DIAGNOSIS OF PLANT NUTRIENT DEFICIENCIES BY MEANS OF SOIL TESTS, PLANT TISSUE TESTS, AND FOLIAR ANALYSIS

by

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J.Q.L.

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INTRODUCTION

Over a century ago Von Liebig established the fact that soil is the source of mineral nutrients for plants. Since that time there have been innumerable investigations searching for factors that govern plant development and composition with particular reference to complex soil relationships. Many early investigators established the fact that plants vary greatly in composition and concluded that evaluation of analytical data dealing with plant composition was precluded because of so many influencing factors (36). Most of these data were concerned with analyses of the total plant including stems, leaves, and fruit (15).

More recently a method using only the physiologically active portion of the plant in a "foliar analysis" has been more successful for indicating plant nutrient status than the method of total analysis. This method is based on quantitative measurements of nutrient elements present in the leaf at the moment of sampling. Interpretations of analytical data are made in terms of "intensity" or critical concentration and in terms of relative content or "balance" of the nutrient elements determined.

Plant tissue tests were introduced simultaneously with the development of "foliar analysis" and tissue testing has also been used extensively for diagnosing nutrient requirements of plants. The theoretical basis for this method rests on the extraction of unassimulated inorganic constituents from plant tissue which is primarily concerned with

nutrient translocation. These determinations are roughly quantitative and test results are interpreted relative to the presence or absence of nutrient ions in sufficient quantity to insure optimum plant growth.

Although both of these methods have received widespread application as diagnostic aids, there is still a difference of opinion as to what part of the plant should be sampled and at what physiological stage the plants should be sampled for analysis. There is controversy as to whether the soluble fraction is a better index of nutritional status than the total quantity of elements present. It is questioned whether differences are sufficiently large to overcome sampling errors and if it is possible to state absolute critical concentrations for each nutrient under different soil and climatic conditions.

This investigation was undertaken to compare foliar analysis and green tissue testing for diagnosing nutrient deficiencies in plants grown in greenhouse and field experiments on soil types of widely differing fertility level.

A chemical study was made of changes in the soil nutrient status of fertilizer-rotation field experimental plots following seven continuous years of experimental work. Plant tissue testing and foliar analysis were used to determine the nutrient status of one crop, corn, in these field experiments during the 1947 and 1948 growing seasons and these results were correlated with soil analysis and crop yield.

REVIEW OF LITERATURE

Diagnosis of plant nutritional status by means of chemical analysis has been an object of study since the early history of agriculture chemistry. As the complexity of the problem became more and more apparent, detailed investigations tended to divide into two separate phases. Physical and chemical studies of the soil were made to determine how improvements could be made to produce more desirable plant growth. Physiological studies of the plant itself were made to determine its needs for maximum growth and production.

In recent years attempts have been made to correlate results of plant tests and soil tests with a view of increasing crop production by detecting and eliminating certain nutrients as factors which may limit plant growth. Toward this end foliar analysis and green tissue testing techniques have been introduced as experimental aids along with soil testing, deficiency symptoms, pot cultures and field plot procedures for interpreting the complex relations of soil fertility and crop yields.

Goodall and Gregory (15) have presented a detailed review of literature concerned with chemical composition of plants as an index of nutritional status. They point out changes in concepts prevalent in past investigations concerning total plant analysis as an indication of soil fertility and furthermore, they emphasize the caution necessary in using the results of soil analysis as absolute

critera for plant needs. These investigators propose an integration of soil analysis, plant analysis, and field experimentation as a means to attain practical solutions to plant nutritional problems.

Foliar Analysis: "Foliar analysis" as a means of determining the nutritional status of plants was suggested by Lagatu and Maume (21) in 1924. This method of approach has since been utilized by many workers and has been applied to both perennial and annual plants. The principals of sampling and analytical procedure have been adapted without major change but controversy now exists as to the interpretation of data from these procedures.

Lundegardh (23) presents the fundamental theory of this concept as being based on the fact that functioning assimilating leaves are "laboratories of nutrition". Their composition reflects the nutritional status of the plant at the moment of sampling as influenced by all factors of soil and climate which govern plant growth.

Thomas (42) has modified the original procedures and has adapted them for several horticultural and agronomic crops. This worker recognizes two factors in interpretating such analyses. First, the quantity or "intensity" of an element may be of significance and critical concentrations may be established for optimum growth. Second, the ratio of nutrients present or "quality" may be governing plant development. Thomas utilizes a system of mathematical interpretation of the ratio of nutrients present in terms of milligram equivalents. Both interpretations

assume that the limiting factor in plant growth will be reflected in foliar analysis as a function of absorption and utilization of nutrient elements. It is pointed out by Thomas that the validity of any system of analysis applied to dynamic systems as those of plants and soils are necessarily comparative in nature.

Ulrich (50) has emphasized that foliar analyses do not reflect the nutritional status of soils but rather the whole complex of environmental factors which influence plant growth. Composition of leaves and changes in their composition during growth provides direct information on the "nutrition" of plants. He further proposes this method as means of determining "critical concentrations" of nutrient elements. He points out that sensitivity of plant tests depends upon the part of the plant analyzed, the particular fraction of nutrient determined, and the position on the plant from which samples are selected. This worker reports very satisfactory results with foliar analysis in determining the potassium needs of grapes.

Macy (26) has proposed quantitative measurement of mineral nutrients in plants as a criterion for the nutritive status of plants. As stated by Macy "Sufficiency of a nutrient is a function of its per cent content in the plant." This is interpreted to mean that there is a critical per cent for each nutrient, above this content there is luxury consumption, below this point there is "poverty adjustment" of the plant to a deficient state where a "minimum" percentage is reached. This minimum

value results in the appearence of deficiency symptoms, senescence, and finally death of the plant.

Nightingale (30) recognized that variations in the critical concentrations of nutrients in plants were influenced by environmental factors of light, temperature, and moisture. He evolved a system of testing for pineapple plants in which consideration was given to these environmental conditions. This method was used for determining optimum nitrogen-carbon ratios within plants at critical stages in their development.

Shear, Crane, and Myers (38) have proposed nutrient-element balance as a fundamental concept in plant nutrition. The principal idea involved is that any element as it decreases or increases from its concentration at optimum intensity may affect plant growth. The maximum growth possible within the new limits of supply of that element can result only when the concentrations of all elements have been brought into balance at the new level of intensity determined by that particular element.

These workers, utilizing foliar analysis as means of determining this balance of elements have carried out intensive investigations on tung trees giving consideration to the nutrient elements nitrogen, potassium, phosphorus, magnesium and calcium. No attempt was made to utilize the concentration of these elements in terms of chemical equivalents as did Thomas (44). It is pointed out that the stage of plant development and position of

the leaf are important factors to consider in sampling for analysis.

Tyner (48) utilized a method of foliar analysis for nitrogen, phosphorus and potassium on a basal leaf of corn (Zea maize) and proposed "critical" concentrations of these elements for maximum yield based on a large number of sample plants grown in seven different experimental areas. Leaf samples were taken once during the growing season when plants were in full tassel and silk.

Plant Tissue Testing: A system of tissue testing proposed by Hoffer (20) has been extensively applied as an aid in the interpretation of field plot results. The tests are made on extracts of fresh tissue from portions of the plant consisting largely of conducting tissue. The concentrations of unassimilated elements are determined semiquantitatively in this procedure and these tests are considered to give a measure of the current rate of nutrient uptake. The interpretation of these tests is concerned with the plant absorption of elements rather than the utilization of nutrients as in the case of foliar analysis. The theoretical basis, testing procedure, and methods of applying results of green tissue tests have been discussed in detail by Scarseth (37).

Some of the ideas of Hoffer were later extended by Thornton (47) who modified the Purdue soil testing procedure for making plant tissue tests. Other workers, who

have developed similar testing systems are Emmert (13), Carolus (6), Hester (19), and Cook and Millar (11). These methods differ mainly in the nature of the extraction solution and in the part of the plant utilized for testing.

Emmert (13) and Carolus (6) utilized 2% acetic acid extracts of plant tissue for making the tests. According to Carolus the concentration of a nutrient in such an extract represented the current balance between its absorption and utilization. He quotes limiting values for the potato plant below which deficiency is to be suspected and found that these values changed during the course of plant development.

Hester (19) has described a system of tissue testing utilizing an acetate buffer solution in which tomato stems were extracted in a mechanical cocktail mixer. He proposes values for deficiency and normal ranges for the content of nitrate-nitrogen, phosphate-phosphorus, and potassium but recommended making a comparison of the composition of the best and the poorest plants in a field rather than relying on limiting values.

Thornton (47) uses the testing reagents in making tissue extractions for potassium and phosphorus. Samples of tissue are treated with diphenylamine-sulphuric acid reagent for an estimation of unassimilated nitrate-nitrogen. Finely chopped tissue from terminal growth is extracted with a solution of ammonium molybdate in 0.1N HCl solution for phosphorus. Finely chopped tissue from a

middle part of the plant is extracted with a solution of sodium cobaltinitrite in acetic acid for a potassium test.

Cook and Millar (11) use the same portions of the plant for testing as Thornton but the extractions are made in distilled water utilizing the reagents of Spurway (40) for the actual chemical tests. Arbitrary values of blank, low, medium, high, and very high are given these test results and interpretations of the tests are based on these values in conjuntion with plant deficiency symptoms and soil tests.

Harrington (18) has made a comprehensive study of factors which influence the reliability of plant tissue tests. He found that the composition of fresh plant tissue was greatly influenced by soil type regardless of fertilizer treatment. The age of the plant, when sampled, had considerable effect on the test results. Concentrations of soluble nutrient elements in the plants decreased with maturity in all cases. This writer concludes that the composition of the conducting tissue of the petiole or stem, is a reliable guide to changes in current nutrient status. He found greater differences between types of tissue on the same plant than between plants having different fertilizer treatments.

PLAN OF STUDY

This investigation was undertaken with the idea of producing plants differing widely in their nutrient content by modifying the soil conditions and to compare foliar analysis and green tissue testing as means for diagnosing these differences.

These two methods were applied to plants produced in the greenhouse in soils of different fertility levels and also to plants grown on one soil type under field conditions. These methods were also compared on a series of rotation-fertilizer experiments.

The plan of study included the following:

I. Description of soil types used

II. Greenhouse experiments for comparative analytical studies

A. Physical and chemical properties of soils used in greenhouse experiments

1. Particle size distribution

2. Aggregate analysis

3. Base exchange capacity

4. Soil reaction, organic matter content, adsorbed phosphorus, exchangeable calcium, potassium, magnesium, hydrogen, and manganese

B. The production of corn (Zea maize) plants of different nutrient status in the greenhouse on Miami, Conover, Brookston, and Plainfield soil types

1. Soil treatments equivalent to applications of 2000 pounds of 10-20-20 fertilizer per acre in the following combinations

a. No treatment (check)

b. 10- 0- 0 (N)

c. 0-20- 0 (P)

d. 0- 0-20 (K)

e. 10-20- 0 (NP)

f. 10- 0-20 (NK)

g. 0-20-20 (PK)

h. 10-20-20 (NPK)

2. Results of foliar analysis and plant tissue testing from two crops on the same soils

3. Yield data (dry weights)

4. Soil pH after plant growth

C. The production of white bean plants of different nutrient status in the greenhouse on Miami and Granby soil types

1. Soil treatments equivalent to the following

a. No treatment

b. 2 tons $CaCO_3$ per acre (Granby), 4 tons $CaCO_3$ per acre (Miami)

c. 500 pounds $MnSO_4$ per acre on both soil types

d. 1000 pounds 10-20-20 fertilizer per acre on both soil types

2. Results of foliar analysis and plant tissue testing of two crops on the same soil

3. Yield data (dry weights)

4. Soil pH after plant growth

D. The production of corn and white bean plants deficient in magnesium with an unbalanced nutrient solution.

III. Field experiment on Miami soil type for comparative study of foliar analysis and plant tissue testing as diagnostic aids

A. Description of soil type, topography and previous cropping

B. Fertilizer treatments applied at the rate of 1000 pounds per acre

1. No treatment (check)
2. 10- 0- 0 (N)
3. 0-20- 0 (P)
4. 0- 0-20 (K)
5. 10-20- 0 (NP)
6. 10- 0-20 (NK)
7. 0-20-20 (PK)
8. 10-20-20 (NPK)

C. Results of foliar analysis and plant tissue tests

D. Yield data (corn grain and fodder yields)

IV. Chemical investigations of fertilizer-rotation field experiments

A. Description of field experiments

B. Chemical investigations of soil samples taken at the start and after seven years

C. Results of plant tissue tests, foliar analysis study, and crop yields for 1947

D. Results of plant tissue tests, foliar analysis study, and crop yields for 1948

E. Greenhouse study of two crops grown on soil from the field plots after the 1947 season

F. Nitrification studies of soil from field plots for 1948

EXPERIMENTAL PROCEDURE

Description of Soils Used*

Five soil types were used in the greenhouse studies. These types represent five different fertility levels so selected as to represent extremes in nutrient content, organic matter, soil reaction, and texture.

Miami Type: This soil type occurs on level to gently rolling topography as grayish-brown mellow granulated sandy loam or loam. The plow layer is underlain by pale-yellow or gray leached material. At depths ranging from 8 to 15 inches this layer grades into brown compact gritty coarsely granular clay loam to depths of 36 to 40 inches. The substratum is comparatively hard gray or pale-yellow clayey calcareous glacial till containing large amounts of free calcium carbonate. The surfact layers are usually slight to strongly acid. Drainage is usually good, the subsoil, although sufficiently friable for root penetration and development, is highly retentive of moisture and is one of the best heavy upland soils for cultivated crops. This soil was collected from the R.L. Cook farm, Clinton County, near Dewitt, Michigan.

Conover Type: A soil type intermediate between Miami and Brookston occurring on gently sloping and nearly level

*Essentially according to Veatch, J. O., Agricultural land classification and land types of Michigan, Mich. Exp. Sta. Special Bul. 213 (1st rev.) 1941

topography as dark grayish-brown mellow loam to depths of 4 to 6 inches. This is underlain by pale-yellow or gray friable gritty loam somewhat mottled at lower depths and at 10 to 15 inches grades into moderately compact but penetrable clay of coarsely granular structure. The substratum consists of a heavy clay calcarous drift to a depth of several feet. Internal drainage is imperfect to poor but when artificial drainage is provided is one of the most productive soils for general crops. This soil type was collected from the R. L. Cook farm, Clinton County, near Dewitt, Michigan.

Brookston Type: This soil type occurs on very smooth level topography as a dark gray to nearly black mellow granular plow soil to a depth of 8 to 12 inches. The subsoil is heavy textured, gray or yellowish-gray coherent clayey material 4 to 8 inches thick, beneath which is a steel gray or bluish-gray more plastic sticky clay slightly mottled with yellow and rust brown. The substratum consists of massive clayey glacial till containing abundant free lime. This soil was obtained from the Lee Ferden farm, Saginaw County, near Chesaning, Michigan.

Plainfield Type: This soil type occurs as dry yellowish-brown loamy sand occurring on nearly level land. Clay is scarcely noticeable in the subsoil and loose dry sand or sand and gravel extend to depths of several feet. The plow layer of 6 to 7 inches consists of light grayish-brown loose loamy sand, low in organic matter and water holding capacity. The

substratum is usually coarse gravelly material containing little carbonaceous material. This soil is too drouthy and low in fertility to be used extensively for cultivated crops. This soil type was obtained from the Rose Lake Wildlife Experimental Farm, Clinton County, near Bath, Michigan.

Granby Type: This soil type commonly occupies poorly drained sinks and swales as a dark wet sandy soil high in organic matter content underlain by sandy clayey material with mottled yellow-gray rust colored subsoil occurring at depths of 1 to 2 feet below the surface. This soil contains substantial amounts of free calcium carbonate through the soil profile and is neutral or alkaline in reaction. When adequately drained this soil is productive for truck and cultivated crops but is not as durable as the more heavy mineral soils under intensive cultivation. This soil type was obtained from the Wagbo farm, Ingham County, Michigan.

Physical and Chemical Determinations of Soils Used

Several physical and chemical determinations were carried out on these soil types in an effort to evaluate their respective fertility levels.

The particle size distribution of each soil is presented in table 1. These soils vary in texture from a heavy clay soil to a coarse light sandy soil.

The results of the aggregate analysis (table 2), when compared with the data of particle size distribution, indicate the relative amounts of aggregation and stability of the structure. The data show that the soils used in these studies differ markedly in their structural characteristics ranging from the well aggregated heavy Brookston to the single grained structure of the light coarse Plainfield soil.

The base exchange capacity, exchangeable ions, pH, and the organic content of the soils used in the greenhouse studies are presented in table 3. It is noted (tables 1 and 3) that a decrease in organic matter is accompanied by an increase in acidity and that the base exchange capacity and the exchangeable calcium diminish with a decrease in the quantity of the clay fraction except in the Granby soil which is dominated by organic matter.

A potentiometric method was used for determining base exchange characteristics of these soils. A detailed description follows:

Twenty five gram samples of air-dried soil were placed in gooch crucibles fitted with filter paper and soaked overnight in distilled water. The soil was then leached with 200 ml. of .05N HCl and washed with two 300 ml. portions of distilled water followed by a washing with 100 ml. of 90 per cent ethanol. After the samples were dried at 80°C for 12 hours they were transferred to beakers, 25 ml. of distilled water added, stirred vigorously, and the reaction determined potentiometrically.

One gram of solid $BaCl_2$ was then added to each hydrogen saturated sample, stirred two minutes and another 25 ml. of water added. Each sample was titrated potentiometrically to a pH of 8.0 using a standard solution of $Ba(OH)_2$. The base was added in 1 ml. increments to a pH of about 5.0 followed by 0.5 ml. increments. The additions of $Ba(OH)_2$ were made precisely at one minute intervals and pH determinations were made just prior to each addition. The milliequivalents of base necessary to adjust the soil to pH 7.0 was calculated and curves were plotted from the titration data of six samples of each soil (figure 1).

These curves, together with the data in tables 1 and 3, show the effects of clay and organic matter in relation to the exchange capacity and buffering properties of the respective soils.

Table 1 The Particle Size Distribution of the Soils Used in the Greenhouse Experiments*

Soil type	Per cent composition Size of particle in millimeters						
	2.0-1.0	1.0-0.5	0.5-.25	.25-.10	.10-.05	.05-.002	< .002
Granby sandy loam	2.32	4.12	15.60	21.92	21.14	30.20	4.70
Brookston sandy clay soil	1.52	3.64	11.00	14.80	15.54	19.30	34.20
Conover sandy clay loam	4.20	7.00	13.60	18.00	8.90	26.50	21.80
Miami sandy loam	3.77	9.51	23.50	17.50	9.90	22.60	14.10
Plainfield loamy sand	5.72	11.88	21.28	36.76	5.56	10.70	8.10

* Determined according to Bouyoucos (4)

Table 2 The Aggregate Analysis of the Soils Used in the Greenhouse Experiments*

Soil type	Per cent composition Size of aggregate in millimeters						
	4.0	4.0-2.0	2.0-1.0	1.0-0.5	0.5-.25	.25-.10	<.10
Granby sandy loam	1.72	6.00	4.00	5.80	13.40	13.36	55.72
Brookston sandy clay soil	6.72	6.56	6.48	11.72	24.52	22.00	22.00
Conover sandy clay loam	3.48	9.85	10.75	9.85	29.70	23.70	20.50
Miami sandy loam	.04	4.00	3.60	5.20	14.00	23.00	38.20
Plainfield loamy sand	-	.20	3.16	5.34	22.28	47.80	21.52

* Determined according to Yoder (52)

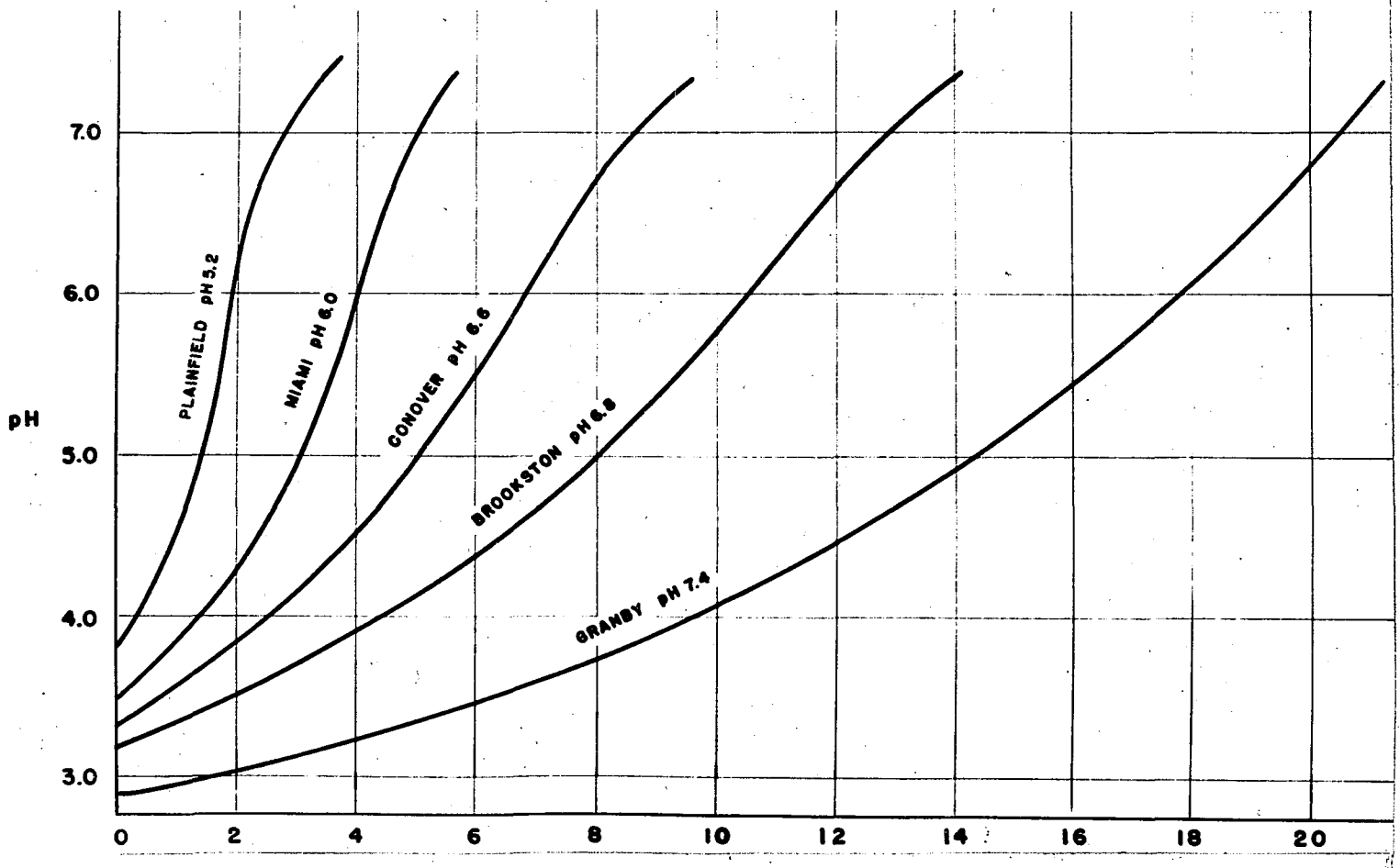
Table 3 The Base Exchange Capacity, Exchangeable Ions, pH, and Organic Matter Content of Soils Used in the Greenhouse Experiments

Soil type	% O.M.[1]	pH[2]	Milliequivalents per 100 grams soil						
			Base Exch. Cap.	Ca^2	K^2	Mg^3	P^4	Mn^2	H^2
Granby sandy loam	14.52	7.4	20.50	13.90	.178	.163	.112	.088	.95
Brookston sandy clay soil	7.76	6.8	13.45	8.95	.325	.165	.169	.105	2.45
Conover sandy clay loam	5.16	6.6	9.30	6.19	.170	.116	.145	.138	2.68
Miami sandy loam	2.34	6.0	5.40	2.83	.134	.105	.103	.201	2.40
Plainfield loamy sand	1.08	5.2	2.25	.71	.063	.042	.079	.075	1.46

1. Determined according to Walkley (51)
2. Determined by methods of Peech, et al (32)
3. Determined essentially by the method of Gillam (14)
4. Determined according to Bray and Kurtz (5)

Figure 1.

ELECTROMETRIC TITRATION FOR BASE EXCHANGE AND BUFFERING CAPACITY OF SOILS USED IN THE GREENHOUSE EXPERIMENTS



Greenhouse Experiments for Tissue Testing and Foliar Analysis

Experiments with Corn: The soil types used for these experiments were collected in the field, screened, and air dried. For the experiments with corn (Zea maize), Miami, Conover, Brookston, and Plainfield soils were placed in four gallon jars, sixteen kilograms per jar. The following fertilizer treatments were applied as mixtures of NH_4NO_3, $CaHPO_4 \cdot H_2O$, and KCl at the rate of 2000 pounds per acre.

No treatment	(check)
10- 0- 0	(N)
0-20- 0	(P)
0- 0-20	(K)
10-20- 0	(NP)
10- 0-20	(NK)
0-20-20	(PK)
10-20-20	(NPK)

These treatments were replicated three times. After sufficient water was added to bring the soils up to their moisture equivalent they were planted to corn. One week after tasseling, the third functioning basal leaf was removed from each plant and the remainder of the plant dried and weighed. The sheaths of the leaves removed were used for green tissue tests and the leaf blade used for a foliar analysis of total nitrogen, phosphorus, potassium, calcium, and magnesium. The yields and analyses of the first greenhouse crop are presented in tables 4, 6, and 8.

Following the first crop, the three replicate jars for each treatment were dumped together, the soil thoroughly mixed, a sample taken and the soil replaced in the jars. The second corn crop was planted and harvested in the same manner as the first crop. Results of the second crop are shown in tables 5, 7, 9, and 10.

Experiments with White Beans: Miami sandy loam and Granby sandy loam were used in these greenhouse experiments to produce plants of varying nutrient status to investigate the application of green tissue testing and foliar analysis on plants particularly responsive to minor elements. Twelve kilograms of air dried soil were placed in three gallon pots and were given the following treatments:

No treatment

2 tons $CaCO_3$ (Granby), 4 tons $CaCO_3$ (Miami) per acre

500 lbs. $MnSO_4$ per acre

1000 lbs. 10-20-20 per acre

White beans were planted and, following full bloom, one of each of the three replicates was harvested for analysis. The stems were used for the tissue tests and the leaves for foliar analysis. The plants in the remaining two pots of each treatment were harvested for yield. Following the first crop, the contents of replicate jars were dumped together, thoroughly mixed, sampled, replaced in the jars and planted to a second bean crop. The yields and results of the chemical analyses for the first crop are presented in table 11 and for the second crop in table 12.

Sand Culture Experiments: In this experiment an unbalanced nutrient solution was supplied to corn and bean plants grown in quartz sand cultures. The nutrient solution suggested by Shive and Robbins (39) was used with the exception that the salts KH_2PO_4 and $Ca(NO_3)_2$ were doubled in amount and only 1/100 of the prescribed amount of $MgSO_4$ was included.

These plants were harvested when in full bloom. The leaf sheath of the third basal leaf of the corn plants was used in foliar analysis. The stems of the bean plants were used for tissue tests and the leaves were used in the foliar analyses. The results are presented in table 13.

Method of Tissue Testing: For this investigation a system of tissue testing was devised which is essentially a laboratory adaptation of the field testing method proposed by Cook and Millar (11).

The portion of the corn plant selected for testing was the leaf sheath, primarily conductive tissue, of the third functioning basal leaf. The basal leaf was selected because it is recognized as a reliable indicator of the nutrient status in plants (43, 45). The leaf blade of this same leaf was used for the foliar analysis study.

The sheath tissue was finely chopped and extracted with distilled water, in a 1:10 ratio, by vigorus shaking for one minute. The extraction equivalent of 1/3 gram of tissue was used for phosphorus determinations using

0.1 ml of molybdic acid and a small grain of stannous chloride. The results were recorded as blank, low, medium, high, and very high depending on the color intensity. These color intensities approach concentrations of 0, 0.5, 1.0, 3.0, and 5.0 ppm of phosphorus.

The extraction equivalent of 1/3 gram of tissue was used for potassium determinations to which was added 1 ml. of 2.5% cobaltinitrite-15% $NaNO_2$ solution and 5 ml. of 95% ethanol. The turbidity was read as blank, low, medium, high, and very high corresponding to the turbidity of solutions with 0, 10, 20, 40, and 60 ppm of potassium respectively.

For the nitrate test, approximately 1/4 gram of finely chopped tissue was placed in a white spot plate and .5 ml. of .2% diphenylamine-conc. H_2SO_4 solution was added. The amount of nitrate nitrogen present was indicated by the amount of blue color which developed. This color was recorded as blank, low, medium, and high corresponding to 0, 2, 5, and 10 ppm of nitrate nitrogen.

The extraction equivalent of 1/6 gram was used for both magnesium and calcium. The arbitrary values of blank, low, medium, and high were assigned to concentrations approximately 0, 1, 3, 6ppm and above for magnesium as determined by the procedures of Peech and English (31) for soil testing. Calcium determinations were made using the stearic-oleic acid procedure of Peech and English (31) and the results assigned arbitrary values corresponding

to blank, 10, 20, 40 ppm and above.

Manganese was determined by the procedure suggested by Cook and Lawton (9). In this method the extraction is made with 3% acetic acid and the permanganic color is developed with sodium periodate.

These procedures were carried out in the laboratory and were used for both the greenhouse and field investigations.

In the course of this investigation a method was devised for making permanent plastic standards for use in rapid soil and tissue testing procedures (25). These standards were made in an effort to give a constant reference for the color and turbidity developed with standard solutions. A commercial liquid casting material "Castolite" was used. This material is a colorless, thermosetting, isotropic material which is easily colored with dyes that are soluble in acetone. Turbidity was produced by the addition of powdered calcium carbonate.

Standards were made for determinations of phosphorus as phosphomolybdic-blue, potassium in the turbidimetric cobaltinitrite test, magnesium as the titan yellow color lake, and calcium in the turbidimetric stearic-oleic soap solution test. These plastics follow Beer's law of light transmission for color intensity and degree of turbidity in the ranges used and are excellent duplications of actual solution standards. Once colored the hardened plastic material is very resistant to fading. They are convenient

and simple to use and are especially adapted for field testing of soil and plant tissue.

Methods of Foliar Analysis: The leaf blade of the same leaf sampled for green tissue tests was used in the foliar analyses. The mid-rib of the leaf was removed and the plant material dried at 80°C for 12 hours and ground in a Wiley micro mill to pass a 20 mesh screen. One-half gram of oven dried tissue was digested according to the wet digestion method of Piper (34) for determining the mineral elements. One gram of oven dried tissue was used for total nitrogen determination by the Kjeldahl-Gunning method (1).

Phosphorus, potassium, magnesium, and calcium were determined on aliquots of the digested material essentially as suggested by Linder (22).

These same procedures were applied to the field experiments reported later in this paper.

Table 4 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of Corn Plants Grown in the First Greenhouse Crop on Plainfield Loamy Sand[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade m.e. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	5.3	14.1	41.6	.31	M	M	-	M	M	129	10.6	34.8	12.6	9.2
10- 0- 0	5.2	10.9	32.6	.32	VH	M	-	L	M	245	23.2	21.5	16.1	8.4
0-20- 0	5.3	21.1	41.7	.35	L	H	-	L	M	107	27.4	28.8	25.8	17.3
0- 0-20	4.7	5.7	27.0	.25	VH	L	L	M	M	115	19.0	20.6	35.6	7.9
10-20- 0	5.4	27.3	55.0	.41	VH	L	-	L	H	280	27.6	17.2	20.6	18.8
10- 0-20	4.8	4.3	20.5	.30	VH	L	L	M	M	250	21.8	22.8	23.2	5.8
0-20-20	4.9	21.6	49.6	.36	L	H	L	H	L	126	29.2	19.2	34.2	15.6
10-20-20	4.9	17.6	45.1	.32	VH	M	L	M	H	291	30.2	24.8	25.6	16.8

1. Data represents the mean of three replications
2. Legend: VH very high, H high, M medium, L low, - blank
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder (22)

Table 5 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of Corn Plants Grown in the Second Greenhouse Crop on Plainfield Loamy Sand[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade m.e. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	5.5	15.5	22	.33	-	M	-	M	M	95	12.8	23.2	19.4	17.7
10- 0- 0	5.6	35.4	20	.44	H	L	-	M	M	159	16.8	24.2	18.1	16.1
0-20- 0	5.6	25.4	23	.46	-	H	-	M	H	91	32.0	11.4	11.2	22.9
0- 0-20	4.7	25.3	21	.48	-	L	M	L	M	142	24.0	21.4	15.6	31.7
10-20- 0	5.5	88.6	42	.68	H	H	-	M	H	145	28.0	17.4	15.0	22.4
10- 0-20	5.4	27.1	18	.42	H	-	L	L	L	148	13.2	31.5	15.7	13.0
0-20-20	5.5	36.6	20	.51	-	H	L	L	L	109	14.0	40.0	11.3	12.0
10-20-20	5.5	180.7	45	.71	H	M	M	L	M	180	20.0	51.0	10.6	8.9

1. Data represents the mean of three replications
2. Legend: H high, M medium, L low, - blank
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder (22)

Table 6 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of the Corn Plants Grown In the First Greenhouse Crop on Miami Sandy Loam.[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade m.e. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	5.9	12.0	48.7	.33	VH	L	-	L	H	191	14.7	30.5	10.3	19.8
10- 0- 0	5.6	9.8	45.8	.23	VH	L	-	L	M	238	21.2	42.0	15.9	11.5
0-20- 0	5.8	28.1	52.0	.51	-	H	-	L	H	167	24.6	22.3	17.4	21.8
0- 0-20	5.6	6.0	27.2	.32	VH	L	M	L	M	212	16.8	71.8	6.4	12.9
10-20- 0	5.6	14.8	35.3	.47	VH	VH	-	L	H	289	30.4	23.6	15.9	22.9
10- 0-20	5.5	5.1	21.2	.28	VH	L	H	L	L	273	13.9	69.0	15.7	9.4
0-20-20	5.5	36.6	60.2	.43	-	H	H	L	H	156	21.4	32.3	14.9	20.2
10-20-20	5.6	27.6	61.3	.40	M	M	M	L	M	234	21.9	43.5	17.6	13.3

1. Data represents the mean of three replications.
2. Legend: VH very high, H high, M medium, L low, - blank.
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder(22).

Table 7 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of Corn Plants Grown in the Second Greenhouse Crop on Miami Sandy Loam[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade m.e. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	5.5	36.2	36	.46	L	L	L	L	M	115	16.0	10.5	17.5	11.7
10- 0- 0	5.4	66.5	35	.57	H	-	M	L	L	219	17.2	22.6	15.0	15.6
0-20- 0	5.6	50.2	39	.48	-	H	L	M	M	88	24.8	12.5	28.1	16.2
0- 0-20	5.3	67.5	34	.51	M	L	H	L	L	159	18.0	43.0	26.2	13.0
10-20- 0	5.8	141.3	65	.69	H	M	L	M	H	187	24.4	15.6	19.1	20.3
10- 0-20	4.8	75.6	34	.54	H	-	H	M	M	226	18.0	51.0	21.9	15.6
0-20-20	5.6	42.3	36	.45	-	M	H	M	-	98	22.0	64.4	15.0	15.1
10-20-20	5.8	230.3	64	.71	H	M	H	M	-	230	25.6	77.6	12.1	19.2

1. Data represents the mean of three replications.
2. Legend: VH very high, H high, M medium, L low, - blank.
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder (22).

Table 8 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of Corn Plants Grown in the First Greenhouse Crop on Conover Sandy Clay Loam[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade m.e. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	6.5	6.3	33.8	.24	VH	L	-	M	M	206	21.2	25.5	10.7	12.7
10- 0- 0	6.4	7.5	31.3	.33	VH	L	-	M	H	284	13.4	30.5	13.4	19.0
0-20- 0	6.5	36.0	53.1	.49	-	VH	-	M	H	130	22.4	20.5	11.3	23.8
0- 0-20	6.3	9.1	36.9	.26	M	L	M	L	H	224	21.2	36.8	18.0	20.4
10-20- 0	6.4	26.0	47.4	.42	VH	H	-	L	M	234	22.4	15.3	17.8	23.1
10- 0-20	6.3	4.1	23.8	.26	VH	L	M	M	H	222	16.8	60.5	11.4	20.2
0-20-20	6.4	41.3	64.9	.46	-	VH	VH	L	M	129	19.0	63.0	11.6	19.6
10-20-20	6.4	31.7	50.7	.41	VH	M	M	M	M	245	15.4	49.5	13.6	24.2

1. Data represents the mean of three replications
2. Legend: VH very high, H high, M medium, L low, - blank
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder (22)

Table 9 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of Corn Plants Grown in the Second Greenhouse Crop on Conover Sandy Clay Loam[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade m.e. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	6.8	62.5	39	.62	H	-	-	M	M	222	18.4	19.6	14.4	20.8
10- 0- 0	6.7	83.3	36	.55	H	-	L	M	M	107	18.7	14.1	17.0	16.5
0-20- 0	6.8	44.1	40	.48	-	H	-	M	M	83	28.0	16.8	31.3	19.2
0- 0-20	6.7	68.8	35	.51	M	-	H	L	L	180	17.6	43.4	15.6	14.6
10-20- 0	7.1	164.1	65	.67	H	H	-	M	H	146	30.0	18.9	26.3	15.1
10- 0-20	6.8	85.4	36	.52	H	-	M	L	L	277	18.0	58.0	19.4	13.0
0-20-20	6.8	53.6	35	.50	-	H	H	L	L	71	24.0	52.6	14.4	23.4
10-20-20	7.0	183.2	66	.77	H	M	M	M	M	253	24.8	57.8	21.9	20.8

1. Data represents the mean of three replications
2. Legend: VH very high, H high, M medium, L low, - blank
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder (22)

Table 10 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test and Foliar Analysis of Corn Plants Grown in the Second Greenhouse Crop on Brookston Sandy Clay Soil[1]

Fertilizer treatment 2000 lbs. per acre	Soil pH	Yield grams	Height inches	Stalk diam. inches	Analysis of third basal leaf									
					Green tissue test leaf sheath tissue[2]					Foliar analysis leaf blade M.E. per 100 grams[3]				
					N	P	K	Ca	Mg	N	P	K	Ca	Mg
none	6.6	40.8	44	.55	H	M	M	H	M	200	10.0	31.2	16.3	19.4
10- 0- 0	6.3	71.5	53	.51	H	M	M	H	H	288	11.2	27.5	17.5	18.7
0-20- 0	6.7	64.2	56	.63	M	H	M	M	H	184	15.8	36.2	22.5	16.2
0- 0-20	6.6	58.8	47	.57	M	M	H	H	M	225	12.9	45.6	18.8	21.8
10-20- 0	7.1	104.1	62	.58	H	H	M	H	H	266	13.2	28.8	17.5	16.5
10- 0-20	6.5	44.2	54	.55	H	L	H	M	H	288	9.3	52.5	20.6	15.0
0-20-20	6.3	92.5	63	.63	L	H	H	M	M	152	18.8	53.7	16.3	13.1
10-20-20	6.5	159.6	58	.76	H	M	H	H	M	306	13.6	41.7	17.5	16.2

1. Data represents the mean of three replications
2. Legend: H high, M medium, L low, - blank
3. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially by methods of Linder (22)

Results with Corn: The first corn crop grown on Conover, Miami, and Plainfield soil types gave a very marked response to phosphate fertilizer (tables 4, 6, and 8, and plates 1, 2, 3, 4, 5 and 6). The absence of phosphorus in the fertilizer treatments resulted in substanial growth depressions with the early appearance of phosphorus deficiency symptoms. Nitrogen deficiency symptoms appeared after six weeks on all treatments not receiving nitrogen fertilizer but were severe only on the PK treatment of all three soil types. The Plainfield soil responded markedly only when phosphorus and nitrogen were applied in combination.

In order to determine if the amount of fertilizer applied was sufficient to cause injury from high concentrations of salts, conductivity measurements were made on all treatments. These measurements determined according to Magistad, et al (27) were less than 40 x $10^{-5}\Lambda$ which is slightly less than tap water used in the laboratory. Injury for sensitive plants under ordinary greenhouse conditions is not expected to occur at values less than 100 x $10^{-5}\Lambda$ (27).

Results of the soil pH determinations (tables 4, 5, 6, 7, 8, and 9) indicate that the fertilizer treatments did not change appreciably the soil reaction.

Of particular interest in this investigation was the precision with which the two methods used for determining nutrient status of the plants indicated differences in soil treatments. For the elements nitrogen, phosphorus, and potassium, tissue tests indicated almost without exception the soil

treatment. Foliar analysis of the same elements was somewhat less conclusive in indicating various treatments.

In general, the calcium and magnesium tissue test results were not in accord with results of foliar analysis for these elements. However, no effort was made to control the supply of these two elements in the various fertilizer treatments and the soil types used in this experiment contained variable amounts of available calcium and magnesium (table 3). It is noted that the concentration of these elements was generally greater in the plants grown on soils with the higher content of exchangeable calcium and magnesium.

A second corn crop was grown on these same soils with no additional fertilizer treatment. Brookston sandy clay soil was included in the experiment for this second corn crop. The growth of this crop followed closely the nitrogen-phosphorus treatments on all soils with the greatest growth occurring only when both elements were applied in combination. Nitrogen deficiency symptoms were severe in all treatments not receiving this element except on the Brookston soil where they appeared only on the PK treatment. Considerable depression of plant growth occurred on treatments which produced a large first crop but received no nitrogen in the fertilizer treatments.

Tissue tests on the second crop revealed rather precisely differences in soil treatments for the elements nitrogen, phosphorus, and potassium. Considerable variability appeared between results of calcium and magnesium tissue tests and results of foliar analysis for these elements. The foliar analysis of

Table 11 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of White Bean Plants Grown in the First Greenhouse Crop on Miami Sandy Loam and Granby Sandy Loam

Soil type	Treatment per acre	Soil pH	Yield grams	Green tissue test[1] plant stems						Foliar analysis[2] m.e. per 100 grams					
				N	P	K	Ca	Mg	Mn	N	P	K	Ca	Mg	Mn
Granby sandy loam	none	7.6	14.1	H	M	M	H	H	M	202	23.2	36.0	23.2	12.3	1.56
	2 tons $CaCO_3$	7.8	20.1	H	M	M	M	M	L	219	35.8	40.0	28.8	11.0	.94
	500 lbs. $MnSO_4$	7.6	21.6	H	L	M	H	H	H	199	14.8	34.0	10.3	11.4	4.17
	1000 lbs. 10-20-20	7.7	43.2	VH	H	H	H	L	-	284	37.2	76.6	24.1	10.2	1.12
Miami sandy loam	none	5.9	18.1	M	M	M	M	H	M	174	27.4	30.6	19.3	10.4	6.25
	4 tons $CaCO_3$	7.5	19.8	H	L	L	H	M	M	161	25.6	27.0	24.5	10.1	4.70
	500 lbs. $MnSO_4$	6.4	4.8	M	M	M	M	H	H	65	20.6	36.6	19.8	19.9	16.51
	1000 lbs. 10-20-20	5.5	34.3	VH	H	H	M	L	M	217	33.6	52.6	13.5	11.9	8.25

1. Legend: VH very high, H high, M medium, L low, - blank
2. Nitrogen determined by the Kjeldahl-Gunning method (1), manganese by method of Peech, et al (32), other elements by methods of Linder (22)

Table 12 The Effects of Various Fertilizer Treatments on the Growth, Tissue Test, and Foliar Analysis of White Bean Plants Grown in the Second Greenhouse Crop on Miami Sandy Loam and Granby Sandy Loam

Soil type	Treatment per acre	Soil pH	Yield grams	Green tissue test[1] plant stems						Foliar analysis[2] m.e. per 100 grams					
				N	P	K	Ca	Mg	Mn	N	P	K	Ca	Mg	Mn
Granby sandy loam	none	7.6	10.6	H	H	M	M	H	M	298	16.0	29.8	8.0	24.0	.95
	2 tons $CaCO_3$	7.8	17.4	H	M	L	M	H	L	262	28.0	29.0	11.0	10.3	.15
	500 lbs. $MnSO_4$	7.6	20.4	H	M	M	L	H	H	299	12.0	14.2	11.8	18.4	1.56
	1000 lbs. 10-20-20	7.7	43.0	H	H	H	L	L	L	328	34.0	36.4	12.3	20.2	.76
Miami sandy loam	none	6.3	17.4	M	H	L	M	M	H	213	20.0	20.8	12.0	12.3	2.43
	4 tons $CaCO_3$	7.9	25.8	M	H	L	M	M	H	248	38.0	24.2	12.5	22.1	2.15
	500 lbs. $MnSO_4$	6.5	13.2	H	H	M	L	M	H	252	22.0	28.4	11.2	14.0	7.93
	1000 lbs. 10-20-20	5.8	36.2	H	M	H	L	L	H	270	48.0	44.2	10.8	18.3	3.46

1. Legend: VH very high, H high, M medium, L low, - blank
2. Nitrogen determined by the Kjeldahl-Gunning method (1), manganese by method of Peech, et al (32), other elements by methods of Linder (22)

nitrogen, phosphorus, and potassium was less sensitive than tissue tests in detecting the soil treatments but in general followed them more closely than the foliar analyses of the first crop.

Results with White Beans: The soil types selected for the experiments with white beans were purposely chosen for their differences in manganese content. White beans are plants notably sensitive to manganese availability and are suitable plants for this type of an investigation.

The crop grown on the Granby soil receiving no fertilizer treatment exhibited typical manganese deficient symptoms from the appearance of the first true leaves. These symptoms were more intense with lime application but symptoms did not appear on the manganese treatment. The application of nitrogen, potassium, and phosphorus resulted in larger plant growth but manganese deficient symptoms were severe. Plant growth and symptoms were nearly the same for both the first and second crops grown on this soil. Tissue tests for manganese indicated the relative total amounts of manganese present in the leaves found by foliar analysis.

The bean plants grown in the Miami soil exhibited no manganese deficient symptoms even with the application of the large amount of $CaCO_3$. This treatment was used in an effort to produce manganese deficiency such as that observed on other soil types receiving high rates of lime (24). A growth depression of both crops grown on this soil type resulted from the addition of $MnSO_4$. The amount of manganese in the leaf

Table 13 The Effect of an Unbalanced Nutrient Solution on Growth, Tissue Test, and Foliar Analysis of Corn and White Bean Plants Grown in Quartz Sand[1]

Plant grown	Green tissue test[4]					Foliar analysis m.e. per 100 grams[5]				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
Corn[2]	H	VH	VH	H	M	215	28.1	58.5	38.2	18.3
White beans[3]	M	VH	VH	H	M	285	37.2	72.1	28.8	16.4

1. Data represents the mean of three greenhouse replications
2. Leaf sheath tissue of the third basal leaf was used for tissue test, leaf blade of this same leaf used for foliar analysis
3. Leaf petiole used for tissue test, leaf blade used for foliar analysis
4. Legend for test results: VH very high, H high, M medium, L low, - blank
5. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined essentially according to Linder (22)

tissue increased considerably with the manganese treatment.

Results with Corn and White Bean Plants in Sand Culture: In this experiment, the nutrient solution was purposely unbalanced in an effort to produce a magnesium deficient condition by increasing the concentrations of other base ions.

The plants grown in this culture exhibited symptoms which have been recognized as specific for magnesium deficiency (17). Tissue tests for nitrogen, phosphorus, potassium, and calcium indicated high concentrations of these elements as did the results of a foliar analysis of the same plants. The magnesium tissue tests were moderately high and did not reflect the low concentration of the element recovered in the foliar analyses.

Field Experiments for Tissue Testing and Foliar Analysis

In order to make a comparative study of foliar analysis and tissue testing on corn plants under field conditions, a field experiment was conducted on the R. L. Cook farm. This farm is located in the south central part of Clinton county, near Dewitt, Michigan. The experimental plots were set up on the same Miami soil type used in the greenhouse experiments. A heavy stand of first year mammoth clover was turned under as a green manure crop when fitting this field for the experimental plots.

Treatments were the same as those in the greenhouse experiment except that fertilizers were added at the rate of 1000 lbs. per acre. One half of the fertilizer was drilled on both sides of the seed at planting and half was applied as a side dressing four weeks after planting. The experimental design and plot arrangement were as follows:

Block I		Block II		Block III	
No treatment	(ck)	10-20- 0	(NP)	10-20-20	(NPK)
10- 0- 0	(N)	10- 0-20	(NK)	0-20-20	(PK)
0-20- 0	(P)	0-20-20	(PK)	10- 0-20	(NK)
0- 0-20	(K)	10-20-20	(NPK)	10-20- 0	(NP)
10-20- 0	(NP)	No treatment	(ck)	0- 0-20	(K)
10- 0-20	(NK)	0- 0-20	(K)	0-20- 0	(P)
0-20-20	(PK)	0-20- 0	(P)	10- 0- 0	(N)
10-20-20	(NPK)	10- 0- 0	(N)	No treatment	(ck)

One block was sampled and tested each week beginning six weeks after planting. The tests were conducted in the same manner as described in the greenhouse experiments. In addition to sampling the third basal leaf, additional foliage samples

were taken just below the tassel as suggested by Thornton (47). Results of these experiments are presented in tables 14, 15, 16, 17, and 18.

In general, tissue tests carried out on the third functioning basal leaf indicated the fertilizer treatment given to each plot. Although the corn plants on all fertilized plots gave a blank test for soluble nitrate nitrogen near the latter part of the season, the plants on plots receiving nitrogen fertilizer gave positive tests for a longer period of time. Phosphorus and potassium tests indicated higher concentrations in the plants from plots receiving these elements in the fertilizer.

In comparing the third functioning basal leaf and the leaf tissue below the tassel (table 17) as to precision for indicating fertilizer treatments, nitrate tests were consistently lower in the new than in the basal tissue. The phosphorus and potassium tissue tests were usually lower in the upper tissue and, in general, did not indicate the fertilizer treatment. These data indicate that the basal tissue of the corn plant is a more reliable indicator of nutrient status than is the younger tissue.

Results of foliar analysis of leaf samples taken through the growing season (table 18) show a general decrease in all of the elements determined as the season progressed. This is of particular interest as one sampling period occurred at the beginning and one at the end of the flowering period indicating

Table 14 The Effects of Various Fertilizer Treatments on Yield and Nitrate Tissue Tests of Corn Plants Grown in the Field Experiment on Miami Sandy Loam, R. L. Cook Farm

Fertilizer treatment 1000 lbs. per acre	Yields bu. per acre	Fodder tons/ acre	Green tissue test[1] third basal leaf sheath							
			July			August				Sept.
			20	26	31	7	14	21	28	4
No treatment	47.2	1.57	H	M	H	L	L	-	-	-
10- 0- 0 (N)	58.1	1.99	VH	H	H	H	M	L	L	-
0-20- 0 (P)	57.5	1.95	M	M	M	M	-	-	-	-
0- 0-20 (K)	52.8	1.64	H	H	M	L	L	-	-	L
10-20- 0 (NP)	56.7	1.62	VH	H	H	H	L	L	L	L
10- 0-20 (NK)	52.0	1.59	M	H	H	M	L	-	-	-
0-20-20 (PK)	52.5	1.77	L	M	L	M	-	-	-	-
10-20-20 (NPK)	58.7	1.62	VH	M	H	H	M	L	-	-

1. Legend: VH very high, H high, M medium, L low, - blank

Table 15 The Effects of Various Fertilizer Treatments on Yield and Phosphorus Tissue Test of Corn Plants Grown in the Field Experiment on Miami Sandy Loam, R. L. Cook Farm

Fertilizer treatment 1000 lbs. per acre	Yields bu. per acre	Fodder tons/ acre	Green tissue test[1] third basal leaf sheath							
			July			August				Sept.
			20	26	31	7	14	21	28	4
No treatment	47.2	1.57	M	H	H	H	L	M	L	L
10- 0- 0 (N)	58.1	1.99	H	M	H	H	M	H	M	M
0-20- 0 (P)	57.5	1.95	VH	VH	VH	VH	H	H	H	H
0- 0-20 (K)	52.8	1.64	H	M	H	H	VH	H	L	M
10-20- 0 (NP)	56.7	1.62	H	VH	VH	VH	M	VH	H	H
10- 0-20 (NK)	52.0	1.59	M	M	H	H	M	H	L	M
0-20-20 (PK)	52.5	1.77	VH	H	VH	VH	H	H	H	H
10-20-20 (NPK)	58.7	1.62	H	VH	H	VH	M	H	M	M

1. Legend: VH very high, H high, M medium, L low, - blank

Table 16 The Effects of Various Fertilizer Treatments on Yield and Potassium Tissue Tests of Corn Plants Grown in the Field Experiment on Miami Sandy Loam, R. L. Cook Farm

Fertilizer treatment 1000 lbs. per acre	Yields bu. per acre	Fodder tons/ acre	Green tissue test[1] third basal leaf sheath							
			July			August				Sept.
			20	26	31	7	14	21	28	4
No treatment	47.2	1.57	M	H	H	VH	L	H	M	M
10- 0- 0 (N)	58.1	1.99	M	H	M	H	M	M	M	M
0-20- 0 (P)	57.5	1.95	M	H	H	M	H	M	M	M
0- 0-20 (K)	52.8	1.64	VH	H	VH	VH	H	H	VH	H
10-20- 0 (NP)	56.7	1.62	H	H	H	L	H	M	M	M
10- 0-20 (NK)	52.0	1.59	VH	VH	VH	H	M	H	H	H
0-20-20 (PK)	52.5	1.77	VH	H	VH	H	H	H	VH	H
10-20-20 (NPK)	58.7	1.62	H	H	H	H	H	H	H	M

1. Legend: VH very high, H high, M medium, L low, - blank

Table 17 The Effects of Various Fertilizer Treatments on Tissue Tests for Nitrogen, Phosphorus, and Potassium of Different Parts of Corn Plants Grown in the Field Experiment, R. L. Cook Farm Miami Sandy Loam

Portion of the plant sampled	Fertilizer treatment 1000 lbs. per acre	Green tissue test*								
		Sampled Aug. 7			Sampled Aug. 14			Sampled Aug. 21		
		N	P	K	N	P	K	N	P	K
Third basal leaf sheath	10-20- 0	M	VH	VH	L	M	H	L	VH	M
	10- 0-20	M	H	VH	M	M	H	-	H	H
	0-20-20	-	VH	VH	-	H	VH	-	H	H
	10-20-20	H	VH	VH	L	M	M	L	H	VH
Leaf sheath below tassel	10-20- 0	L	H	M	-	VH	M	-	H	H
	10- 0-20	-	M	H	-	H	VH	-	H	H
	0-20-20	-	H	VH	-	VH	H	-	H	H
	10-20-20	L	VH	VH	-	M	VH	-	VH	VH

* Legend: VH very high, H high, M medium, L low, - blank

Table 18 The Effects of Various Fertilizer Treatments on the Yield and Foliar Analyses of Third Basal Leaves Sampled at Three Periods During Growth of Corn Plants in the Field Experiment, Miami Sandy Loam, R. L. Cook Farm[1]

Fertilizer treatment	Yield bu./ acre	Milliequivalents per 100 grams[2]														
		Sampled July 20					Sampled July 26					Sampled August 7				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
No treatment	47.2	211	18.8	20.6	31.8	12.5	200	11.6	23.8	26.3	13.8	178	16.4	17.5	21.5	9.4
10- 0- 0	58.1	238	16.0	19.4	25.0	11.9	240	14.0	15.8	20.0	11.3	184	17.0	15.0	27.0	8.0
0-20- 0	57.5	215	12.8	17.5	18.8	18.1	234	14.6	20.0	16.3	10.6	161	13.0	25.0	35.0	8.7
0- 0-20	52.8	196	19.8	23.1	12.5	17.5	196	16.6	28.4	25.0	7.5	176	18.6	29.0	30.0	9.4
10-20- 0	56.7	152	12.6	18.1	37.5	11.3	199	10.4	16.9	19.0	16.0	167	15.6	18.0	28.0	8.1
10- 0-20	52.0	135	17.6	42.5	15.0	18.8	236	12.8	32.5	17.5	18.8	166	19.6	25.3	25.3	12.5
0-20-20	52.5	182	16.4	28.1	15.5	18.1	190	17.8	24.3	15.0	18.1	138	12.4	11.9	35.0	18.6
10-20-20	58.7	189	17.8	29.8	25.0	19.3	220	10.6	25.0	33.0	11.9	157	10.4	20.0	38.5	11.9

1. Data represents the mean of three plot replications
2. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined according to Linder (22)

that the time of sampling within this period may have considerable influence on composition of leaves.

Chemical Investigations of Fertilizer-Rotation Field Experiments

This study was undertaken to investigate certain chemical changes that may have taken place in a Brookston soil following seven years of continuous fertilizer and rotation experiments. Tissue tests and foliar analyses were carried out on the corn crop in these experiments during two growing seasons, 1947 and 1948, to determine the nutrient status of the corn plants as affected by the experimental treatments.

This field experiment is located on the Lee Ferden farm, Saginaw county, near Chesaning, Michigan. It was established in the spring of 1940 as a cooperative project of the Farmers and Manufacturers Beet Sugar Association and the Michigan Agriculture Experiment Station under the supervision of the Soil Science Department, Michigan State College. Cook, et al (10) have presented a detailed outline of this field experiment.

The soil is classed as a Brookston sandy clay soil and occupies very level topography with naturally imperfect drainage. The field is tiled but internal drainage is slow. This soil is high in organic matter and inorganic plant nutrients with a pH near neutral and, when properly drained, is one of the most productive soils in Michigan. A detailed description of this soil is presented in the description of soils used on page 14.

Seven five year rotations are included in this experiment as follows:

1. Barley, alfalfa, alfalfa, corn, sugar beets
2. Barley, alfalfa, alfalfa, sugar beets, corn
3. Barley, alfalfa, alfalfa, beans, sugar beets
4. Barley, oats, alfalfa, corn, sugar beets
5. Barley, oats, clover-timothy, corn, sugar beets
6. Barley, beans, wheat, corn, sugar beets
7. Barley, sweet clover, beans, wheat, sweet clover, corn, sugar beets (sweet clover is seeded with barley and is plowed under the next spring for beans and is seeded in the spring on wheat to be plowed under the next spring for corn)

This study included all rotations except number 3 in which corn does not appear.

The plots are arranged in a split-plot randomized block design and the treatments are replicated four times with each individual plot being 28 by 90 feet. The rotations were arranged at random in each block, with each rotation occupying five plots in each of the four blocks. Each plot is further divided into two 14 by 90 feet sub-plots. One sub-plot receives 2-16-8 fertilizer at the rate of 1,000 pounds per acre in five years while the other receives 400 pounds of the same fertilizer during the rotation period. In both cases one-half of the fertilizer is applied for sugar beets and the other one-half for grain, all for barley in rotations 1, 2, and 3 and divided between the two grain crops in the other rotations.

The use of manure and the disposition of crop residues are regulated according to the systems of farming which might be practiced with the different rotations. In rotations 1, 2,

and 3, manure at the rate of 10 tons per acre is applied for corn or beans. In rotations 4 and 5, with only 20 per cent of the land in hay production, the manure application is 7 tons per acre. No manure is applied in rotations 6 and 7.

Corn stover is left on all plots. Grain straw, bean straw, and sugar beet tops are returned to the plots in rotations 6 and 7. Barley in rotations 1, 2, and 3 and oats in rotation 4 serve as nurse crops for alfalfa. A red clover and timothy mixture is seeded with oats in rotation 5. Sweet clover is seeded with both grain crops in rotation 7 and is plowed under for beans and corn.

The plots were further divided in 1946 with half of each plot fall plowed and half spring plowed.

The soil in each individual plot was sampled in the spring of 1940 with the installation of the field experiment. The method of sampling consisted of taking eighteen samples in a diagonal line across each plot. These individual samples were composited, air dried, screened through a 4mm screen and stored in sealed half gallon jars.

For this study the plots in which corn appeared were again sampled in 1947. Four samples were taken from each of the three mid row spaces of the four rows of corn on each plot. The twelve samples thus obtained were composited, screened, and a kilogram sample taken for analysis.

The 1947 and the 1940 soil samples were subjected to a partial chemical analysis for a comparative study of the changes which had taken place in the seven years. The data from these analyses are presented in table 19.

Table 19 The Soil pH and Exchangeable Ions of Field Experimental Plots on Brookston Soil, Ferden Farm[1]

Rotation[2]	Pounds of 2-16-8 per rotation	Milliequivalents per 100 grams									
		1940 Soil samples					1947 Soil samples				
		pH[3]	K[3]	P[4]	Ca[3]	Mg[5]	pH[3]	K[3]	P[4]	Ca[3]	Mg[5]
1.	1000	6.75	.372	.183	11.03	.316	6.58	.415	.206	10.61	.385
	400	6.77	.355	.197	10.96	.330	6.50	.403	.191	9.97	.391
2.	1000	6.90	.385	.200	11.59	.377	6.48	.407	.183	9.37	.328
	400	6.80	.340	.182	10.77	.293	6.51	.355	.179	9.68	.372
4.	1000	6.85	.368	.171	11.35	.384	6.54	.360	.178	8.80	.385
	400	6.80	.372	.197	11.13	.243	6.53	.378	.189	9.81	.357
5.	1000	6.81	.340	.149	10.02	.335	6.52	.351	.164	9.36	.315
	400	6.95	.352	.173	10.25	.298	6.65	.382	.172	10.10	.341
6.	1000	6.78	.370	.187	9.55	.281	6.71	.377	.149	10.82	.354
	400	6.80	.378	.194	10.41	.288	6.73	.392	.151	10.91	.341
7.	1000	6.70	.382	.214	11.22	.356	6.61	.370	.159	9.18	.306
	400	6.78	.312	.205	11.64	.317	6.59	.405	.178	8.93	.313

1. Data represents the mean of three replicate plots
2. Rotation crops and sequence are presented on page 50
3. Determined by methods of Peech, et al (32)
4. Determined according to Bray and Kurtz (5)
5. Determined essentially by the method of Gillam (14)

Soil Test Results: According to the soil test results no great chemical change had taken place in these soils which could be detected by the testing methods used. The rotation plots receiving the high amounts of fertilizer do not indicate any appreciable accumulation of the fertilizer elements.

Exchangeable calcium and pH values of the 1947 soil samples are lower than those of the 1940 samples. It should be pointed out that the 1940 samples were taken in the spring of that year. The 1947 samples were taken in the fall from plots growing an intensively cultivated row crop. It is noted that the soil from rotation 6 has a higher pH and a slightly higher content of calcium than the soil from the other rotations. This difference was noted in all four plot replications and is also reflected in the soil samples collected in 1948 for nitrification experiments (table 25). This may be due to the fact that crop yields were consistently lower in this rotation with less removal of plant nutrients than in the other rotations. Furthermore rotation 6 does not include legume hay crops which are notoriously heavy users of calcium.

From these data it was concluded that differences in plant growth and crop yields could not be accounted for by chemical soil tests alone. Another method of approach to this problem has been undertaken by Robertson (35) in a study of the physical characteristics of the soil in these field experiments to determine structural changes that have taken place as a result of experimental treatment.

Plant Tissue and Foliar Analysis Studies: A study of the nutrient status of corn plants by tissue test and foliar analysis was made during the 1947 and 1948 growing seasons in order to determine the relationship of these test results and the effect of the different fertilizer-rotation treatments on corn yield.

Tissue tests were applied on the sheath tissue of the third basal leaf of composite samples from six plants taken weekly from each plot during the growing season. The remaining portion of the sampled leaf was dried, ground to pass a 20 mesh screen and used in a foliar analysis.

Results of the 1947 analyses are presented in tables 20 and 21 and figures 2 and 3, the 1948 analyses in tables 22 and 23 and figures 4 and 5.

Results of 1947 Analyses: During the 1947 growing season tissue tests for phosphorus and potassium were high and very high on all samples from all plots. Results of the nitrogen tests are presented in table 20. It is observed that concentrations of nitrogen decreased in all instances as the season progressed. Although all plants tested blank near the end of the growing season, it should be noted that the more rapid the disappearence of soluble nitrate nitrogen the smaller were the corn yields.

From these data it was concluded that phosphorus and potassium were not limiting nutrients because of their relatively high concentrations in a water soluble form within the plant throughout the growing season. The nitrate tissue tests

Table 20 The Yield and Nitrate Tissue Tests of Corn Plants During 1947 on the Field Experimental Plots Brookston Soil, Ferden Farm

Rotation[1]	Pounds of 2-16-8 per rotation	Yield bu. per acre	Green tissue test[2] third basal leaf sheath								
			July			August				Sept.	
			12	19	26	2	9	16	23	15	22
1.	1000	49.4	H	H	H	M	L	L	-	L	-
	400	42.0	H	H	H	H	M	-	L	L	-
2.	1000	36.4	H	M	M	L	L	L	-	L	-
	400	35.9	H	M	M	L	L	-	-	-	-
4.	1000	44.1	H	H	H	M	L	L	L	L	-
	400	45.3	H	H	M	L	L	-	L	-	-
5.	1000	43.3	H	H	H	M	L	L	-	L	-
	400	44.5	H	H	H	M	L	L	-	L	-
6.	1000	28.5	L	L	-	-	-	-	-	-	-
	400	31.1	L	-	-	-	-	-	-	-	-
7.	1000	31.5	M	L	L	L	-	-	-	-	-
	400	35.5	M	L	L	-	-	-	-	-	-

1. Rotation crops and sequence are presented on page 50
2. Legend: VH very high, H high, M medium, L low, - blank

Figure 2

THE RELATION OF CORN YIELDS TO TOTAL NITROGEN CONTENT OF THE THIRD BASAL LEAF DURING BLOOM STAGE ON FERTILIZER-ROTATION EXPERIMENTS FOR 1947. BROOKSTON SOIL

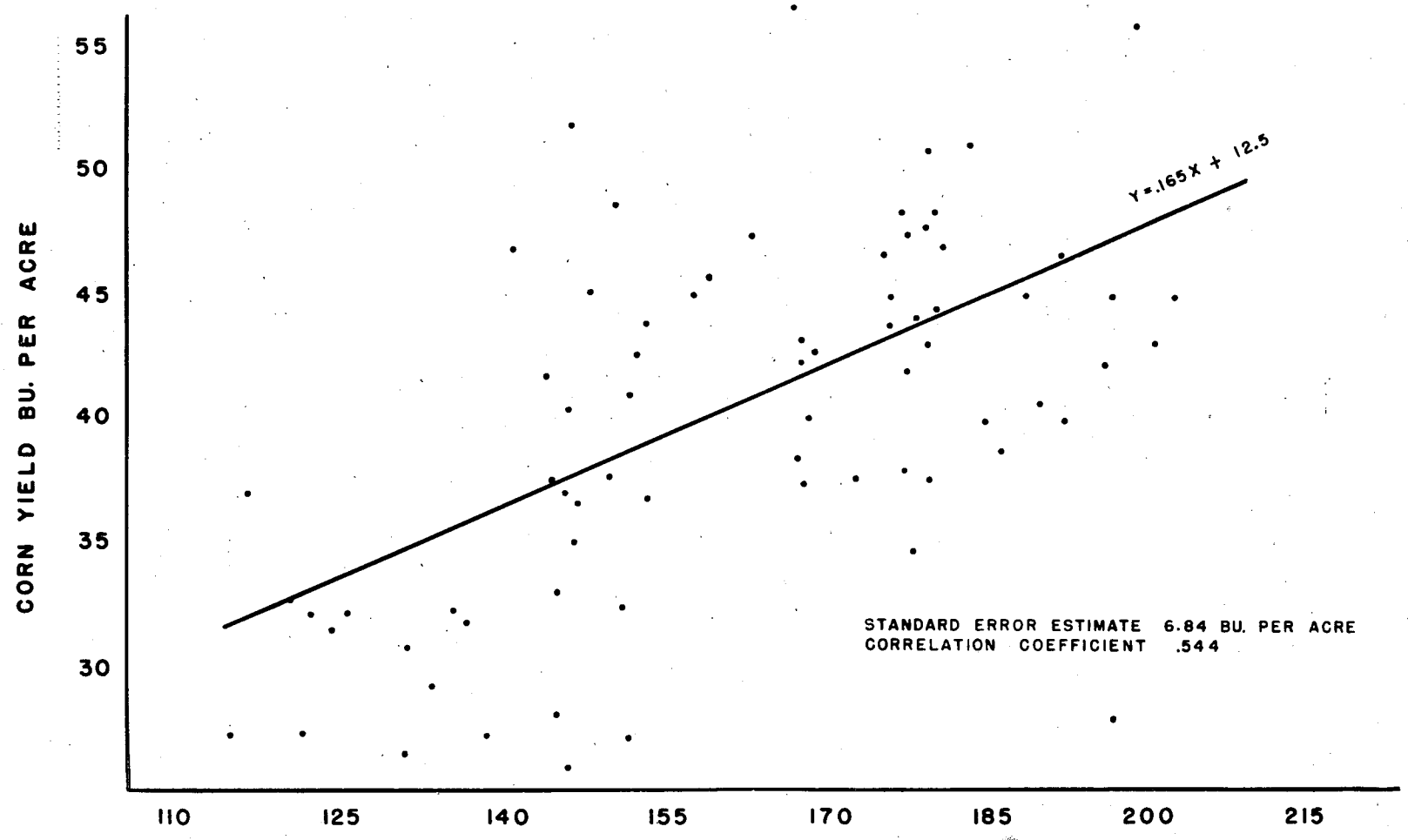


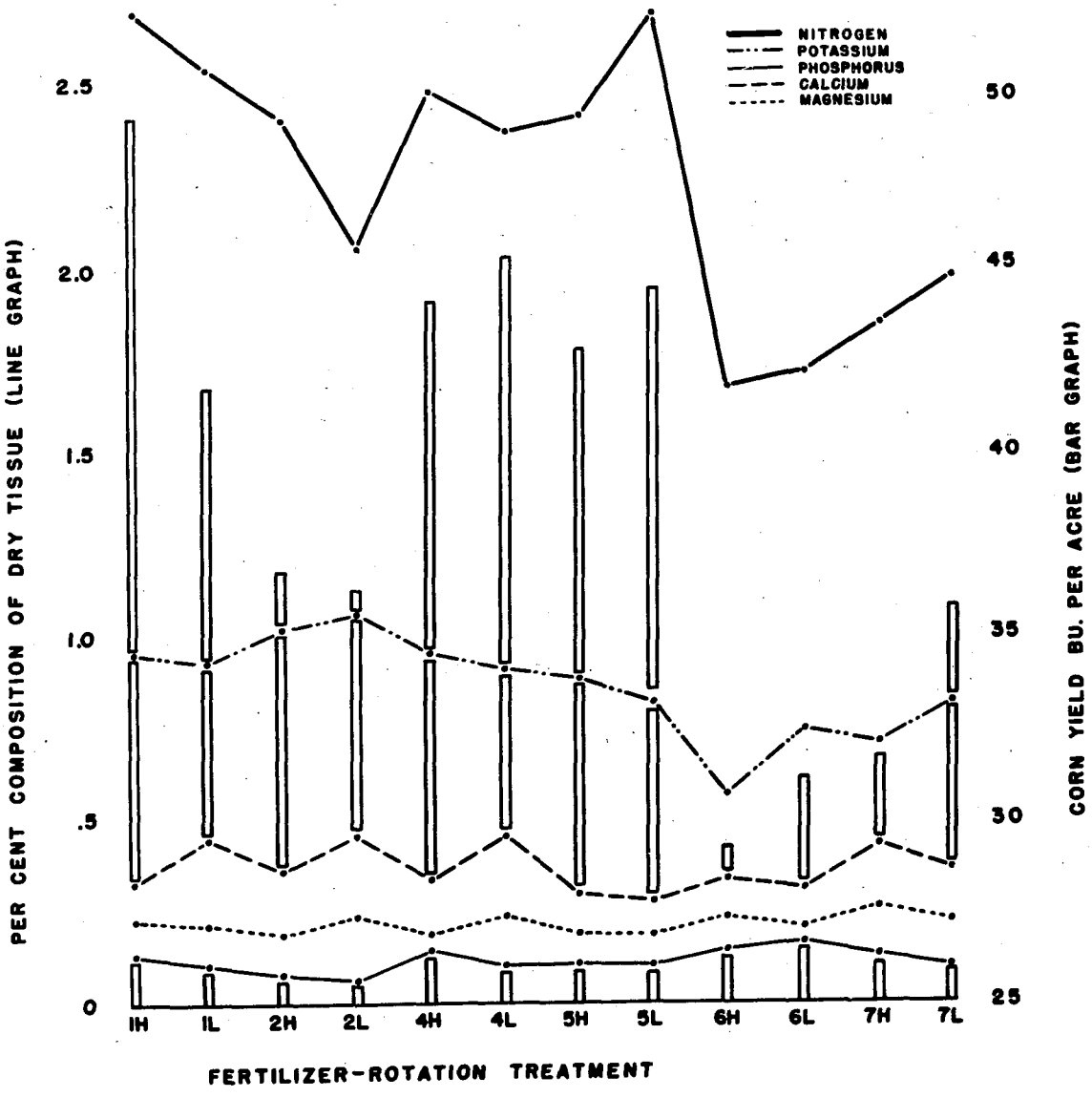
Table 21 Foliar Analysis of 1947 Corn Leaf Samples Having Least Regression from Predicted Values for Nitrogen Content-Yield Correlation, Brookston Soil, Ferden Farm

Rotation[1]	Pounds of 2-16-8 per rotation	Yield bu. per acre	m.e. per 100 grams[2]				
			N	P	K	Ca	Mg
1.	1000	48.0	192	12.6	23.1	14.7	17.4
	400	41.8	183	10.2	22.5	18.4	15.9
2.	1000	37.0	175	8.6	25.6	17.2	12.3
	400	36.1	152	6.8	28.1	19.4	17.7
4.	1000	44.2	178	12.0	23.1	14.7	14.3
	400	45.5	172	11.0	22.5	19.4	19.5
5.	1000	42.2	174	11.6	21.8	14.1	13.8
	400	44.4	193	10.2	18.7	13.4	12.5
6.	1000	29.1	121	13.2	14.4	16.3	18.4
	400	30.6	126	15.2	16.9	15.9	12.5
7.	1000	32.1	131	12.0	15.6	18.1	19.7
	400	36.3	137	8.2	17.5	14.1	18.4

1. Rotation crops and sequence are presented on page 50
2. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined according to Linder (22)

Figure 3.

FOLIAR ANALYSIS OF 1947 CORN LEAF SAMPLES HAVING LEAST REGRESSION FROM PREDICTED VALUES FOR NITROGEN CONTENT-YIELD CORRELATION. BROOKSTON SOIL



Rotation crops and sequence are presented on page 50
Fertilizer additions per rotation: H 1000 lbs. per acre (2-16-8)
L 400 lbs. per acre

strongly suggested that nitrogen was the limiting nutrient. In view of this, a foliar analysis for total nitrogen content was made on leaf samples obtained during the three week flowering period. This period appeared to be critical in the growth of the plant by the definite change in nitrate content for all treatments. From the time the first tassel began to form until all plants were in full tassel and silk covered almost a three week period, from July 19 to August 9. The correlation of the nitrogen content, as milliequivalents per 100 grams of dry leaf tissue, and yields of corn in bushels per acre is presented in figure 2.

In order to investigate the concept of nutrient balance as proposed by Shear and Crane (38) it was assumed that this nitrogen-yield correlation was valid. Since only one plot replication was sampled each week, it was necessary to use some means to determine which plot sample to use in a foliar analysis. This was done by selecting the plot sample taken in the three week flowering period which had the least regression from predicted values for the nitrogen content-yield correlation. A total analysis for phosphorus, potassium, calcium, and magnesium was made on these samples. The samples from the fall and spring plowed portions of each plot were composited for this analysis. These data are presented in table 21 and figure 3.

Results of 1948 Analyses: The results of the tissue tests on corn for the 1948 growing season were similar to those from the previous year. Phosphorus and potassium were

Table 22 The Yield and Nitrate Tissue Tests of Corn Plants During 1948 on the Field Experimental Plots Brookston Soil, Ferden Farm

Rotation[1]	Pounds of 2-16-8 per rotation	Yield bu. per acre	Green tissue test[2] third basal leaf sheath									
			July				August				Sept.	
			10	17	24	31	7	14	21	28	4	11
1.	1000	47.6	VH	M	M	M	-	-	-	-	-	-
	400	47.8	VH	M	H	L	-	L	-	-	-	-
2.	1000	61.0	VH	H	H	M	L	L	-	L	-	-
	400	62.0	VH	H	H	M	L	L	L	L	-	-
4.	1000	51.9	H	H	H	M	-	L	-	-	-	-
	400	54.6	H	H	M	M	-	L	-	-	-	-
5.	1000	34.7	M	M	M	L	-	-	-	-	-	-
	400	36.3	H	H	M	-	-	-	-	-	-	-
6.	1000	26.0	-	-	L	-	-	-	-	-	-	-
	400	20.5	L	-	-	-	-	-	-	-	-	-
7.	1000	45.6	H	H	M	M	-	L	-	-	-	-
	400	47.9	H	H	H	L	-	-	-	-	-	-

1. Rotation crops and sequence are presented on page 50
2. Legend: VH very high, H high, M medium, L low, - blank

Figure 4.

THE RELATION OF CORN YIELDS TO TOTAL NITROGEN CONTENT OF THE THIRD BASAL LEAF DURING BLOOM STAGE ON FERTILIZER - ROTATION EXPERIMENTS FOR 1948. BROOKSTON SOIL

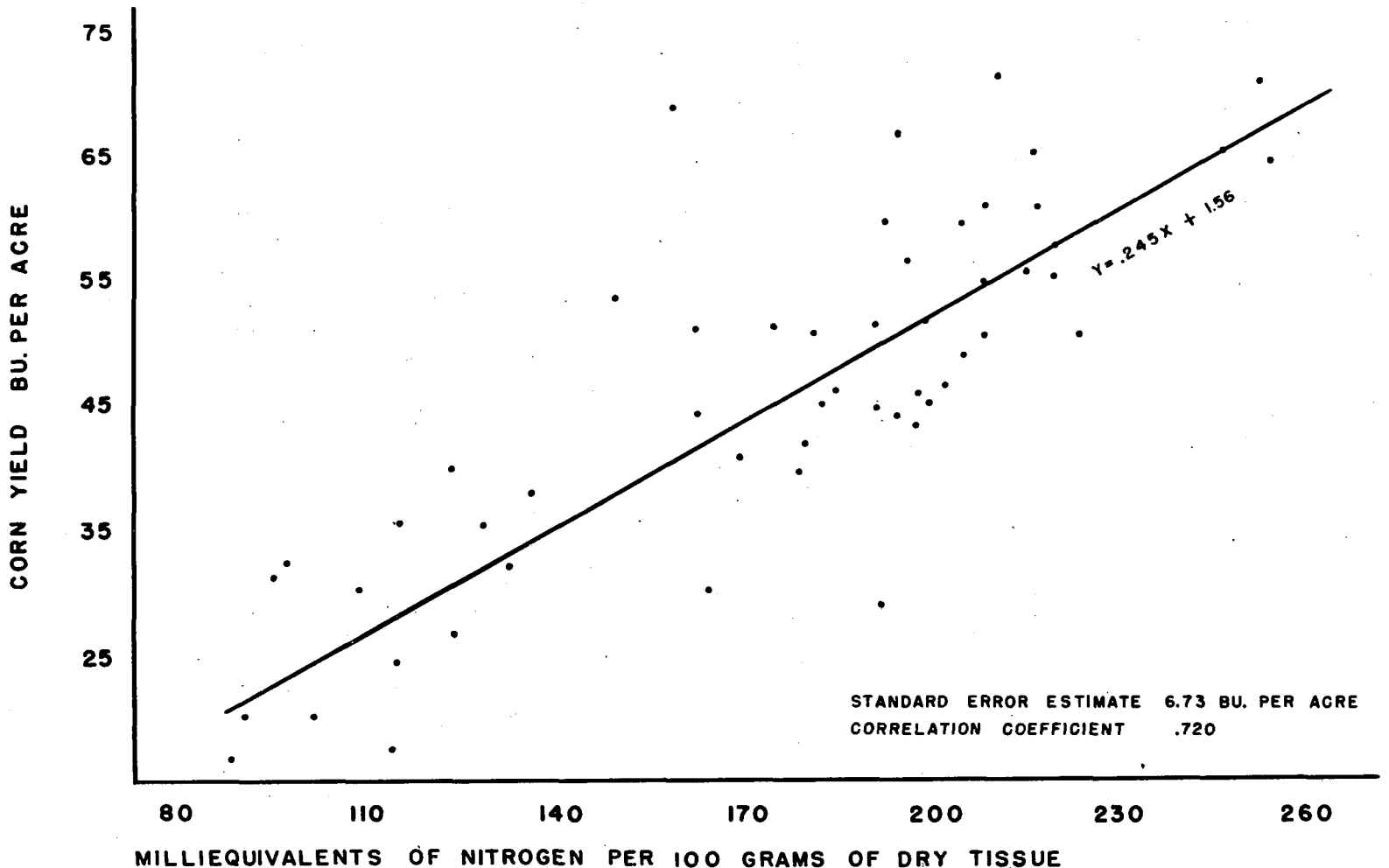


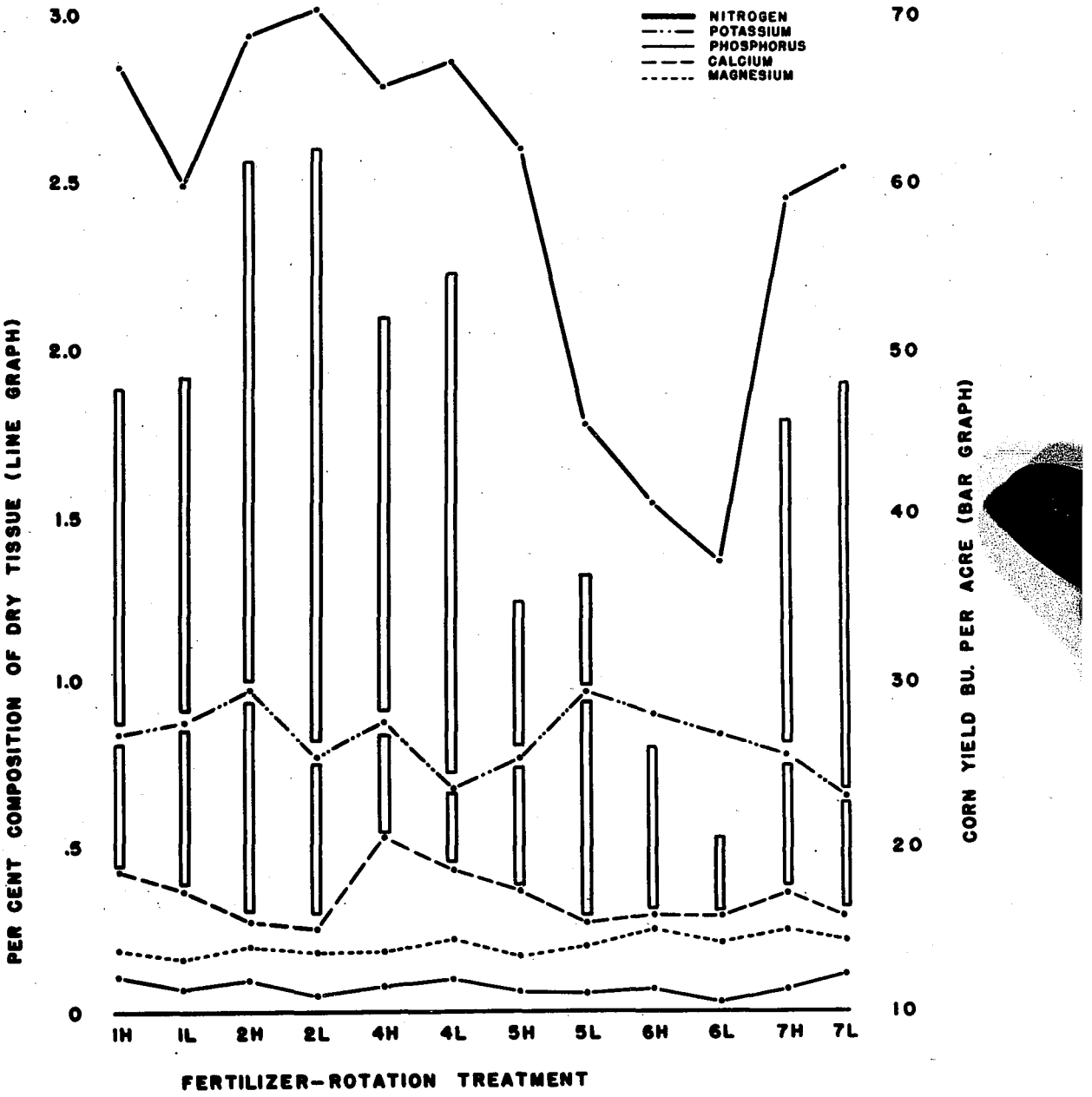
Table 23 Foliar Analysis of 1946 Corn Leaf Samples Having Least Regression from Predicted Values for Nitrogen Content-Yield Correlation, Brookston Soil, Ferden Farm

Rotation[1]	Pounds of 2-16-8 per rotation	Yield bu. per acre	m.e. per 100 grams[2]				
			N	P	K	Ca	Mg
1.	1000	46.9	202	10.9	19.4	20.3	15.7
	400	47.2	179	9.0	19.8	18.2	13.3
2.	1000	60.8	213	10.3	23.1	12.5	16.7
	400	62.2	216	8.4	17.5	11.9	15.7
4.	1000	51.0	197	9.2	18.8	27.5	15.8
	400	53.6	204	9.5	16.3	21.9	18.2
5.	1000	33.4	183	8.2	16.9	18.8	15.8
	400	36.3	124	6.9	23.1	12.2	16.7
6.	1000	25.6	102	8.1	22.5	12.5	19.3
	400	21.0	94	6.2	21.3	13.2	17.2
7.	1000	45.5	175	8.0	18.8	16.9	19.2
	400	48.7	179	8.7	14.5	14.4	18.2

1. Rotation crops and sequence are presented on page 50
2. Nitrogen determined by the Kjeldahl-Gunning method (1) other elements determined according to Linder (22)

Figure 5.

FOLIAR ANALYSIS OF 1948 CORN LEAF SAMPLES HAVING LEAST REGRESSION FROM PREDICTED VALUES FOR NITROGEN CONTENT - YIELD CORRELATION. BROOKSTON SOIL



Rotation crops and sequence are presented on page 50
Fertilizer additions of 2-16-8 per rotation are:
H 1000 lbs. per acre
L 400 lbs. per acre

were consistently high on all samples from all treatments throughout the year. Soluble nitrogen decreased as the season progressed up to the flowering period after which the corn plants from all treatments gave a blank test. In general, the more rapid the disappearance of soluble nitrogen the smaller were the corn yields (table 22).

Each plot was sampled each week during the two week flowering period of July 24 to August 7 and total nitrogen determinations were made on leaf samples obtained during this period. Equal portions of the plot sample were taken from the spring and fall plowed portions of the plot. These data, expressed as milliequivalents of nitrogen per 100 grams of dry tissue, were correlated with corn yields as bushels per acre (figure 4). The plot samples within this sampling period having least regression from predicted values for nitrogen content-yield correlation were analyzed for total phosphorus, potassium, calcium, and magnesium. These data are presented in table 23 and figure 5.

The 1947 season was characterized by a long cold wet spring which resulted in a sugar beet crop failure on all rotation plots of this field experiment for that year. As a result, the 1948 corn crop in rotation 2 actually follows a fallow year after turning under second year alfalfa. This was undoubtedly influentual in producing the very high yields from this rotation for the 1948 crop year.

Greenhouse Study of Rotation-Fertilizer Experiments

Soil from the experimental plots (Ferden Farm) were collected from three replicate blocks and replicate treatments were composited. Each composite sample was screened, air-dried, and placed in four gallon pots, sixteen kilograms per jar, with three replicate jars for each treatment. Sufficient water was added to bring the soils up to their moisture equivalent and planted with corn. One week after tasseling the plants were harvested and the dry weight of the plants recorded. Following the first crop, replicate pots were dumped, thoroughly mixed, replaced in jars and planted to a second crop.

The results of the first greenhouse crop indicated that treatments which produced the smallest yield in the field actually produced the largest growth in the greenhouse. This was particularly evident in rotations 6 and 7. This may be explained on the basis that the higher yielding plots had removed more available plant nutrients from the soil before the soil samples were taken. Nitrogen deficiency symptoms, which were so evident in the field, did not appear on the corn growing in soil from rotations 6 and 7.

These results indicated that the treatment these soils received in the greenhouse of being screened, dried, aerated, and maintained at optimum moisture content may have resulted in nitrification of the easily nitrifiable material present. This would have resulted in making available considerable nitrogen for plant growth.

Table 24 The 1947 Field Experiment Corn Yields and Greenhouse Yields on Soil taken from the Field Plots with no Additional Treatment Brookston Soil, Ferden Farm

Rotation[1]	Pounds of 2-16-8 per rotation	Yield bu. per acre[2]	Greenhouse yields[3]			
			1st crop		2nd crop	
			Height inches	Dry wt. grams	Height inches	Dry wt. grams
1.	1000	49.4	47	10.3	39	48.6
	400	42.0	44	8.8	38	42.9
2.	1000	36.4	68	7.4	41	57.4
	400	35.9	52	11.4	44	66.7
4.	1000	44.1	48	9.7	43	69.1
	400	45.3	39	4.7	40	50.9
5.	1000	43.3	48	8.8	42	66.0
	400	44.5	43	6.8	43	64.8
6.	1000	28.5	59	15.9	36	34.3
	400	31.1	53	12.0	31	33.3
7.	1000	31.5	66	13.6	37	52.9
	400	35.5	55	9.9	32	50.1

1. Rotation crops and sequence are presented on page 50
2. Field plot yields represent the mean of four replications
3. Greenhouse yields represent the mean of three replicate jars

The results of the different soil treatments on the growth of the second crop agreed rather closely with the field results of the previous year. Severe nitrogen deficient symptoms appeared on the plants growing on the soil from rotation 6, indicating that the nitrogen supply was rapidly depleted.

Nitrification Studies of Soil from the Fertilizer-Rotation Field Experiment

Soil samples were collected from the corn plots of the fertilizer-rotation field experiment three times during the 1948 growing season. These samples were taken from all four plot replications at the time of seed emergence, tasseling, and at the glazed stage of the corn plant. At each sampling period the replicate samples were screened, composited, and the moisture content determined.

The equivalent of 100 grams of oven dry soil was placed in covered tumblers after being brought to 20% moisture content. At the end of each incubation period of 3, 6, and 8 weeks, duplicate samples were leached with 300 cc of 4% KCl. The soluble nitrogen (including both ammonia and nitrate nitrogen) content of a 250 cc aliquot of this leachate was determined by the reduction method using Devarda's Alloy. Results of these determinations are presented in table 25.

These data indicate no significant differences in the nitrogen supplying capacity of the soils from the various plots. Increases in the amounts of extractable nitrogen were

Table 25 The Soluble Nitrogen Supplying Power of Soils Taken from the Corn Plots of the Rotation-Fertilizer Field Experiments, Brookston Soil, Ferden Farm[1]

Rotation[2]	Pounds of 2-16-8 per rotation	Extractable nitrogen, m.e. per 100 grams dry soil[3]														
		Sampled June 22					Sampled July 23					Sampled August 14				
		pH	start	3 wks	6 wks	8 wks	pH	start	3 wks	6 wks	8 wks	pH	start	3 wks	6 wks	8 wks
1.	1000	6.5	.29	.33	.40	.51	6.3	.05	.17	.44	.27	6.3	.05	.23	.37	.45
	400	6.5	.25	.28	.38	.46	6.4	.04	.14	.30	.23	6.5	.03	.17	.28	.39
2.	1000	6.5	.23	.25	.81	.32	6.3	.06	.16	.19	.36	6.6	.02	.18	.32	.48
	400	6.4	.28	.29	.37	.38	6.4	.09	.20	.20	.19	6.3	.03	.16	.16	.38
4.	1000	6.6	.23	.27	.25	.27	6.3	.02	.19	.24	.14	6.4	.05	.19	.36	.28
	400	6.5	.20	.27	.36	.32	6.5	.11	.17	.26	.16	6.6	.03	.15	.27	.41
5.	1000	6.6	.25	.30	.50	.26	6.5	.03	.15	.19	.20	6.5	.21	.17	.25	.29
	400	6.6	.22	.26	.48	.31	6.6	.02	.14	.21	.28	6.5	.05	.15	.30	.25
6.	1000	6.8	.23	.22	.27	.23	6.7	.04	.12	.24	.25	6.6	.06	.13	.25	.21
	400	6.8	.22	.21	.42	.39	6.7	.07	.11	.18	.19	6.7	.06	.09	.18	.24
7.	1000	6.6	.23	.28	.21	.45	6.5	.06	.15	.20	.20	6.4	.02	.12	.22	.27
	400	6.5	.18	.22	.21	.34	6.5	.09	.13	.17	.25	6.5	.04	.15	.20	.30

1. Data represents the mean of duplicate samples incubated at 20% moisture content.
2. Rotation crops and sequence are presented on page 50
3. Nitrogen determined in a soil extract of 4% potassium chloride

obtained after six weeks of incubation for all three sampling periods. In general, the soil of the first sampling produced the largest amounts of soluble nitrogen.

DISCUSSION

In this experiment soil testing procedures were applied to soil samples taken from rotation-fertilizer field experiments at their initiation and after seven years of continuous experimental treatment. Results of these soil analyses did not reflect sufficient deviation between treatments to account for the large differences in yields. Results of microbiological testing procedures did not show conclusive evicence as to differences in the nitrogen supplying status of the experimental plots. Tissue testing and foliar analysis studies were undertaken on the corn crop of these field experiments in an effort to account for crop yields in terms of plant nutrient status.

Greenhouse and field experiments were undertaken to compare tissue testing and foliar analysis as to precision in detecting soil fertilizer treatment. Especial effort was made to determine which portion of the plant is a reliable indicator of its nutrient status and to determine the time of sampling most suitable for these tests.

The third functioning basal leaf was selected to indicate the nutrient status of the plant at the time of sampling. This third functioning basal leaf may actually be numerically the fourth, fifth, or even the sixth leaf, in cases of severe deficiency, from the base of the plant. By this method the "morphologically homologous" leaves, described by Thomas (42) as desirable for such an analysis, are approached by disregarding those basal leaves whose physiological functions are affected by severe necrosis resulting from nutrient deficiency.

The sheath of this sampled leaf was used in tissue tests and the leaf blade was used in foliar analysis.

This selection was based on the fact that deficiencies of nitrogen, phosphorus, and potassium affect a more rapid senescence of older leaves (6, 28). Such deficiencies are related to a change of nutrient status at the initiation of the reproductive phase (16). These are the same basic considerations given by Thomas (43, 44, 46) and Chubb and Atkinson (6) in selecting the portion of the corn plant to use in foliar analysis. Tyner and Webb (49), however, used the sixth basal leaf in foliar analysis studies by reason of its ease in taxonomic location and its freedom from severe nutrient deficiency necrosis.

An investigation was carried out in the field on the R. L. Cook farm comparing tissue test results of the third functioning basal leaf and the leaf just below the tassel. The results showed a lower content of nitrates in the younger than in the older tissue but little difference was found in the contents of phosphorus and potassium. Harrington (18) and Thomas (45) concluded from similar experiments that meristematic tissue was less indicative of nutrient status than were mature leaves.

Weekly tissue tests were carried out on corn plants grown in the field in order to follow the nutrient status of the plants throughout the growing season. This sampling procedure was adapted in an effort to overcome the objections to tissue testing when interpretations of data are based on only one or

a few sampling periods such as that of Atkinson, et al (2) and Drake (12).

Because of the limited amount of plant material available for testing in the greenhouse experiments, the plants were sampled only once. These samples were taken when the plants were in full tassel and silk. The tissue test results were compared with foliar analysis of the same leaf samples.

Data from both the greenhouse and field experiments indicate that tissue testing is a dependable indicator of soil fertilizer treatment for the elements nitrogen, phosphorus, and potassium. Atkinson, et al (2) using Thornton's method (47) obtained similar results for nitrogen and potassium but were unable to detect phosphorus fertilizer treatments in their experiments.

In this study the results of tissue tests for calcium and magnesium were incomplete and inconclusive because the experiments were not designed for a detailed study of these elements. These results were incidental to the principal portion of the investigation.

Results of foliar analysis of corn plants from the field experiments on the R. L. Cook farm indicated that the physiological age at the time of sampling exerts considerable influence on leaf composition, even during the period deemed critical for sampling by Tyner (48). This would indicate that there must be a suitable and valid physiological basis, other than visual estimation, of obtaining foliar samples if interpretation is to be given practical consideration.

It is proposed in this investigation that green tissue testing be utilized for determining the period of sampling for foliar analysis, for indicating possible limiting factors in plant growth, and for substantiating the interpretation of analytical results.

Tissue tests were made on corn plants from experimental plots of the rotation-fertilizer experiments on the Ferden farm throughout the growing seasons of 1947 and 1948. These test results indicated that sufficient amounts of phosphorus and potassium were available throughout both growing seasons. Nitrate tests indicated that the nitrogen supply was the limiting factor in corn production on these experimental plots and that a sudden change in nitrate content took place during the period of flowering. All treatments did not flower on the same date nor is it to be expected that all plants were physiologically the same on different fertilizer-rotation plots on any one sampling date. However, it is obvious that plants will attain the same physiological stage within a certain period. The tissue tests indicated that a critical physiological stage of plant growth occurs within the period from the time the most advanced plants began to form tassels until all plants are in full tassel and silk.

From these data it was evident that the more rapid the disappearance of soluble nitrate-nitrogen the lower was the corn yield. This was true for both years investigated.

From the results of the tissue tests it might be assumed that corn yields on these experimental plots were a function

of nitrogen supply. To test the validity of this conclusion, the leaf samples collected within this critical period were analyzed for content of total nitrogen. It should be pointed out that this Brookston soil has a relatively high fertility level as compared with other leading agricultural soils in Michigan (table 3).

Correlations of total nitrogen content of leaf samples and corn yields gave positive coefficients considered reliable for two factor differential correlations (29).

The assumption is made from these data that this correlation is valid and that corn yields in this experiment were a function of nitrogen supply and utilization. By this reasoning the foliage samples having least regression from predicted values for nitrogen content-yield correlation were used for analysis of total phosphorus, potassium, magnesium, and calcium. Data from these analyses thus have a physiological basis for the application of such diagnostic interpretations as nutrient-element balance (38), critical element concentrations (50), and intensity of nutrition (44). None of these interpretations are attempted on the data herein reported.

In sampling for foliar analysis, Thomas (43, 44, 45) sampled corn plants four times during the growing season. From these data he interprets the "course of nutrition" as reflected by the amount of ions present, the concentrations of elements in relation to each other, and the changes in leaf composition at the different sampling periods. Chubb and Atkinson (8) sampled corn plants twice within a five

day period and interpreted the mean value of the analytical results in the manner of Thomas (44). Tyner and Webb (49) sampled corn plants at four stages of development and found a general decrease in the amount of elements present in the sampled leaf with increase in plant maturity.

The most general criticism of plant analysis studies has been directed toward the interpretation of analytical results (15, 41). Shear et al (38) have discussed in detail the critism for using leaf content of nitrogen alone as a criterion of nutritional status of plants. This investigation does not take issue with this viewpoint, nor with that of Thomas (42) who critizes tissue testing as a measure of element accumulation rather than utilization. This investigation actually supports the methods and concepts of both of these workers by placing foliar analysis interpretation, as advanced by these investigators, on a more sound physiological basis.

SUMMARY

The principal objective of this investigation was to make a comparative study of soil tests, foliar analysis, and plant tissue tests for indicating the nutrient status of corn plants.

Greenhouse experiments were conducted in which plants were produced varying greatly in their nutrient status by growing them on soils of widely varying fertility levels. A comparison of foliar analysis and tissue testing was made on corn, sampled periodically through the growing season, grown under field conditions.

A partial chemical analysis was made on soils taken from rotation-fertilizer field experiments at the beginning and after seven years of continuous treatment. Plant tissue tests and foliar analyses were made on the corn plants of these field experiments during the growing seasons of 1947 and 1948. Soils from these field plots were used for greenhouse studies and for nitrification experiments in the laboratory.

These studies may be summarized as follows:

1. Tissue testing is a reliable method for detecting soil fertilizer treatments for the elements of nitrogen, phosphorus, and potassium on soil types varying widely in physical and chemical characteristics.

2. A method for determining manganese in fresh tissue was found to give a reliable indication of the total manganese present in the foliage of white bean plants.

3. The third functioning basal leaf of corn plants, as used in the tissue tests and foliar analyses, was found to be a reliable indicator of plant nutrient status.

4. Soil analysis of samples taken from the fertilizer-rotation field experiments at the beginning and after seven years of continuous experimental treatment did not show differences sufficiently great to account for the differences in corn yields.

5. Experiments with soil from the fertilizer-rotation field experimental plots indicated that the lowest yielding plots in the field were temporarily capable of relatively higher production if given treatment suitable for nitrate production in the greenhouse.

6. Laboratory experiments measuring the nitrifying capacity of soil samples from the field experimental plots indicated no conclusive evidence as to accumulation of soluble nitrogen compounds.

7. Tissue tests throughout two growing seasons on these fertilizer-rotation field experiments indicated that nitrogen is the limiting factor in plant growth and that a definite change took place in the nitrogen status of plants with the initiation of the flowering period.

8. Foliar analysis for total nitrogen in leaf samples taken during the flowering period indicated positive correlation with corn yields.

9. The interpretation of foliar analysis data in terms of tissue test results is proposed.

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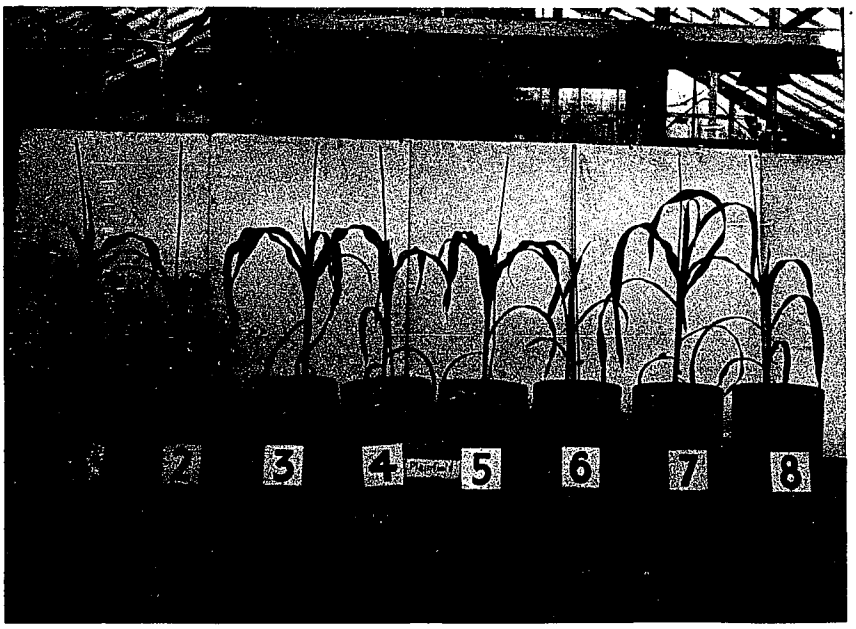

Plate 1. The effects of various fertilizer treatments on the growth of corn plants at 8 weeks, first greenhouse crop on Plainfield loamy sand. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K 5 NP, 6 NK, 7 PK, 8 NPK.

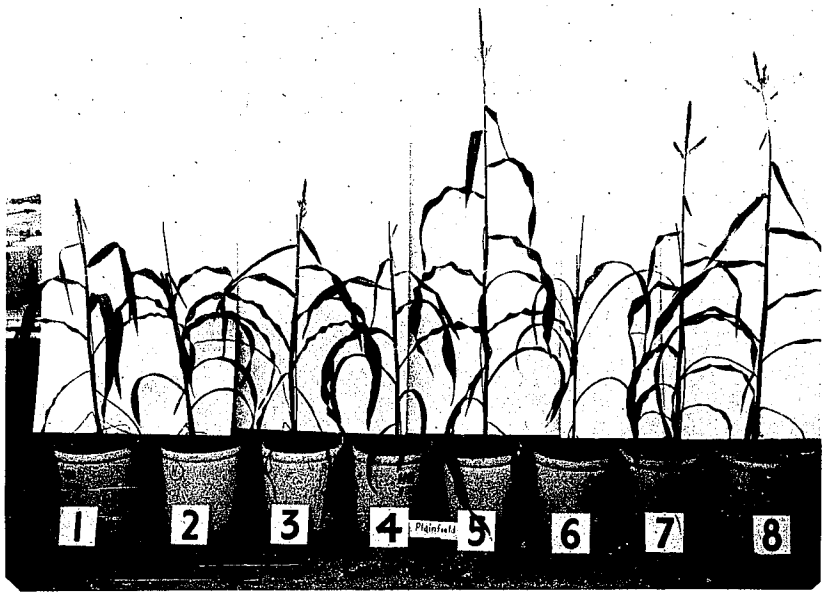

Plate 2. The effects of various fertilizer treatments on the growth of corn plants at 11 weeks, first greenhouse crop on Plainfield loamy sand. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K, 5 NP 6 NK, 8 PK, 7 NPK.

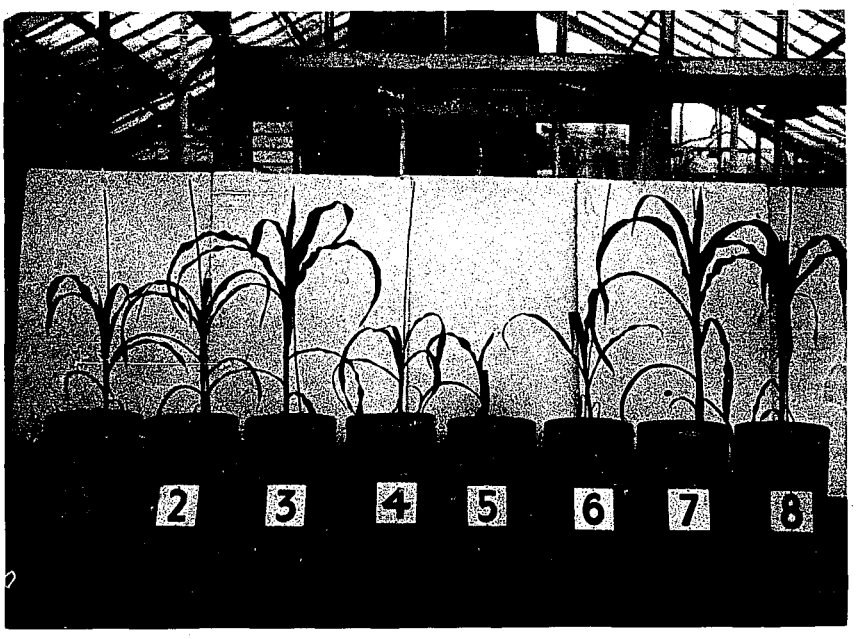

Plate 3. The effects of various fertilizer treatments on growth of corn plants at 8 weeks, first greenhouse crop on Miami sandy loam. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K, 5 NP, 6 NK, 7 PK, 8 NPK.

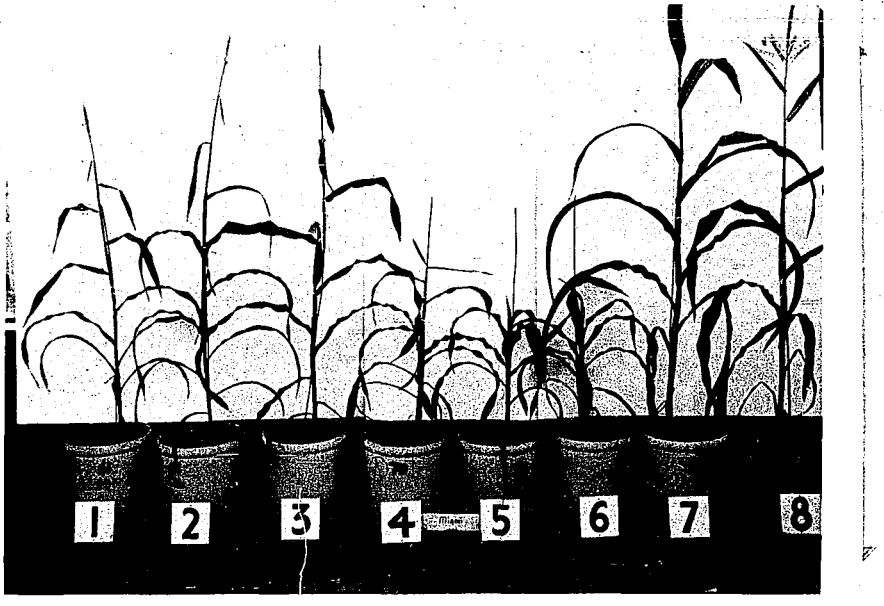

Plate 4. The effects of various fertilizer treatments on growth of corn plants at 11 weeks, first greenhouse crop on Miami sandy loam. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K, 5 NP, 6 NK, 7 PK, 8 NPK.

Plate 5. The effects of various fertilizer treatments on growth of corn plants at 8 weeks, first greenhouse crop on Conover sandy clay loam. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K, 5 NP, 6 NK, 7 PK, 8 NPK.

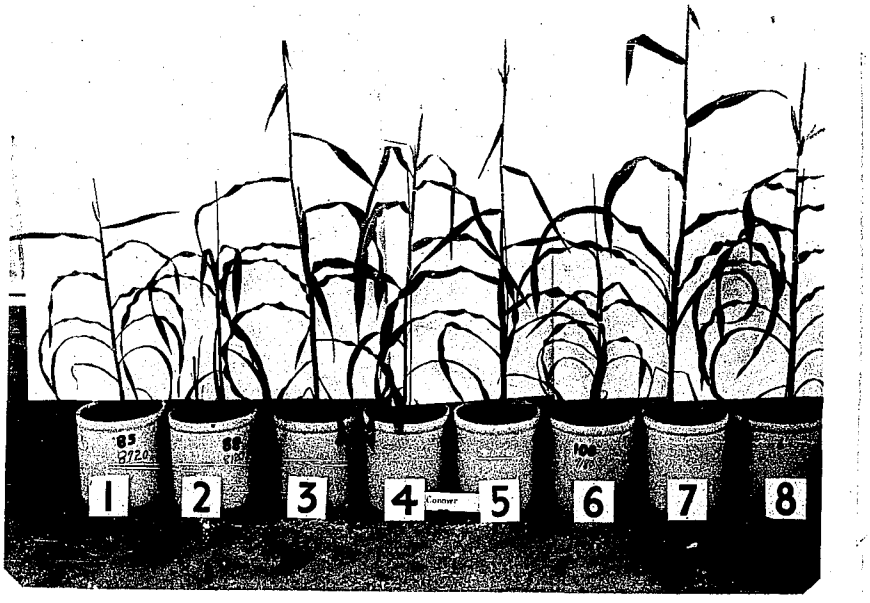

Plate 6. The effects of various fertilizer treatments on growth of corn plants at 11 weeks, first greenhouse crop on Conover sandy clay loam. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K, 5 NP, 6 NK, 7 PK, 8 NPK.

Plate 7. The effects of various fertilizer treatments on growth of corn plants at 8 weeks, second greenhouse crop on Miami sandy loam. Fertilizer treatments are: 1 none, 2 N, 3 P, 4 K.

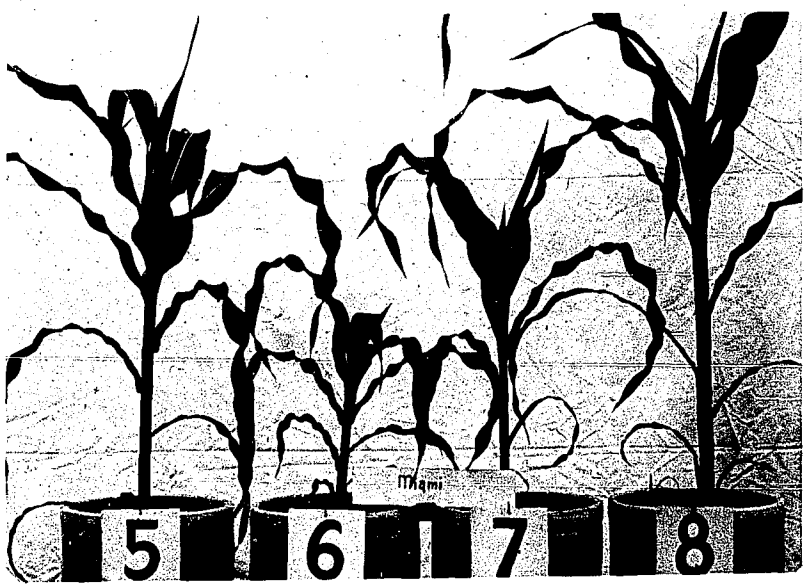

Plate 8. The effects of various fertilizer treatments on growth of corn plants at 8 weeks, second greenhouse crop on Miami sandy loam. Fertilizer treatments are: 5 NP, 6 NK, 7 PK, 8 NPK.

Plate 9. The effects of various fertilizer treatments on growth of corn plants at 8 weeks, second greenhouse crop on Conover sandy clay loam. Fertilizer treatments are: 5 NP, 6 NK, 7 PK, 8 NPK.

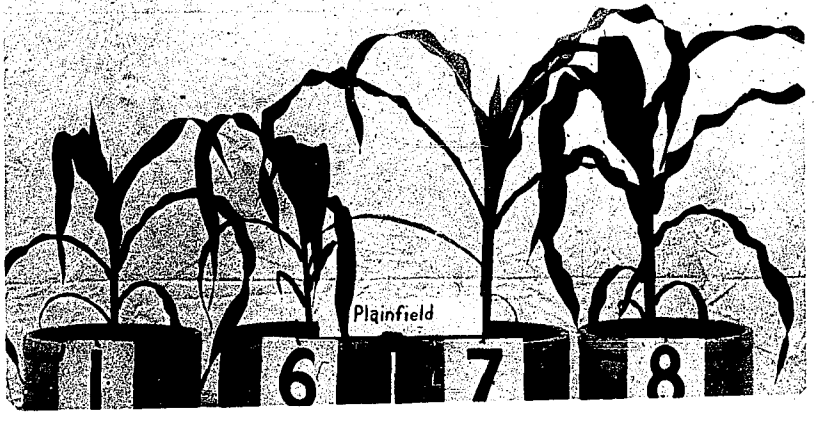

Plate 10. The effects of various fertilizer treatments on growth of corn plants at 8 weeks, second greenhouse crop on Plainfield loamy sand. Fertilizer treatments are: 1 none, 6 NK, 7 PK, 8 NPK.

Plate 11. The effects of various soil treatments on the growth of white bean plants at 8 weeks, first greenhouse crop on Granby sandy loam. Treatments are: 1 none, 2 lime, 3 $MnSO_4$; 4 NPK.

Plate 12. The effects of various soil treatments on the growth of white bean plants at 8 weeks, first greenhouse crop on Granby sandy loam. Treatments are: 1 none, 2 lime, 3 $MnSO_4$.
Note the manganese deficiency symptoms on jars 1 and 2.

Plate 13. The effects of various soil treatments on the growth of white bean plants at 8 weeks, first greenhouse crop on Granby sandy loam. Treatments are: CK none, L 2 tons $CaCO_3$, Mn 500 lbs. $MnSO_4$, NPK 1000 lbs. 10-20-20 per acre. Note the severe manganese deficiency symptoms on all plants except those receiving the $MnSO_4$ treatment.

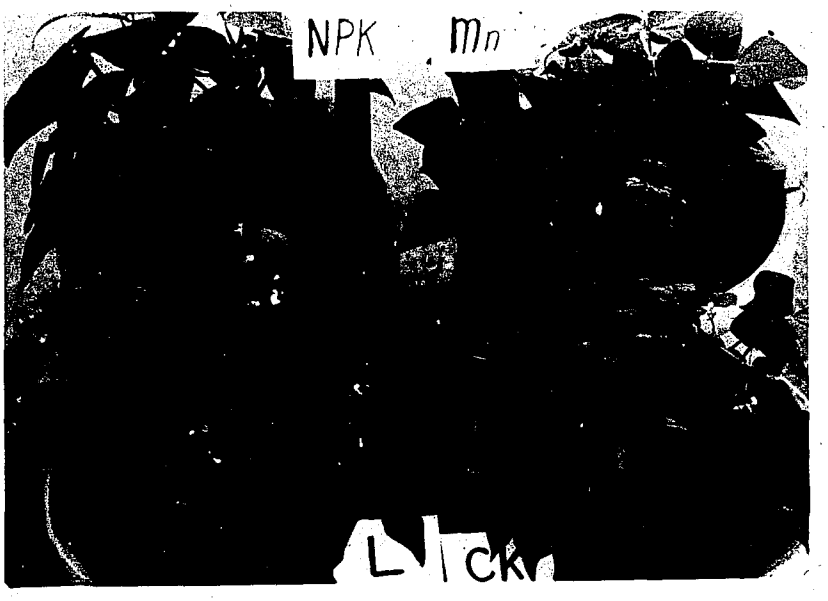

Plate 14. The effects of various soil treatments on the growth of white bean plants at 8 weeks, second greenhouse crop on Miami sandy loam. Treatments are: CK none, L 4 tons $CaCO_3$, Mn 500 lbs. $MnSO_4$, NPK 1000 lbs. 10-20-20 per acre. Note the severe manganese toxicity symptoms where the $MnSO_4$ was applied.

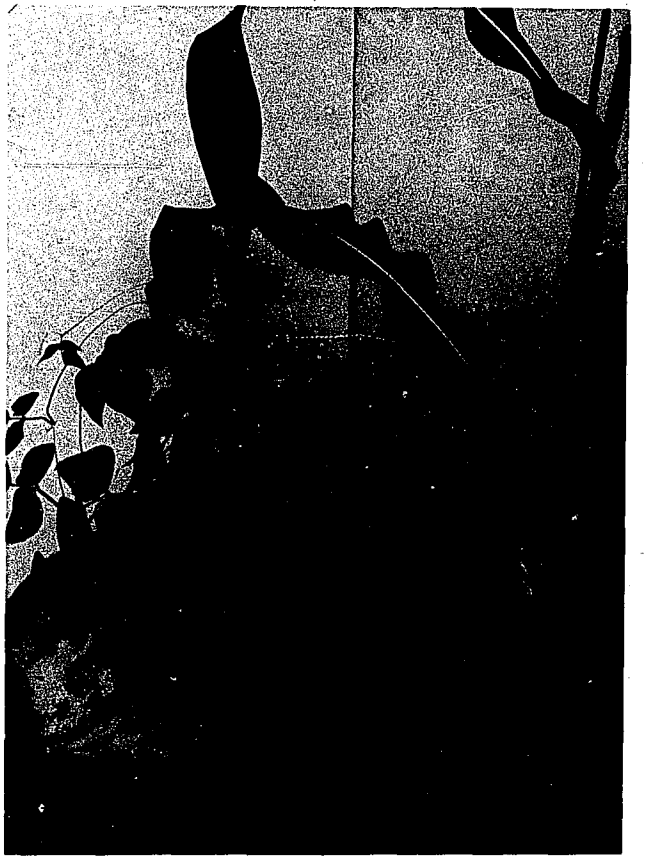

Plate 15. The effects of an unbalanced nutrient solution on growth of corn and white bean plants at 8 weeks. See table 13 for treatments and analytical results.
Note the magnesium deficiency symptoms on both plants.