

# THE RELATION BETWEEN A NITRATE-NITROGEN INCUBATION SOIL TEST AND GREENHOUSE AND FIELD RESPONSE OF CROPS TO ADDED NITROGEN

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# THE RELATION BETWEEN A NITRATE-NITROGEN INCUBATION SOIL TEST AND GREENHOUSE AND FIELD RESPONSE OF CROPS TO ADDED NITROGEN

Ву

Toshiaki Kinjo

# AN ABSTRACT

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### ABSTRACT

This study was undertaken to determine if a rapid laboratory nitrate-nitrogen incubation soil test could be correlated under Michigan conditions with crop response to added nitrogen and from different crop rotations.

Eight soils of varying texture and organic matter content were collected from central Michigan. Three of these soils were from plots of a rotation involving the use of green manure crops in growing sugar beets and field beans. The preliminary analyses were made for texture, soil reaction, phosphorus, potassium, residual nitrate, and organic matter content. The procedure of the nitrate-nitrogen incubation soil test developed in Iowa was followed with slight modification. Soils were incubated in the laboratory for a period of eight weeks under controlled conditions of moisture and temperature, and tests for nitrate-nitrogen were made every two weeks.

The quantity of nitrate-nitrogen released on moist incubation was found to be closely related to the organic matter content of the soils. There was a direct relation-ship between soil texture and release of nitrate-nitrogen on incubation; namely, the finer the texture, the greater the amount of nitrate produced.

In the greenhouse the response of the tomato, wheat, and field bean plants in terms of increased dry weight to added nitrogen was not significantly related to nitratenitrogen released on incubation. In addition, the correlation between the nitrate-nitrogen test values and the dry
weight of crops grown on soils receiving no nitrogen was
not significant.

The nitrate-nitrogen released from the soils on moist incubation was more closely related to the quantity of nitrogen taken up by the several crops than to their dry weights. However, only in the case of the wheat crop of the first series was a significant positive correlation coefficient obtained.

A negative relationship was found between laboratory nitrate-nitrogen incubation tests and nitrogen absorption response on all crops. However, correlation was not significant when all the soils were considered.

Yield response of corn grown on three different rotations to added nitrogen in the field in 1954 was not related to the nitrate-nitrogen incubation tests of soils from these rotation plots.

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#### INTRODUCTION

Nitrogen, an essential element for plant growth, plays a most important role in the fertility of soils and thereby in crop production. The need for additions of nitrogen to soil for continued high yields of crops has been emphasized in the last twenty-five years. For more than a century it was known that much of the benefit derived from animal manures and other organic residues applied to soils was due to their nitrogen content. During this time a general relationship between soil organic matter and plant requirements for nitrogen in the form of organic or mineral fertilizers was noted. Although numerous exceptions are evident, crop response to nitrogen is generally greater on soil low in organic matter.

Because ammonification and nitrification, the processes leading to the production of nitrates, are biochemical in nature, no great degree of success has been achieved using chemical soil tests to estimate the nitrogen supplying power of soils. Even biological tests are subject to considerable error since microbial activity in soils is influenced to a great extent by moisture and temperature. Thus, seasonal fluctuations of these factors are important reasons why empirical tests for nitrogen needs of field soils are often inaccurate.

Nevertheless, agronomists have spent considerable effort in attempting to develop reliable methods of determining nitrogen availability in soils. A reasonably rapid nitrogen incubation test has recently been developed by the Iowa Agricultural Experiment Station. The results of this test have been correlated with the response of corn to added nitrogen.

The purpose of this study is to determine whether such a nitrogen incubation test under Michigan conditions can be correlated, first with crop response to added nitrogen fertilizers, and secondly, with different crop rotations in which the nitrogen needs of specific crops are quite different.

# REVIEW OF LITERATURE

Many studies of the influence of nitrate-nitrogen on crop production have been carried out since the beginning of the twentieth century.

In 1917, Lipman and Burgess (19), working on the problem of ammonification versus nitrifiability as a test for the relative availability of nitrogenous fertilizers, concluded that the nitrate form of nitrogen was of paramount importance for the nutrition of most plants. Agronomists both in Europe and America at that time came to this same conclusion. Hence nitrifiability was adopted as the criterion of availability of organic nitrogenous fertilizers rather than ammonifiability in normal soil conditions. As Gainey (13) pointed out, ammonia accumulates under conditions such as poor drainage or water-logging.

Experimenting on Hawaiian soils in 1918, Burgess (8) attempted to determine the possibility of predicting, with some degree of accuracy, crop yields from microbiological data. Criteria used in routine and comparative tests were ammonification, nitrification, and nitrogen fixation. Results of these experiments showed that the nitrification process was by far the most accurate available biological measure for evaluating soil fertility conditions and

Several types of procedure for determining nitrogen availability in soils have been advocated. The method Woodruff (29, 30) has described is based on the amount of easily oxidizable soil organic matter. He stated that each percentage of organic matter in the surface plow depth of soil corresponded to about 1000 pounds of total nitrogen per acre. If all organic matter were alike, Woodruff concluded that the liberation of nitrogen from organic matter in a form available to plants would be proportional to the amount of organic matter in the soil. The fraction that decomposes to liberate nitrogen for a particular crop

depends upon such factors as the season of the year in which the crop grows, the temperature and moisture condition of the soil throughout the growing period of the crop, and the textural and structural condition of the soil.

Thus, it is necessary to know how much nitrogen is released from organic matter under specific environmental conditions.

Another group of methods involves the chemical extraction of a fraction of the soil nitrogen. Truog (26) proposed a procedure involving alkaline oxidation of soil organic material.

A third procedure, which has received recent widespread attention, is that of biological mineralization of nitrogen from soil during a controlled incubation period (10, 25).

Since nitrification is biochemical in nature, many complex and interacting factors have an influence on the rate of nitrate production. Such factors are amount and kind of organic matter, nature of the residue of the previous crop, moisture and temperature fluctuation patterns, nature of soil microflora, aeration, soil reaction, mineral nutrient status, and other biotic properties of soils.

One of the most important factors which influences the production of nitrates is the presence of nitrifying bacteria as well as ammonifying bacteria. Fortunately, it has been shown by Gainey (13) and others that all cultivated soils under normal conditions contain active nitrifying

organisms. Specific organisms responsible for nitratenitrogen formation from ammonia are two groups of bacteria
called Nitrosomonas and Nitrosococcus. Bacteria of the
group known as Nitrobacter carry out the formation of
nitrate from nitrite compounds.

Nitrifying bacteria are aerobic organisms. They obtain their energy by the oxidation of certain elements and compounds. Thus, the amount of nitrate formed is affected in part by the amount of oxygen available in soil air. Waksman (27) pointed out that the quantity of oxygen consumed during nitrification was closely related to the amount of nitrogen nitrified. The data obtained by Fathi and Bartholomew (9) from studies on influence of oxygen concentration of oxygen for nitrification in soil is about that contained in ordinary air, that is, approximately 21 percent by volume. Approximately half as much nitrate was produced when the oxygen concentration was maintained at 2.1 percent as at 20 percent. Reducing the oxygen concentration from about 20 to 11 percent had only a negligible influence upon the rate of nitrification. Jodidi and Wells (17) found that several Iowa soils, when soil air was analyzed in July, 1911, contained 20.39 percent of oxygen in the soil air at 7-inch depth, while data by Boynton (5) on a New York soil showed it to have 11.60 percent of oxygen by volume at 6-inch depth when tests were made after frequent showers in July.

Thus, oxygen is not a limiting factor for nitrification under normal soil conditions.

Another major factor affecting the numbers and activities of nitrifying organisms is soil moisture. Major fluctuations in soil moisture primarily govern soil aeration. The nitrification process is retarded by very low as well as by very high moisture conditions (20). According to Fitts, Bartholomew, and Heidel (12), 100 centimeters of water tension provided the optimum moisture for the production of nitrates under laboratory conditions. Depending on the texture of the soils the tension resulted in 25 to 35 percent moisture. This was found to be slightly above the field capacity. These Iowa workers also pointed out that adjustment to 25 percent moisture gave lower and less consistent results than wetting and removal of excess water by tension. With soils of widely varying texture, the uniform percentage adjustment would be expected to be even less favorable.

The effect of temperature on production of nitrates is also very striking. According to Waksman (27) nitrate formation was noticeable at 5°C, became prominent at 12°C, and reached a maximum at 37°C. Higher temperatures, such as 45°C, exerted an injurious effect. Panganiban (21) and Russell, Jones, and Bahrt (22) found the optimum temperature for nitrification to be 35°C.

It is known that activities of the nitrifying organisms are to some extent dependent upon the pH of the medium in which they function. The limiting acidity for the development of these organisms, as Waksman (27) concluded, is pH 3.7 to 4.0, whereas their optimum reaction is at pH 6.8 to 7.3. Calcium carbonate is generally used as the compound to neutralize acid soils. However, Brown and Hitchcock (7) found that presence of an excess of calcium carbonate in concentrations up to 1.5 to 6.0 percent proved toxic in normal Wyoming soils. Halvorson and Caldwell (15), studying factors affecting the nitrate producing power of some Minnesota soils, also came to the conclusion that the presence of a large amount of calcium carbonate inhibited nitrification.

Baldwin, Walters, and Schmidt (3) noted that nitrification in some normal soils was stimulated by the addition of phosphate and potash. This stimulation was still greater when nitrogen was used in combination with phosphorus and potassium.

In regard to nitrification of organic matter, Whiting (28) stated that, even with carbon in a resistant condition, nitrate was formed rapidly if organic or inorganic nitrogen was added. About the same time Brown and Gowda (6) recognized that sodium nitrate increased the nitrate content of the soil and enhanced its nitrifying power.

Organic matter, applied as animal manure or green manure, definitely influences nitrification process, according to Brown and Gowda (6), and Kubota, et al (18). In studies by Brown and Gowda (6), the nitrifying power of a soil was increased by manure applications up to 36 tons per acre, but nitrate accumulation was not proportional to the amount of manure used. These investigators found that clover hay had little effect on the nitrate content of the soil, but it increased the nitrifying capacity.

Gainey (14) reported a specific instance wherein the ability of a soil to accumulate nitrate-nitrogen rapidly under laboratory conditions appeared to be more intimately associated with a certain small fraction of soil nitrogen than with the total quantity. He worked on the problem of total nitrogen as a factor influencing nitrate accumulation in soils. Gainey (14) Yound that when the nitrogen content and the nitrate producing power of a large number of soils were determined and the data grouped on the vasis of the nitrogen content of the soils, and averaged, an almost perfect direct relationship appeared to exist between total nitrogen content and the accumulation of nitrates. when the data were studied in detail, it was evident that there were a large number of irregularities in the direct relationship between total nitrogen and nitrate production. It was found that the nearly perfect correlation coefficient of 0.988 + 0.006 obtained from average values was reduced

to a non-significant value of 0.368 + 0.052 when it was calculated from the individual samples. Jensen (16) stated that no correlation could be established between total nitrogen and nitrate content in soils. Gainey (14) concluded that as long as the quantity of easily nitrifiable nitrogen varied in soils, a perfect correlation could not exist between total nitrogen and nitrate accumulation, at least during a limited incubation period. Yet, Allison and Steeling (1) maintained that wide variations between total nitrogen and production of nitrates of many individual soils were due, in part, to the use of natural soils without additions of microorganisms, lime, phosphate or other substances that may be limiting for microbial growth. In their work, nitrate formation from soil organic matter was directly correlated with total soil nitrogen at all incubation periods in both limed and unlimed soils. Allison and Steeling (1) emphasized that the quantity of organic matter in a soil was a more important factor than either quality or source of organic matter.

Recently, a nitrate-nitrogen incubation soil test was developed at Iowa Agricultural Experiment Station as a means of evaluating the nitrogen supplying power of a soil and predicting the magnitude of crop response from nitrogen application. Although there was considerable seasonal variability, the data obtained from the work of Fitts, Bartholomew, and Heidel (11) showed that nitrification rate,

as determined by their incubation soil test, gave results that were correlated with response to application of nitrogen fertilizer on corn when the stand count was considered. The regression coefficient between nitrification rate and response of corn to nitrogen fertilizer on fields with thin stands was found to be -0.630, significant at the one percent level.

#### MATERIALS AND METHODS

#### Soils Used

Eight different surface soils varying in organic matter content and texture were used for this experiment. including Oshtemo sand, Hillsdale sandy loam, Conover loam, Miami sandy loam, Brookston loam, and three samples of Sims The three Sims soils were obtained from different experimental rotation plots at the Ferden farm in Saginaw County. The purpose of the rotation experiment on the farm was to compare seven systems of farming at two fertility levels. One-half of each rotation plot was at low fertility level, and 800 pounds of 5-20-10 fertilizer per acre was applied in 1951. The other half of each rotation plot was at high fertility level, and 1600 pounds of 5-20-10 fertilizer per acre was applied in 1951. The five-year crop sequence for rotation 1 was alfalfa brome, alfalfa brome, corn, beets, and barley. Ten tons of manure per acre for corn or beans was applied in rotation on plots of rotation 1. In rotation 4, the crop sequence was oats, alfalfa brome, corn, beets, and barley, and seven tons of manure per acre was applied for corn. The crop sequence for rotation 6 was beans, wheat, corn, beets, and barley. This is a cash crop rotation without green manure crops, and with no manure

applied, although straw was plowed under. All the remaining soils were collected from farm areas in lower Michigan and were in storage in an air dry condition at the college barn. Oshtemo sand, Conover loam, and Miami sandy loam soils came from the Rose Lake Experiment Station, the R. Miller farm, and the R. L. Cook farm, respectively, in Clinton county.

A sample of Brookston loam was taken from the Britton farm in Lenawee county. Hillsdale sandy loam soil was collected from the K. Lawton farm in Ingham county.

The texture of each soil was determined with the hydrometer method developed by Bouyoucos (4). The results are shown in Table I. The soils were analyzed for pH, available phosphorus, available potassium, nitrate, and organic matter. The results are shown in Table II.

# Laboratory Studies

Laboratory experiments consisted of three parts:

(a) preliminary analyses of the eight soils, (b) nitrateni trogen incubation soil tests, and (c) determination of
the total nitrogen content of greenhouse crops by the

Kjeldahl procedure.

Soil reaction values were determined using a 1:1 soil to water suspension and a Beckman pH meter. Available phosphorus and potassium were measured according to the reserve method of Spurway and Lawton (24). Estimates

TABLE I

THE SAND, SILT, AND CLAY CONTENT OF SOILS

USED IN THE EXPERIMENT

Soil Series	Sand percent	Silt percent	Clay percent	Textural Class
Oshtemo	89.67	9.30	1.03	sand
Hillsdale	69.31	23.12	<b>7.</b> 57	sandy loam
Brookston	46.01	34.62	19.37	loam
Conover	52.40	32.39	15.21	loam
Miami	56.16	31.22	12.62	sandy loam
Sims (rotation 1)	48.22	29•93	21.85	loam
Sims (rotation 4)	46.71	29.42	23.87	loam
Sims (rotation 6)	46.21	29•39	24.40	loam

TABLE II

ANALYSES OF EIGHT SOILS FOR SOIL REACTION, PHOSPHORUS,

POTASSIUM, NITRATE-NITROGEN, AND ORGANIC MATTER

Soil Type	р <b>Н</b>	P* (pound	K*	NO <sub>3</sub>	Organic Matter (percent)
Oshtemo sand	5.8	43.6	<b>1</b> /1/1	1.2	1.30
Hillsdale sandy loam	5.3	28.0	156	4.4	1.86
Brookston loam	6.4	247.5	224	7.2	6.89
Conover loam	7.2	47.5	104	27.6	3.92
Miami sandy loam	6.5	19.0	90	27.6	2.48
Sims loam (rotation 1)	6.6	81.5	188	1.44	4.86
Sims loam (rotation 4)	6.6	127.5	194	1.20	4.67
Sims loam (rotation 6)	6.7	110.0	170	0.48	4.18

<sup>\*</sup>Spurway reserve test

of nitrate-nitrogen were obtained using the phenoldisulfonic acid procedure, which was modified by Fitts, et al.(12) to speed up the operations of the old procedure. Organic matter content was determined by dry combustion method. The results of these analyses are in Table II.

The nitrate-nitrogen incubation tests were carried out using the procedure developed by Hanway and Stanford (25). at Iowa State College. Thirty-five milliliter Gooch crucibles were used instead of thirty milliliter glass tubes. Gooch crucibles were kept in a desiccator, which had one inch of water at the bottom in order to maintain optimum moisture of the soil-vermiculite mixtures. desiccator was placed in an oven maintained at a temperature of 35° C + 2° C. The soils were aerated three times a week to supply sufficient oxygen for optimum nitrification. After leaching and suction, these soils were stirred with a pin to prevent compaction and to maintain soil structure. By so doing, it was much easier to leach out soils without any puddling after a two-week period of incubation. The total incubation period was limited to eight weeks because the production of nitrate-nitrogen was negligible after this time. Production of nitrate-nitrogen was measured every two weeks by the phenoldisulfonic acid method. Duplicates of all soil samples were used, and the average results calculated.

For the determination of the total nitrogen content of greenhouse crops, Gunning's modified method of the Kjeldahl procedure was followed. One gram portions of ground plant tissue dried at 65° C were used for the determination of total nitrogen. The amount of total nitrogen absorbed by the plants in each pot was calculated by multiplying the value of dry weight by percent of total nitrogen.

## Greenhouse Studies

Two pot experiments were carried out in the greenhouse to determine the response of several crops to added nitrogen. Three levels of nitrate were obtained by using two rates of added nitrogen, 50 and 150 pounds of nitrogen per acre and a no nitrogen treatment. Ammonium nitrate, applied at the rate equivalent to a two million pounds per acre of 6-inch depth soil, was used to supply the nitrogen. An amount equal to two hundred pounds per acre of  $P_2O_5$  and  $R_2O$  in the form of 0-20-20 fertilizer was thoroughly mixed with the soil in each pot to supply optimum amounts of phosphorus and potassium.

Three replications of each treatment were set up in one-gallon glazed jars. The position of the jars was rotated several times during the experiment. These jars were filled with screened, air-dry soil to within one inch of the top. The amount of soil of each type varied, and

fertilizers were applied accordingly, on a two million pounds per acre surface soil basis.

Spring wheat and tomato seeds were planted on the 10th of November, 1954, for the first series of greenhouse tests. Eight plants of spring wheat per pot and one plant of tomato per pot were grown. Since vegetative growth was more important than seed or fruit formation, the wheat plants were cut close to the soil when they started to head out, and the tomato plants were harvested at about the time flowering began. Plants harvested from each pot were put in separate bags and dried in an oven at 65° C.

In the second series of greenhouse studies, field beans were grown in those soils which had previously grown spring wheat, and spring wheat was grown in those soils which had grown tomatoes. The same rates of nitrogen application as in the first series of tests were used. However, the amount of phosphorus and potassium was reduced to one-half of the previous application. Before planting the second series, soils from all pots were dumped out, and the large roots were removed to avoid addition of organic matter to the soils.

Eight plants of spring wheat and four plants of field beans per pot were allowed to grow until they flowered, at which time the entire above ground parts of the plants were harvested. Before the second series of greenhouse experiments were set up, the soils, in which the first series of crops were grown, were analyzed for nitrate-nitrogen left in the soil.

#### RESULTS AND DISCUSSION

The results obtained from the first series of nitratenitrogen incubation soil tests are given in Table III.

Easily nitrifiable nitrogen was mineralized to a great extent in a two-week period of incubation under controlled
optimum conditions. After this period, nitrogen in more
resistant compounds was nitrified slowly in small amounts
without much variation over the rest of the incubation period.
The Brookston loam, which contained a relatively large amount
of organic matter, provided the only exception. It did not
fit into the pattern of nitrification of the rest of the
soils. It can be noted that a rather large amount of
nitrate-nitrogen was liberated from Brookston loam soil
over the period of four weeks. Figure 1 shows the trend of
nitrification of each soil during an eight-week period of
successive incubation.

In order to evaluate the relationship between the organic matter content and the production of nitratenitrogen in each soil after eight weeks of success incubation, a graph (Figure 2) was plotted with the values of
total nitrate-nitrogen produced in terms of pounds per
acre against the organic matter content of each soil. A
close relationship between the organic matter content and

TABLE III

THE PRODUCTION OF NITRATE-NITROGEN IN SOILS OF FIRST
SERIES DURING SUCCESSIVE MOIST INCUBATION PERIODS

Soil Type	Incubation Period							
	2nd week	4th week	6th week	8th week	Total Nitrate in 8 weeks			
	(pou	nds per a	cre of ni	trate-ni	trogen)			
Oshtemo sand	9•2	9.6	4.8	4.8	28.4			
Hillsdale sandy loam	13.6	5.6	5.6	7.8	32.6			
Brookston loam	55.2	29.6	12.4	12.4	109.6			
Conover loam	20.0	8.0	8.4	6.4	42.8			
Miami sandy loam	28.4	9.6	6.8	5.2	50.C			
Sims loam (rotation 1)	33.2	7.6	3.2	7.2	51.2			
Sims loam (rotation 4)	29.2	6.4	5.2	8.8	49.6			
Sims loam (rotation 6)	25.6	8.0	1.6	4.8	40.0			

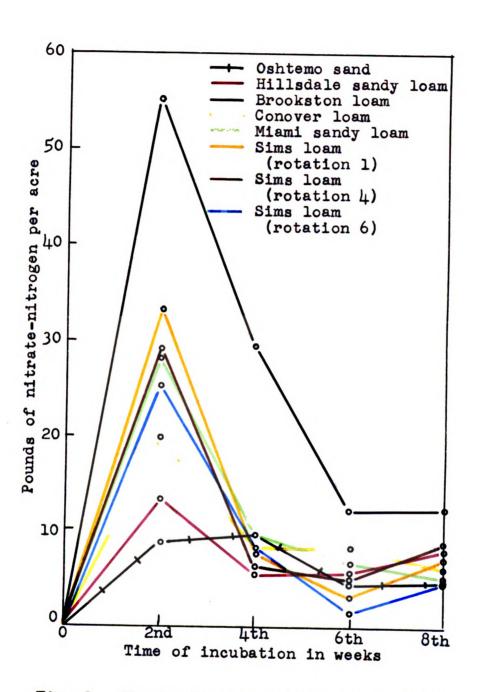


Fig. 1. The production of nitrate-nitrogen in soils of first series during successive moist incubation periods.

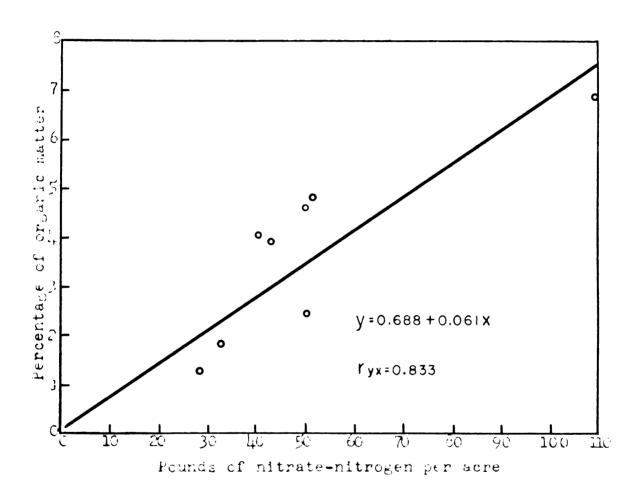


Fig. 2. Relationship between organic matter contents of the eight solls and pounds per acre of nitrate-nitrogen produced in eight weeks.

apparent. A correlation coefficient of 0.833, significant at the one percent level, verifies the relationship. In their studies of Marshall silt loam in Iowa, Andharia, Stanford, and Schaller (2) found a highly significant correlation between the nitrate-nitrogen and the organic matter content.

Table IV shows the results of the first series of the greenhouse experiment on wheat plants, including the average dry weight per pot, average nitrogen content per gram of plant sample, average total nitrogen absorbed per pot, and the response of wheat plants in terms of nitrogen absorption to additions of 50 pounds and 150 pounds of nitrogen per acre.

Wheat plants grown on all soils, except Miami sandy loam with 150 pounds per acre of nitrogen application, and Sims loam (rotation 1) with 50 pounds per acre of nitrogen, responded in yield to added nitrogen, though the degree of response varied with each soil.

The restricted root development due to compactness of the soil may have resulted in the non-response in yield of the above-stated two soils. But in the case of Sims loam soil (rotation 1), treated with 50 pounds per acre of nitrogen, the nitrogen absorption was greater than for the non-treated soils.

Growth and nitrogen absorption response to 150 pounds per acre of nitrogen application for Hillsdale sandy loam,

TABLE IV

THE DRY WEIGHT, NITROGEN CONTENT, TOTAL NITROGEN
ABSORBED, AND NITROGEN ABSORPTION RESPONSE
OF WHEAT PLANTS OF FIRST SERIES

Soil Type	Rate of Nitrogen	Average Dry Weight	Average Nitrogen Content	Average Total Nitrogen Absorbed	Nitrogen Absorption Response
	lbs/acre	gms/pot	mg/gm	mg/pot	mg/pot
Oshtemo sand	0 50 150	8.63 9.15 9.52	16.496 21.968 26.967	142.360 201.007 256.726	58.647 114.366
Hillsdale sandy loar	0 n 50 150	4.73 5.38 4.78	25.694 28.700 29.136	121.533 154.729 139.270	33.196 17.737
Brookston loam	0 50 150	11.39 12.06 11.83	25.457 26.165 26.213	289.955 315.549 310.100	25·594 20·145
Conover loam	0 50 150	5.23 6.19 5.85	24.845 26.071 24.986	129.940 161.379 146.188	31.439 16.248
Miami sandy loar	0 n 50 150	6.11 6.68 5.64	25.174 26.401 25.882	153.813 176.359 245.974	22.546 -7.839
Sims loam (rotation	0 1) 50 150	5.51 5.23 7.20	23.807 28.335 28.807	131.177 148.192 207.410	17.015 76.233
Sims loam (rotation	0 4) 50 150	6.82 7.19 7.77	17.959 27.250 30.128	122.480 195.928 234.095	73.448 111.615
Sims loam (rotation	0 6) 50 150	4.85 6.05 6.29	17.062 23.854 29.090	82.751 144.317 182.976	61.566 100.225

Brookston loam, Conover loam, and Miami sandy loam soils, were less than that of 50 pounds per acre of nitrogen application. Wheat on the remaining four soils showed a greater response to an application of 150 pounds of nitrogen per acre than to the lower rate.

A study of the relationship between the production of nitrate-nitrogen and the total nitrogen absorbed by wheat plants in the check pots of the first series was made by plotting pounds of nitrates produced during an eight-week incubation period against total nitrogen recovered by wheat plants. This relationship, which is graphically presented in Figure 3, does not appear to be entirely satisfactory though the correlation coefficient of 0.9126, statistically significant at the one percent level, was obtained. Values for the Oshtemo sand and Sims loam (rotation 6) did not fall close to the line of best fit. However, a greater number of soils with a wider range in nitrate incubation values are needed to establish the validity of the relationship.

experiment with tomato plants are given in Table V, including dry weight, nitrogen content, total nitrogen absorbed, and nitrogen absorption response. The dry weight of tomato plants grown on all soils increased with added nitrogen. In Conover loam and three Sims loam soils (rotations 1, 4, and 6), the response of plant growth to 150 pounds per acre

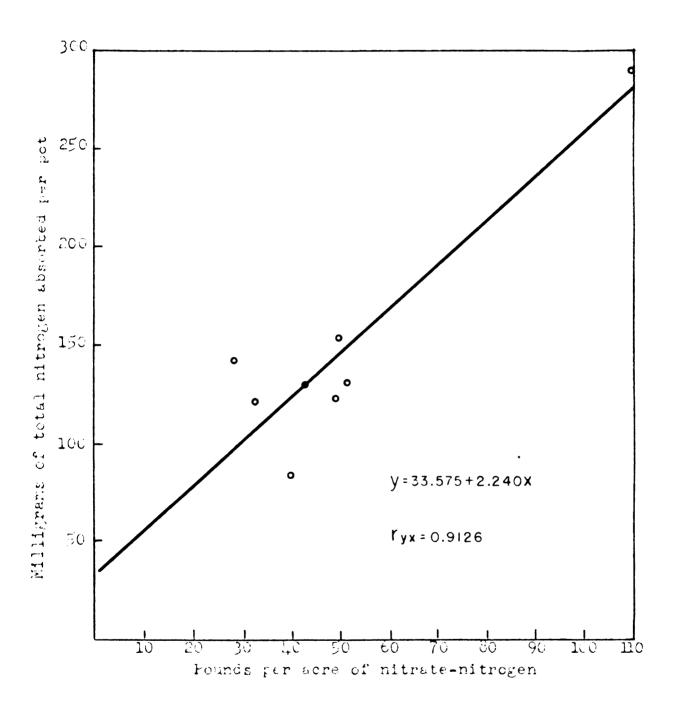


Fig. 3. Relationship between the production of nitrate-nitrogen and total nitrogen absorbed by wheat plants of check pots of first series.

TABLE V

THE DRY WEIGHT, NITROGEN CONTENT, TOTAL NITROGEN
ABSORBED, AND NITROGEN ABSORPTION RESPONSE
OF TOMATO PLANTS OF FIRST SERIES

Soil Type	Rate of Nitrogen	Average Dry Weight	Average Nitrogen Content	Average Total Nitrogen Absorbed	Nitrogen Absorption Response
	lbs/acre	gms/pot	mg/gm	mg/pot	mg/pot
Oshtemo sand	0 50 150	9.912 11.639 11.444	13.833 19.348 31.384	137.113 225.191 359.158	88.078 222.045
Hillsdale sandy loar	0 n 50 <b>1</b> 50	10.137 10.668 7.869	10.778 20.263 37.700	109.257 216.166 296.661	106.909 187.404
Brookston loam	0 50 <b>1</b> 50	13.984 18.654 14.013	21.224 18.890 30.103	296.796 352.374 421.833	55.578 125.037
Conover los	am 0 50 150	13.899 12.835 16.013	18.752 23.833 28.363	260.634 305.897 454.177	45.263 193.543
Miami sandy loar	0 n 50 150	14.733 16.486 15.588	19.898 22.689 30.790	293.157 374.051 479.955	80.894 186.798
Sims loam (rotation	0 1) 50 150	7.735 8.905 10.534	14.496 19.050 31.796	112.127 169.640 334.939	57.513 222.812
Sims loam (rotation	0 4) 50 150	8.150 12.105 12.190	13.672 12.941 22.232	111.427 156.651 271.008	45.224 159.581
ims loam (rotation	0 6) 50 150	5.987 10.459 9.372	14.519 16.396 24.154	86.925 171.486 226.371	84.561 139.446

of nitrogen application was greater than that of 50 pounds per acre of nitrogen application. In the rest of the soils, the growth response of tomato plants was less with 150 pounds per acre of nitrogen treatment than with 50 pounds per acre of nitrogen treatment. However, in every instance, the nitrogen absorption response of tomato plants to 150 pounds per acre of nitrogen application was far greater than that of 50 pounds per acre of nitrogen application. One explanation for this phenomenon would be that a greater dry weight production was obtained using tomato plants, thereby increasing the absorption of nitrogen at the 150 pounds per acre level.

The relationship between the production of nitratenitrogen and the total nitrogen absorbed by tomato plants
grown at the zero level of nitrogen in the first series
was poor. In this case the correlation coefficient was 0.593,
not significant at the five percent level. The data from
the Miami sandy loam and Conover loam soils, especially,
appear to be at variance with the relationship as it is
shown in Figure 4. Tomato plants absorbed as much nitrogen from Miami sandy loam as from Brookston loam soil,
though the amount of nitrate-nitrogen produced during incubation in Miami sandy loam was slightly less than one-half
of that of nitrate-nitrogen produced in the Brookston soil.
A high total intake of nitrogen by tomato plants grown on

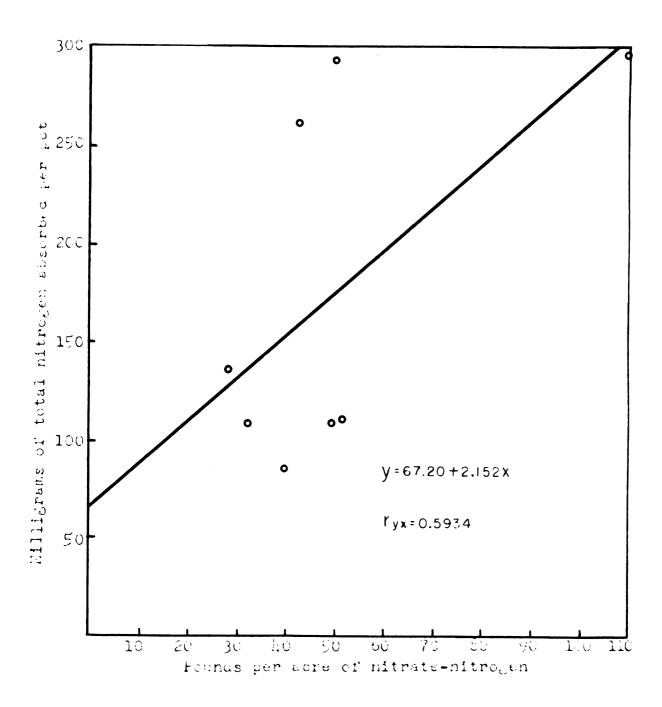


Fig. L. Relationship between the production of nitrate-nitrogen over an eight-week period and total nitrogen absorbed by tomato plants of check pots of first series.

Conover loam soil was noted. According to the data in Table II, Miami sandy loam and Conover loam soils contained large amounts of residual nitrates. It is possible that this accounts for lack of relationship in Figure 4. This nitrate fraction was leached out before incubation studies were started.

Correlation between the nitrate-nitrogen incubation soil tests and the increase in nitrogen absorption by wheat and tomato plants for the first series when 50 pounds and 150 pounds per acre of nitrogen applications were compared with no nitrogen application was not satisfactory in all The coefficient of correlation between the amounts Cases. of nitrate-nitrogen produced in the first two-week incubation period and the values of nitrogen absorption response of wheat plants to 50 pounds of nitrogen application in eight soils was -0.344, non-significant at the five percent level. In the case of 150 pounds per acre of nitrogen application for wheat plants, the correlation coefficient was -0.238, also non-significant at the five percent level. When the total amount of nitrate-nitrogen produced in an eight-week incubation period was correlated with the response of wheat plants to 50 pounds per acre of nitrogen application, the correlation coefficient was -0.364. With response of wheat plants to 150 pounds of nitrogen application, the coefficient

of correlation was -0.332. None of these coefficients are significant at the five percent level.

The reasons for lack of significance are not evident unless nutrient intake may have been restricted in such soils as Hillsdale sandy loam, Miami sandy loam, and Conover loam by a surface crust formation. The caking of soil and the formation of a puddled surface layer may have reduced air diffusion to a level where oxygen was deficient for root respiration and microbial activity.

A higher degree of correlation was obtained from the experiment with tomato plants. The amounts of nitratenitrogen produced in a two-week incubation period were correlated with the nitrogen absorption response of tomato plants to 50 and 150 pounds of nitrogen applications. correlation coefficients were -0.547 and -0.674, respectively. When the total amount of nitrate-nitrogen produced in an eight-week incubation period was correlated with the response of tomato plants to the 50 and 150 pounds per acre of nitrogen applications, the correlation coefficients were -0.470 and -0.629, respectively. These values are not significant, since with only eight pairs of figures a significant correlation coefficient must be above -0.707 at the five percent level. One of the reasons for the lack of significance in the correlation coefficients may be the small number of samples. Fitts, et al. (11) correlated the nitrate-nitrogen

produced during a three-week incubation under controlled laboratory conditions with the yield response of corn to the nitrogen application in field experiments. These workers found a correlation coefficient of -0.630 to be significant at the one percent level.

When wheat and tomato plants of the first series were harvested, the nitrate-nitrogen left in the soils was determined. The results of these analyses are shown in Table VI. The amount of nitrate-nitrogen recovered from the soils on which tomato plants were grown was much lower than that from the soils planted to wheat. This situation would be expected since tomato plants absorbed more nitrogen than did wheat plants, except in the case of the Sims soils from the Ferden rotation plots. Approximately 30 to 40 pounds per acre of nitrate-nitrogen was recovered from the soils where wheat plants were grown after receiving 150 pounds per acre of nitrogen, except in the case of Oshtemo sand. The residual nitrate-nitrogen in Oshtemo sandy soil was lower than that found in other soils mainly because of a greater uptake by the wheat plants.

The second series of experiments were carried out with the same soils which had been used in the first experiment with wheat and tomato plants. The amounts of nitrate-nitrogen produced from each soil, which had previously received no nitrogen, was much less than that obtained during

TABLE VI

ANALYSES OF SOILS FOR NITRATE-NITROGEN LEFT IN SOILS

AFTER GROWTH OF WHEAT PLANTS AND

TOMATO PLANTS OF FIRST SERIES

Soil Type	Rate of Nitrogen	Pounds per acre of nitrate-nitrog		
	Applied lbs/acre	after wheat harvest	after tomato harvest	
Oshtemo sand	0	0.6	0.80	
	50	0.6	0.40	
	150	0.4	1.28	
Hillsdale sandy loam	0	1.2	0.72	
	50	8.4	1.00	
	150	40.8	16.80	
Brookston loam	0	6.8	4.00	
	50	<b>6.</b> 4	5.60	
	<b>1</b> 50	<b>29.</b> 5	4.24	
Conover loam	0	14.2	0.80	
	50	18.8	4.00	
	150	31.2	11.20	
Miami sandy loam	0	13.2	0.60	
	50	19.8	0.72	
	150	32.0	18.40	
Sims loam (rotation 1)	0	12.6	21.80	
	50	8.2	15.60	
	150	32.0	9.20	
Sims loam (rotation 4)	0	5•4	2.20	
	50	2•4	2.88	
	<b>1</b> 50	38•4	23.20	
Sims loam (rotation 6)	0	0.8	2.88	
	50	1.0	1.48	
	<b>1</b> 50	38.0	4.40	

the first series of experiments (Table VII). This decrease in production of nitrate-nitrogen was undoubtedly due to the mineralization of some of the organic nitrogen during the growth of wheat and tomato plants, and its subsequent absorption. The only exception to this trend was with Oshtemo sand, which produced as much nitrate-nitrogen as in the first series of incubations. A graphic presentation of the production of nitrate-nitrogen for each soil is given in Figure 5. The pattern of nitrification closely follows that of the first laboratory experiments.

Data of dry weight, nitrogen content, total nitrogen absorbed, and nitrogen absorption response of wheat plants of the second series are listed in Table VIII. The application of nitrogen fertilizer at 50 and 150 pounds per acre rates increased the yields of wheat plants of the second series in all soils except in Hillsdale sandy loam. In this instance the yield of wheat plants decreased as the application of fertilizer increased. This phenomenon may be attributed to the effect of the rather large quantity of residual nitrate-nitrogen in the soil. The Hillsdale sandy loam soil, which received 150 pounds per acre of nitrogen fertilizer, had the highest residual nitrate-nitrogen (Table VI).

Nitrogen absorption response of wheat plants to 150 pounds per acre of nitrogen application was generally more

TABLE VII

THE PRODUCTION OF NITRATE-NITROGEN IN SOILS OF SECOND

SERIES DURING SUCCESSIVE MOIST INCUBATION PERIODS

Soil Type	Incubation Period					
••	2nd week	4th week	6 th week	8th week	Total Nitrate in 8 week	
	ро	unds per	acre of	nitrate-	nitrogen	
Oshtemo sand	17.40	4.40	3.88	3.12	28.80	
Hillsdale sandy loam	16.60	2.40	2.80	1.88	23.68	
Brookston loam	36.40	8.40	6.32	3.20	54.32	
Conover loam	21.40	4.56	5.48	1.92	33.36	
Miami sandy loam	24.40	6.20	3.52	1.28	35.40	
Sims loam (rotation 1)	28.20	11.28	5.80	0.88	46.16	
Sims loam (rotation 4)	12.20	5.60	5.40	1.72	24.92	
Sims loam (rotation 6)	17.40	4.60	2.20	0.72	24.92	

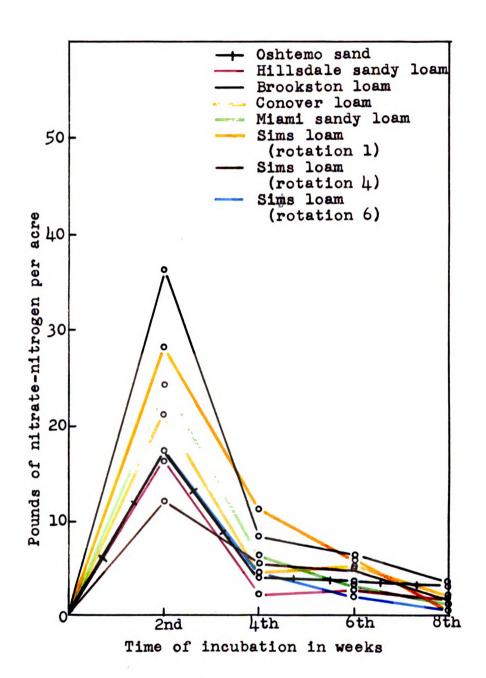


Fig. 5. The production of nitrate-nitrogen in soils of second series during successive moist incubation periods.

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TABLE VIII

THE DRY WEIGHT, NITROGEN CONTENT, TOTAL NITROGEN
ABSORBED, AND NITROGEN ABSORPTION RESPONSE
OF WHEAT PLANTS OF SECOND SERIES

Soil Type	Rate of Nitrogen	Average Dry Weight	Average Nitrogen Content	Average Total Nitrogen Absorbed	Nitrogen Absorption Response
	lbs/acre	gm/pot	mg/gm	mg/pot	mg/pot
Ostemo sano	1 0 50 150	7.025 8.088 8.122	13.780 20.389 25.798	96.805 164.906 209.531	68.101 112.726
Hillsdale sandy loar	0 n 50 <b>1</b> 50	5.572 4.807 3.807	15.188 23.856 27.694	84.628 114.676 105.431	30.048 20.803
Brookston loam	0 50 <b>1</b> 50	8.019 9.615 12.057	15.233 21.267 27.185	122.153 204.482 327.770	82.329 205.617
Conover los	am 0 50 150	3.047 4.630 4.586	22•723 21•752 26•446	69.237 100.712 121.281	31.475 52.044
Miami sandy loam	0 1 50 150	4•492 5•676 4•767	14.240 26.121 29.705	63.966 148.263 141.604	84.297 77.638
Sims loam (rotation	0 1) 50 150	7.082 8.115 8.660	16.598 24.643 23.995	117.547 199.978 207.797	82.431 90.250
Sims loam (rotation L	0 50 150	6.852 10.570 9.352	13.824 16.945 26.561	94.722 179.109 248.398	84.387 153.676
Sims loam (rotation	0 6) 50 150	5.618 6.990 9.453	13.176 20.343 26.723	74.023 142.198 252.613	68.175 178.590

than twice that of the 50 pounds per acre of nitrogen application, with the exception of Hillsdale sandy loam and Miami sandy loam.

The relationship between the production of nitratenitrogen and the total nitrogen absorbed by wheat plants
from soils not treated with nitrogen is shown in Figure 6.
A rather poor relationship is observed. The correlation
coefficient of 0.649 is non-significant at the five percent
level.

In Table IX, the dry weight, nitrogen content, total nitrogen absorbed, and nitrogen absorption response of field bean plants in the second series are shown. The increase in yield of field bean plants due to the nitrogen fertilizer application was rather remarkable in Brookston soil. But for the rest of the soils, the yield of field bean plants at three levels of nitrogen application showed little variance. Field bean plants responded in terms of nitrogen absorption to nitrogen application in all soils with the exception of Sims loam, rotation 4, which was treated with 50 pounds of nitrogen per acre. In all soils, the nitrogen absorption response of the 150 pounds per acre of nitrogen application was greater than that of the 50 pounds per acre of nitrogen application.

The relationship between the production of nitratenitrogen and total nitrogen absorbed by field bean plants

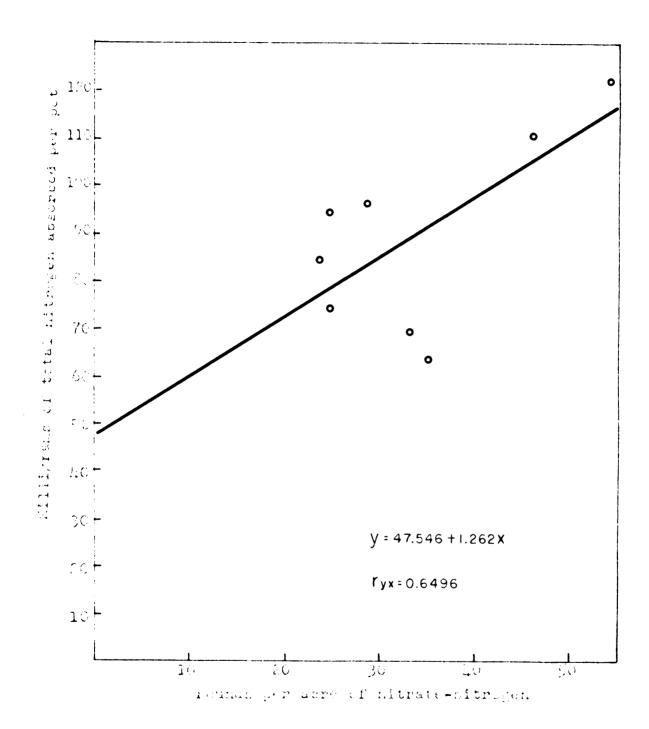


Fig. 6. Actabionship between the production of hitrate-mitropen even as eight-week period and total hitraten absorbed by wheat plants of check pote of second period.

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TABLE IX

THE DRY WEIGHT, NITROGEN CONTENT, TOTAL NITROGEN
ABSORBED, AND NITROGEN ABSORPTION RESPONSE
OF FIELD BEAN PLANTS OF SECOND SERIES

Soil Type	Rate of Nitrogen	Average Dry Weight	Average Nitrogen Content	Average Total Nitrogen	Nitrogen Absorption Response
	lbs/acre	gm/pot	mg/gm	Absorbed mg/pot	mg/pot
0shtemo sand	0 50 <b>1</b> 50	6.088 8.001 6.413	24.245 21.776 30.144	147.604 174.230 193.313	26.626 45.709
Hillsdale sandy loam	0 n <b>50</b> 150	4.134 4.713 4.336	14.286 23.000 40.837	59.058 108.399 177.069	49.341 118.011
Brookston loam	0 50 150	10.368 12.306 15.457	26.121 23.070 25.012	270.823 283.899 386.610	13.076 115.787
Conover loam	0 50 150	5.264 5.249 6.264	23.070 28.110 32.040	121.440 147.549 200.699	26.109 79.259
Miami sandy loam	0 n 50 150	6.640 6.465 6.202	21.960 28.711 31.762	145.814 185.617 196.988	39.803 51.174
Sims loam (rotation	0 1) 50 150	6.165 6.522 7.048	22.913 <b>2</b> 5.059 30.930	141.370 163.435 217.995	22.065 76.625
Sims loam (rotation	0 4) 50 150	7.783 8.223 9.614	28.618 23.672 31.439	222.734 194.655 302.255	-28.079 79.521
Sims loam (rotation	0 6) 50 150	6.158 6.907 7.420	26.353 23.995 32.085	162.282 165.734 238.071	3.452 75.789

in the check pots of the second series was very slight as shown in Figure 7. The correlation coefficient was 0.513, which is non-significant at the five percent level. Lack of relationship may have been due to differences in fixation of atmospheric nitrogen by field beans in each soil.

The correlation coefficients between the nitrate-nitrogen incubation tests and the nitrogen absorption response of wheat and field bean plants in the second series to the added nitrogen resulted in positive figures, whereas in the first series of experiments, negative correlation coefficients were obtained. In the case of the first two-week incubation test and the nitrogen absorption response of wheat plants to 50 and 150 pounds per acre of nitrogen applications, the correlation coefficients were 0.322 and 0.422, respectively. When the total nitrate-nitrogen produced in an eight-week incubation period was correlated with the response of wheat plants to 50 and 150 pounds of nitrogen per acre, the coefficients were 0.271 and 0.319, respectively. For field bean plants, the correlation coefficients between the first two-week incubation soil test and the nitrogen absorption response to 50 and 150 pounds of nitrogen application per acre were 0.245 and 0.284, respectively. When the total nitrate-nitrogen produced in a period of eight weeks was correlated with the response of field bean plants to 50 and 150 pounds of nitrogen per

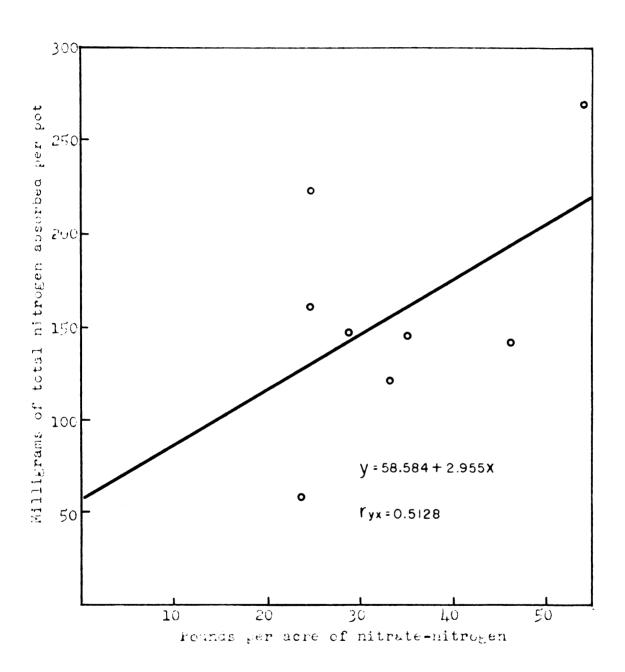


Fig. 7. Relationship between the production of nitrate-nitrogen over an eight-week period and total nitrogen absorbed by field bean plants of check pots of second series.

acre, the coefficients of correlation were 0.085 and 0.235, respectively. All the correlation coefficients obtained from the second series of experiments were non-significant at the five percent level.

In Table X, the corn yields in 1954 and the nitratenitrogen incubation test values of soils from rotation 1. 4, and 6 are shown. Since composite soil samples were taken from high and low levels of fertilizer application for the experiments, corn yields were calculated by averaging yields obtained from high and low fertility levels for each plot. The average corn yield of high and low fertility levels is regarded as that receiving no nitrogen. The increase in yield due to 40 pounds of nitrogen per acre as a sidedressing is considered to be the yield response to added nitrogen. The corn yield from plots of rotation 6 was about one-half of that of the other two rotations. This decrease in yield was due to the exclusion of green manure in rotation 6. Plots of rotation 4, which had one year alfalfa-brome, produced a little more corn than those of rotation 1, which had two years of alfalfabrome. The yield response of corn from rotation 1 was greater than that of the other two plots, though the yield response to 40 pounds of nitrogen per acre as a sidedressing was rather small in three plots. It is believed that the lack of precipitation after nitrogen sidedressing was largely

TABLE X

CORN YIELD, NITRATE-NITROGEN PRODUCED IN A 8-WEEK INCUBATION

PERIOD, AND YIELD RESPONSE OF CORN TO ADDED

NITROGEN FROM ROTATION 1, 4, AND 6

	Nitrate-Nitrogen	Corn	Yield	Yield
Rotations	Produced in an 8-week Period	No N#	40# N sidedressi	Response Ingto Added Nitrogen
	pounds/acre	bush	el/acre	- Nitrogen
1	51.2	86.8	89.1	2•3
4	49.6	92.9	91.9	-1.0
6	40.0	47.4	48.4	1.0

<sup>\*</sup>Mean yield of high and low fertility plots

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responsible for the lack of response in 1954. In 1955, a very significant yield increase was found for sidedressed nitrogen, especially for rotation 6.

When the nitrate-nitrogen incubation tests of these soils were compared with the differences in yield between the plots sidedressed with 40 pounds of nitrogen and the plots receiving no sidedressing, little relationship could be observed. However, it should be pointed out that these soil samples were taken after the corn was harvested. Therefore it may have less validity than if it were taken before planting time. No effort was made to determine a correlation coefficient simply because the number of samples was small.

## SUMMARY AND CONCLUSIONS

The purpose of this study was to determine whether a nitrogen incubation soil test developed by Hanway and Stanford (25) at Iowa State College could be correlated under Michigan conditions with crop response to added nitrogen fertilizers and different crop rotations in which the nitrogen needs of specific crops are quite different.

Greenhouse experiments and laboratory incubation studies were conducted using eight field soils varying in organic matter content and texture. Three of these soils were obtained from different experimental rotation plots at the Ferden farm in Saginaw county. From this study the following conclusions were drawn:

1. Marked differences in the quantity of nitratenitrogen produced after a two-week period were obtained
from the eight soils. A similar trend was evident for
total nitrate-nitrogen produced during an eight-week period.
In general, the higher the organic matter content and the
finer the texture, the greater was the nitrogen released
from soils during moist incubation. A highly significant
correlation coefficient of 0.83 was obtained between
nitrate-nitrogen released on incubation and the organic
matter content of the soils.

- 2. No significant relationship was observed between the dry weight yield of the wheat and tomato plants of the first series where no nitrogen was added and the nitrogen released upon incubation. Lack of such a relationship was also noted for the wheat and field bean crops in the second greenhouse series.
- 3. The yield response of wheat, field beans, and tomato plants to either 50 or 150 pounds per acre of nitrogen in greenhouse experiments was not significantly related to the nitrate-nitrogen released during incubation. Lack of response to added nitrogen on specific soils is believed to be due to soil physical conditions detrimental for plant growth.
- 4. A significant direct relationship was found between the amount of nitrogen absorbed by wheat plants of the first series when no nitrogen was applied and the nitrate-nitrogen released on incubation. In the case of tomato plants of the first greenhouse series and both crops of the second series, non-significant correlation coefficients were obtained.
- 5. Correlation between the nitrogen absorption response to the added nitrogen fertilizer by wheat and tomatoes of the first greenhouse series and the nitrate-nitrogen released from soil during laboratory incubation was negative, but less than that required for significance

at the five percent level. For the wheat and field bean crops of the second series, the coefficients were of low positive value, indicating a direct relationship between the nitrate incubation values and the yield response to added nitrogen fertilizer.

- 6. The yield response of corn grown in the field under three crop rotations to sidedressed nitrogen was not correlated with nitrate-nitrogen released by the incubation test.
- 7. It is apparent that the nitrate-nitrogen incubation soil test as conducted did not clearly reflect the need for or the ability of crops to absorb nitrogen under greenhouse conditions. A preliminary observation also indicates it was not correlated with the yield response of corn to the added nitrogen under field conditions. The small number of soils and crops studied suggests that further experiments are needed to evaluate the present nitrate-nitrogen incubation test or some modified procedure.

Since the nitrification test is an empirical one, it is possible that an incubation temperature of 35°C is too high to reflect the nitrogen supplying power of soils for crops grown under Michigan conditions. In addition, it is possible that soil physical conditions limited crop response to added nitrogen in both greenhouse and field experiments. An empirical nitrogen incubation test

can be evaluated only if all growth factors except nitrogen are not limiting.

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