

Appendix B

PHOSPHORUS FRACTIONS IN PLANTS

1. Preparation of the extract

2. Development of color with ammonium molybdate

3. Phosphorus Fractions

a. Inorganic phosphorus

b. Labile ester phosphorus

c. Resistant ester phosphorus

d. Residual phosphorus

e. Total phosphorus

1. Preparation of the extract

REAGENTS

Trichloroacetic acid. 7.5 percent solution.

Norit. Activated carbon.

PROCEDURE

Place 1 gram of dried, finely ground plant material in a 250 milliliters beaker. Add 60 milliliters of ice-cooled 7.5 percent trichloroacetic acid.

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

Filter into a 250 milliliters beaker. Add 10 milliliters of 7.5 percent trichloroacetic acid to the residue, stir and filter into the same beaker through the same filter paper. Wash

the beaker which contained the plant material twice with 5 milliliters of water, and add the washings to the filtrate.

Save the residue for the determination of residual phosphorus.

Add about 0.2 gram of Norite to the combined filtrate and washings, shake well and let it stand for about 5 minutes.

Filter into a 100 milliliters volumetric flask and dilute to volume with distilled water. This solution contains 5.25 percent trichloroacetic acid.

Designate the solution as "Extract 1".

NOTES

The clarification of the solution with activated carbon is necessary if the solutions are colored in order to permit the later colorimetric determination of phosphorus with ammonium molybdate. Phosphate compounds are not adsorbed to a significant extent by the carbon.

2. Development of color with ammonium molybdate

REAGENTS

Ammonium molybdate-sulfuric acid solution.

Dissolve 5.0 grams of c.p. ammonium molybdate in approximately 90 milliliters of warm, 50° C., water. Add 2.3 milliliters of concentrated sulfuric acid to the cooled solution and dilute to 100 milliliters with distilled water. This solution should not be used if a white residue has settled. Do not prepare big quantities. Use fresh reagent.

Hydroquinone solution.

Dissolve 0.5 gram of hydroquinone in 100 milliliters of distilled water made slightly acid with a drop of concentrated sulfuric 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. Keep in refrigerator.

Standard phosphate.

0.4394 gram of KH_2PO_4 diluted to 1000 milliliters. One milliliter contains 0.1 milligram phosphorus.

Ammonium hydroxide. 1:1.

Boric acid.

Fifty grams of c.p. boric acid crystals are made up to 1000 milliliters with distilled water. (About 0.8 molar)

Bromo-phenol blue.

Dissolve 0.05 gram of bromo-phenol blue in 2.4 milliliters of 0.01 N sodium hydroxide and dilute to 125 milliliters with distilled water.

PROCEDURE

To a 25 milliliters volumetric flask add an aliquot of up to 10 milliliters containing not more than 0.2 milligrams of phosphorus. If fluorides are present at this point, add 5 milliliters of 0.8 M boric acid. If the solution is acid,

add 5 drops of the brono-phenol blue and neutralize 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 blue color development is 5 minutes, provided blanks are used.

1. 2 milliliters of ammonium molybdate
2. 2 milliliters of hydroquinone solution
3. 2.5 milliliters of sodium succinate solution

Make up to volume with distilled water. Allow to stand 30 minutes and measure the color intensity. The color intensity is constant from 0.5 to 4 hours. Determine the phosphorus concentration in a colorimeter using 775 mμ wave length and PC-5 (Coleman).

A blank is run containing all the reagents used in the samples and made up to volume.

Effective range of this method is 0.002 milligrams to 0.3 milligram of phosphorus.

3. Phosphorus Fractions

a. Inorganic phosphorus

PROCEDURE

Pipette a 5 milliliters aliquot of "Extract 1" into a 25 milliliters volumetric flask. Add 5 milliliters of distilled water.

Develop color and read optical density as indicated in section 2.

Prepare standard phosphorus solution containing the same concentration of trichloroacetic acid (approximately 2.6 percent).

Standards. 0.01, 0.05 and 0.10 milligrams phosphorus per flask.

b. Labile Ester Phosphorus

REAGENT

Hydrochloric acid. 1:1

PROCEDURE

Pipette a 5 milliliters aliquot of "Extract 1" into a 25 milliliters volumetric flask. Add 5 milliliters of distilled water plus 2 milliliters of 1:1 hydrochloric acid. This solution will be about 1 N with respect to hydrochloric acid.

Place flask into boiling water bath, stir mechanically with especially prepared glass rod for 7 minutes. Cool in cold water bath or refrigerator.

Develop color and read optical density as indicated in Section 2.

Prepare standard phosphorus solutions as in Section 3a, and add 2 milliliters of 1:1 hydrochloric acid to each flask.

CALCULATIONS

Percent Labile Ester phosphorus = percent phosphorus found - percent inorganic phosphorus.

c. Resistant Ester Phosphorus

REAGENTS

Sulfuric acid. Concentrated.

Nitric acid. Concentrated.

Nitric acid. Approximately 0.5 N.

PROCEDURE

Pipette a 5 milliliters aliquot of "Extract 1" into a 30 milliliters beaker. Add 2 milliliters of nitric acid, concentrated, and 1 milliliter of concentrated sulfuric acid. Place on hot plate, heat at intermediate temperature to dryness. To the cooled beaker add 4 milliliters of 0.5 N nitric acid, warm and transfer quantitatively into a 25 milliliters volumetric flask. Wash beaker three times with 2 milliliters of distilled water. Add washings to the volumetric flask.

Develop color and read optical density as indicated in Section 2.

Prepare standard phosphorus solutions in distilled water using the same concentrations as in Section 3a.

CALCULATIONS

Percent Resistant Esters phosphorus = percent phosphorus found - (percent inorganic phosphorus + labile ester phosphorus)

d. Residual Phosphorus

REAGENTS

Digestion mixture. Sulfuric-Nitric acid.

Add 100 milliliters of concentrated sulfuric acid to 200 milliliters of concentrated nitric acid.

Nitric acid. 1:4.

PROCEDURE

Transfer the residue of the plant material obtained as described under "Preparation of the Extract" to a 250 milliliters beaker. Add 10 milliliters of the digestion mixture. Place the beaker on the hot plate and heat at moderate temperature until all the organic matter has been oxidized and a white precipitate results. Use more digestion mixture if necessary.

Dissolve the precipitate with 10 milliliters of 1:4 nitric acid, add 60 milliliters of hot distilled water and filter into a 100 milliliters volumetric flask. Make up to volume.

Pipette a 5 milliliters aliquot into a 25 milliliters volumetric flask and add 5 milliliters of distilled water.

Develop color and measure optical density as indicated in Section 2.

Prepare standard phosphorus curve. Use the following concentrations: 0.01, 0.05, 0.1 and 0.2 milligrams phosphorus per volumetric flask in 0.1 N nitric acid.

e. Total Phosphorus

REAGENTS

Digestion mixture. As in Section 3d.

Nitric acid. Concentrated.

Kerosene.

PROCEDURE

Place a one-gram sample of plant material in a micro-Kjeldahl flask. Add 10 milliliters of digestion mixture plus 2 drops of Kerosene. Let it stand over night. Heat on micro-Kjeldahl digestion unit. When digestion is complete a colorless liquid of about 1-3 milliliters remains in the flask. Use more digestion mixture if necessary.

Add to the digest 10 milliliters of 1:4 nitric acid and 10 milliliters of distilled water. Warm, filter into a 100 milliliters volumetric flask, make up to volume.

Develop color and measure optical density as indicated.

Prepare standard phosphorus solutions as in Section 3d.

Appendix 9

TOTAL CARBOHYDRATES IN PLANTS

A. Preparation of extract by acid hydrolysis

This method when used with plants includes all sugars and all types of acid hydrolyzable substances.

REAGENTS

Hydrochloric acid. 25 percent.

Neutral lead acetate. 25 percent.

Potassium oxalate. 25 percent.

Methyl Red indicator. 0.1 percent in 60 percent ethyl alcohol.

Sodium hydroxide. 25 percent.

PROCEDURE

Weigh 1 gram of the dried plant material into a 500 milliliters reflux flask. Add 200 milliliters of distilled water, 20 milliliters of 25 percent hydrochloric acid. Swirl thoroughly to assure mixing.

Digest for two and a half hours, swirling the material around the flask occasionally to wash down the material which accumulates on the glass.

Remove the flask at the end of two and a half hours from the digestion rack. Let it cool, transfer it into a 400 milliliters beaker and neutralize by the addition of 25 percent sodium hydroxide with the use of a glass electrode. To the neutral suspension add 20-30 milliliters of the 25 percent neutral lead acetate, and mix. Test for complete precipitation

by the addition of a few drops of the acetate solution. After a few minutes add about 10 milliliters of 25 percent potassium oxalate. Test for complete precipitation.

Filter into a 500 milliliters volumetric flask, using a fluted filter funnel. Bring to volume with distilled water and mix.

B. Determination of reducing sugars

(Heinze, P. H. and Murneek, A. E. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Mo. Agr. Exp. Sta. Research Bulletin. 314. 1940)

REAGENTS

Copper Reagent (Cu)

This solution is prepared by dissolving 25 grams of sodium carbonate and 25 grams of sodium potassium tartrate (Rochelle Salt) in about 600 milliliters of distilled water. 75 milliliters of a 10 percent copper sulphate are then added through a pipette extending below the surface of the liquid. This is followed by the addition of 20 grams of sodium bi-carbonate, 1 gram of potassium iodide, and 200 milliliters of a solution of potassium iodate containing 3.567 grams of pure KIO_3 per liter. The solution is thoroughly mixed, rinsed into a liter volumetric flask and made to volume. If kept in a stoppered Pyrex bottle and protected from strong light the solution will remain unchanged for months. If the

reagents are made as directed, any quantity less than 1.00 milligrams of glucose per 10 milliliters of solution may be determined.

Potassium iodide-Potassium oxalate solution.

8.5 grams of potassium iodide and 8.5 grams of potassium oxalate are dissolved in distilled water and made up to 100 milliliters. A new solution should be prepared every week and preserved in a dark colored bottle in order to avoid errors due to deterioration products. Free iodine present will cause considerable error in the determination.

Sodium thiosulphate. 0.0200 N (standard).

Prepare 0.1 N sodium thiosulphate as a stock solution as follows:

Dissolve 24.82 grams of $Na_2S_2O_3.5H_2O$ to about 800 milliliters boiled water. Add 10 milliliters of 0.1 N NaOH. Dilute to 1 liter and let stand several days.

Standardize the 0.1 N sodium thiosulphate as follows. Weigh into 250 milliliters Erlenmeyer flasks about 0.12-0.17 gram of KIO_3. Dissolve in 50 milliliters of boiled distilled water. Add 2 grams of potassium iodide to the solution and as soon as this has dissolved, add 1 milliliter of concentrated hydrochloric acid diluted to 10 or 15 milliliters. Titrate the iodine immediately with the thiosulfate solution. Add starch as indicator when the solution has become a faint yellow.

Calculate the normality of the thiosulfate. A milliequivalent of potassium iodate is 0.03567 gram.

Sulphuric acid solution. 1 N.

Make by diluting concentrated 28.4 milliliters (Sp. Gr. 1.835 at 15° C.) to 1 liter.

Starch solution.

1 percent starch solution in a saturated NaCl solution.

PROCEDURE

The size of the aliquot must be determined by experiment so that all the copper is not reduced.

Three milliliters of sugar solution are measured into a 8 x 1 inch test tube and 10 milliliters of reagent "C" are added quantitatively. These solutions are thoroughly mixed and the tubes closed with test tube funnels or by small beakers. The tubes are placed in a vigorously boiling water bath for 15 minutes. They are firmly held in metal racks during heating to avoid agitation. At the end of the heating period, the tubes are removed to a container with running water and cooled for 3 minutes to about 30° C. Two milliliters of the potassium iodide-potassium oxalate solution are added to each tube by volumetric pipette, followed by 10 milliliters of 1 N H_2SO_4. The tubes are rotated to insure complete mixing of the contents and dissolution of the cuprous oxide, or are stirred by means of a glass rod. Let stand 5 minutes and titrate with

0.02 N standard thiosulphate solution until the straw color has almost disappeared. Add three drops of starch solution and continue the titration till the blue color disappears. Blanks are run with each set of determinations.

CALCULATIONS

The blank titration minus the titration value of the sample gives the milliliters of thiosulphate proportional to the amount of sugar in the sample. Previously constructed graphs having milligrams of glucose against milliliters of thiosulphate solution are used to calculate the amount of sugar in the aliquot.

Appendix 10

DETERMINATION OF CRUDE FATS

Ether Extraction

PROCEDURE

Weigh a 2-gram sample of dried powdered plant material into a clay thimble.

Extract in Goldfish extraction unit with 50 milliliters of ether for 4 hours.

Determine the increase in weight, before and after the extraction, of the Goldfish beaker.

Express results as percentage of dried material.

Appendix II

TOTAL NITROGEN IN PLANT MATERIAL (MICRO METHOD)

REAGENTS

Reducing Solution.

30 grams of salicylic acid per liter of concentrated H_2SO_4.

Sodium thiosulphate. 33 percent.

50 grams of sodium thiosulphate pentahydrate per 100 milliliters of distilled water.

Boric acid. 2 percent.

10 grams of boric acid crystals in 500 milliliters of boiling distilled water.

Sodium hydroxide. 30 percent.

150 grams of sodium hydroxide pellets in 350 milliliters of distilled water. Store in bottle with rubber stopper.

Selenium metal. Powdered.

Pulverized mixture of potassium sulphate (1 part) and copper sulphate pentahydrate (5 parts).

Hydrochloric acid. 0.01 N.

Mixed Indicator.

Prepare separately 0.1 percent bromcresol green and methyl red in 95 percent ethyl alcohol. Mix these solutions in the proportions of 5:1 bromcresol green to methyl red. (pH around 5.1)

Methyl-Purple Indicator.

Add 1 cc. of the concentrated form of the indicator furnished by the Fleischer Chemical Company to 200 cc. of 2 percent boric acid.

PROCEDURE

Weigh 10 to 50 milligrams sample of grass and transfer it into the bottom of the micro-Kjeldahl flask. Use a long handled, specially constructed weighing tube. Add 2 milliliters of Reuter's solution, mix with the tissue and allow to stand in the cold for 30 minutes. Add 5 drops of the 30 percent Thiosulphate solution to reduce the nitro group and warm gently over a micro burner. Add about 3 milligrams of powdered selenium and 5 milligrams of copper sulphate-potassium sulphate mixture. Place the flask on digestion stand and boil the mixture until clear. The reaction usually is complete in 1 hour. After cooling, add 2 milliliters of distilled water, mix and cool again.

Transfer the content of the digestion flask to the micro-Kjeldahl still. Rinse the digestion flask with about 2 milliliters of distilled water, then introduce 2 to 10 milliliters of 30 percent NaOH. Wash funnel with distilled water. Distill the ammonia into a 50 milliliters Erlenmeyer flask containing 5 milliliters of the boric acid. Add 4 drops of the mixed indicator and titrate to a pink color.

CALCULATIONS

$$\% \text{ N in plants} = \frac{\text{milliliters of 0.02 N HCl} - \text{milliliters blank}}{\text{weight of sample in milligrams}} \times 28$$