A STUDY OF THE NITROGEN CONTENT OF THE EXPRESSED SAP AND THE TISSUES OF WHEAT, RYE, BARLEY AND OATS

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By
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A STUDY OF THE NITRATE CONTENT OF THE EXPRESSED SAP AND THE TISSUES OF WHEAT, RYE, BARLEY AND OATS.

INTRODUCTION

An examination of the available literature reveals considerable information regarding the effect of fertilization and stage of growth on the nitrogen content of wheat, rye, barley and oats, but few data as to the nitrate content of the expressed sap of these plants. In view of this situation it was deemed advisable to study the effects of soil type, soil fertilization, and stage of growth on the nitrate content of the sap of these plants. Since it appears that at least a part of the nitrogen entering the roots of plants is in the nitrate form there may be some correlation between the rate of growth, stage of growth, and the nitrate content of the cell sap of plants.

REVIEW OF LITERATURE

Nitrate Nitrogen in Soils

Smith (22), during a study of the distribution of nitrate in fallow soils found very little loss of available nitrogen over the whole season, but changes in the weather and activities of soil micro-organisms caused decided temporary disappearances of nitrate. He found further that nitrate leached from the surface soil was often retained by the lower soil horizons.

King (9) presented data which show that the nitrate nitrogen content of soil under oats, barley, peas, and rye was much lower than was fallow soil. Some differences were found to a depth of four feet, although the greatest differences occurred in the first foot of soil.

Bizzell (1) found that cultivation increased nitrification in a clay loam but not in a sandy loam soil. He also found that the nitrate content of soil under corn steadily increased during the growing season but that millet or soy beans, growing alone or with corn, caused a decided decrease in the nitrate content of the soil.

In their lysimeter experiments at Cornell University, Lyon and Bizzell (10) discovered that with some crops the sum of the nitrogen in the drainage water and that in the crop was less than in just the drainage water from a bare tank. They have shown further (11) that there is a characteristic relationship between the kind of crop at different stages of growth and the nitrate content of the soil. They suggested that the great differences in the nitrate content of the soil under different crops might be due to a stimulating or depressing influence of the crop on nitrate formation. These investigators (12) have also shown that of two soils which had previously grown alfalfa and timothy the rate of nitrification was greater in the alfalfa soil but that the total the total amount of nitrate formed was equal for the two soils. They pointed out that the rate of nitrification is important in the field because nitrate is being constantly removed from the soil.

Lyon, Bizzell, and Wilson (13) have presented results which show that, under the same conditions of soil and treatment, soil which had grown clover contained more available nitrogen than did that which had grown timothy. They have shown further (14) that wheat has a greater depressive influence than has maize, and have pointed out that the composition of the organic matter liberated by living or by decomposing plant roots has an influence on the activity of the nitrate consuming organisms.

Doryland (4) suggested that the disappearance of nitrate from a soil is due in part to the influence of crop residues which increase the activities of the soil micro-organisms.

Smith (23) found that the curves of nitrate accumulation declined rapidly during the growing season in a cropped and uncropped soil. Although the curve on the fallow was at a higher level than on the cropped soil they both attained the same low level late in the season. He found no correlation between nitrate levels and yield of crops.

Deatrich (5) reported the nitrate in soils under maturing soy beans to be very low.

Jones (9) has shown that added nitrate disappears more completely under timothy sod than in a bare soil. He stated that, with the exception of the largest application of nitrogen, this disappearance was accounted for by the nitrogen taken up by the tops and roots of the crop.

<u>Nitrate Nitrogen in Plants</u>

McCool and Weldon (16) have shown that when the growth of plants is limited, due to the lack of available phosphorus or potassium in the soil, that element which is plentiful in the soil becomes more concentrated in the sap.

Workers at the Rhode Island Experiment Station (20) have found the amounts of nitrate nitrogen, phosphorus, and potassium in the juice of plants to correlate with the amounts of these substances added to the soil. Shortly after a period of high rainfall resulting in a decrease of nitrate in the soil, very low concentrations of nitrate in the sap were obtained. At the same time high concentrations of phosphorus and potassium were found.

Gilbert and Hardin (6), working with cabbage, lettuce, beets, and celery, found the nitrate nitrogen in the sap to increase with the nitrate additions to the soil. The total nitrogen content of the plants correlated quite well with the nitrate nitrogen present.

Determinations of nitrate nitrogen in weeds by earlier workers have shown the concentration of this form of nitrogen

to change considerably during different stages of growth. [illegible]oo (25) observed that the nitrate content of Amaranthus retroflexus increased from early stages until blooming. He assumed from this that it would continue to increase until maturity. Campbell (6) however found the nitrate content of this weed to be highest at early blooming but it contained none at maturity. He obtained similar results with rye and six other species of weeds growing with the rye.

Density of Plant Sap

The consensus of opinion appears to be that fertilizer salts affect the density of the sap of plants. McCool (16) has shown that the concentration of the cell sap of sugar beets, table beets, carrots, and onions grown on muck soil is increased by fertilization. McCool has also shown (17) that on a muck soil additions of phosphate did not increase yields and did not increase the density of the sap of sugar beets, carrots, and swiss chard. When potassium was applied, however, on this same field, increases in yields were obtained with corresponding increases in the density of the sap.

Total Nitrogen

Daley and Miller ([illegible]) reported the per cent of nitrogen in corn plants to be higher when the plants were receiving large amounts of nitrogen as fertilizer. In cases where a liberal amount of nitrogen available there was an accumulation of it in the plants. The per cent of nitrogen in the leaves, roots, and stalks decreased gradually from the first to the last part of the growth period.

Wilfarth, Roemer, and Wimmer (24) reported decreases in the nitrogen content of wheat, barley, and potatoes during successive stages of growth.

Ince (7) and Schweitzer (21) reported similar results from their studies with corn.

PLAN OF PROCEDURE

These investigations were carried out on two outlying soil fertility fields and on special plots receiving varying amounts of nitrate of soda.

On the plots growing wheat and oats nitrogen was applied in the form of urea, phosphorus as twenty per cent superphosphate and potash in the form of chloride. All plots were limed with medium ground limestone. On the barley plots the carriers were the same except that ammonium sulfate was used instead of urea, and no lime was applied. Twenty per cent of the nitrogen was applied on the wheat plots before seeding and the rest early in the spring.

The wheat samples were taken late in the fall of 1927 and three times in 1928, just after growth started, about one week before heading, and just before maturity. The spring grains were sampled three times at similar stages of growth. The data obtained in this investigation were expected to show the influence of soil type, fertilization, and stage of growth on the nitrate content of the expressed sap and the total nitrogen content of the dried tissue.

An attempt was made to gather and care for the samples so that a correct determination of the nitrate content of the sap at the time of sampling would result. Nightingale (19) working with tomato plants found that the period of daily illumination caused variations in the carbohydrate and nitrogen content of the plants. From this one might expect the hour of the day at which samples were taken to influence the nitrate content of the sap. The samples were therefore gathered at as nearly the same hour of the day as possible.

To insure further uniformity throughout the investigations the samples were not gathered during rainy days or when wet with an excessive amount of dew.

All samples were cut at the surface of the ground and placed at once into glass jars. During warm weather the containers were packed in ice immediately after the samples were cut.

ANALYTICAL METHODS

As soon as possible after the samples were taken the material on which nitrate determinations were to be made was ground in a food chopper. The ground tissue was then wrapped in a single layer of new cheese cloth, placed in a steel cylinder of 8 cm. diameter and subjected to a pressure of one ton until the juice ceased to flow.

As soon as the juice was obtained it was centrifuged for ten minutes. This was done to get out small pieces of tissue and other solid particles. Density determinations were made on the juice at this time, using a hydrometer for this purpose.

All nitrate determinations were made as follows. A portion of the centrifuged juice, usually 10cc, was measured into a 100 cc centrifuge tube. An amount of saturated lead subacetate solution equal to 40 per cent of the amount of sap was then added and the volume made up to 100 cc. After thorough stirring this liquid was centrifuged for ten minutes to remove chlorophyll and other organic materials.

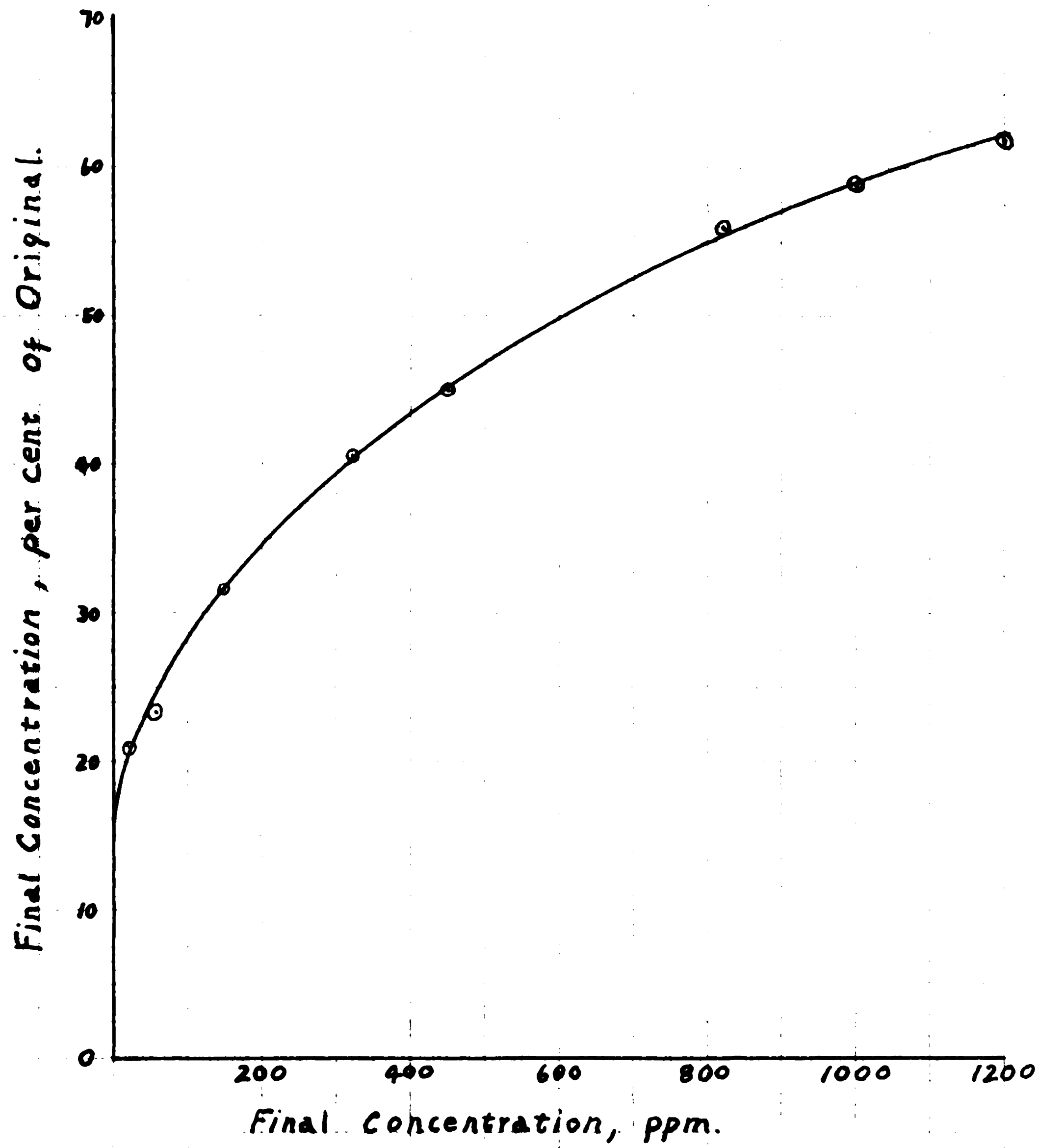
After centrifuging, the supernatant liquid was poured off, shaken with 5 grams of "norit", a wood charcoal, and filtered. The filtrate was treated once more with 5 grams of "norit" and passed again thru the same filter. Fifty cubic centimeters of the filtrate were placed in an 80cc test tube and heated to boiling with 0.5 cc of saturated copper sulfate solution, 25cc of saturated silver sulfate solution, and enough magnesium carbonate and calcium oxide to make a saturated solution.

After cooling, the solution was made up to volume, shaken with one gram of "Norit" and filtered through a hard filter paper. This was sufficient treatment to remove organic matter and chlorides. Duplicate 25cc portions of the filtrate were then evaporated to dryness on the steam bath. Nitrate was determined by the phenoldisulfonic acid method.

It was found that during this procedure considerable nitrate was removed from solution. A series of known solutions containing varying concentrations of nitrate were therefore analyzed. These results showed that the per cent of nitrate recovered varies directly with the concentration of the original solution. The following graph, constructed from these data, was used to correct the results presented below.

Moisture in the green sample was determined by weighing out a large sample, usually 100 grams, drying in a well ventilated oven at 45° to 55°, and reweighing.

Curve Showing the per cent of Nitrate Recovered



EXPERIMENTAL RESULTS

The plants employed in these studies were grown on the general fertility plots and plots treated with varying amounts of sodium nitrate.

Nitrate Nitrogen

Oats

The data in table 1 were obtained from oats grown on Miami loam. It is to be noted that the applications of phosphate and potash reduced the nitrate content of the sap. The greatest effect occurred during the periods of rapid growth as evidenced by the June 1 and June 27 samplings. On June 1 the sap of the plants treated with phosphorus and potash contained only about one-half as much nitrate as that from the plants receiving no fertilizer. It is also apparent that nitrogen applications actually increased the nitrate content of the sap. In all cases there was more nitrate in the sap of the plants treated with nitrogen than in those receiving only the non-nitrogenous fertilizers.

The results presented in table 2, obtained from another field of Miami loam, show up in the effect of applications of phosphate and potash to this soil type. On July 24 the nitrate content of the sap of oats plants was 75 parts per million, whereas it was 610 parts per million in that of the plants produced on the untreated soil. Similar results were obtained in the two earlier samplings. the effects of the nitrogen in some of the mixtures were not consistent, due, no doubt, to the fact that the fertilizers were applied to the crop preceding the oats.

Very striking results were obtained from nitrate treatments applied to oats grown on Miami loam. As shown by the data given in table 3 the yield of grain was high on all plots including the checks, thus showing that nitrogen was not a limiting factor on this field. Regardless of the plentiful supply of available nitrogen the application of sodium nitrate increased the nitrate in the sap from 34 to 815 parts per million. This would seem to show that this plant can accumulate a large amount of nitrate in addition to that which is needed.

Fertilizers caused variations in the nitrate content of the sap of plants grown on Hillsdale sandy loam. According to field trials the yields of several crops on this soil are increased by applications of potash. An examination of the data in table 4 shows that in every case the plants treated with potash contained less nitrate in the sap than did plants receiving no fertilizer or fertilizers other than potash. As shown above with plants on Miami soil, the addition of nitrogenous fertilizers caused increases in the nitrate content of the sap.

Table 5 presents results obtained on a depleted Hillsdale sandy loam. These data are in contrast with those obtained on the Miami soil in that nitrate applications did not increase the nitrate content of the sap. This shows that the plants cannot accumulate nitrate in the sap unless there is an excessively large supply of available nitrogen in the soil.

Barley

Barley, as illustrated by tables 6 and 7, gave results similar to those presented above. It is to be noted that wherever growth, as indicated by the yields, was increased by fertilizer applications other than nitrogen, the nitrate content of the expressed sap was decreased. An exception to this was found when the complete fertilizer treatment increased the nitrate content of the sap, showing again the effect of applications of available nitrogen.

Soil conditions may have considerable influence on nitrogen relationships, as evidenced by the results presented in tables 8 and 9. Nitrogen treatments caused increases in the nitrate content of the sap of barley grown on each of these fields. On the Miami loam, however, the differences in the sap did not appear until the second sampling while on the Hillsdale soil there were large differences in the nitrate content of the sap at the first sampling and very little later in the season. It is difficult to say what caused this, but as the two soils vary widely in texture, soil moisture may have had something to do with it. The spring of 1[illegible] was very cold and wet. Excess water held in the Miami soil might have delayed nitrification of the organic matter of the soil.

<u>Wheat</u>

The data in table 10 were obtained from wheat grown on Miami loam. It is to be noted that with the exception of the autumn sampling the applications of phosphate and potash reduced the nitrate content of the sap. As with oats the greatest effect occurred during the period of very rapid growth.

Investigations with wheat grown on Hillsdale sandy loam as indicated by the data presented in table 11 verify the results obtained from plots on that soil. The nitrate content of the sap tended to be low on all plots treated with potash in all stages of growth, although the differences were larger during the early stage. This is to be expected in the light of Campbell's work on rye and weeds, to which reference was made above. It is also apparent that nitrogen applications, on the other hand, invariably increased the nitrate content of the sap.

Table 12 indicates that applications of sodium nitrate increased the yield of wheat grown on Miami loam. The nitrate content of the sap was likewise augmented. It was less obvious in the later stages, however, than it was in the early stage. This was due, no doubt, to the fact that the period of rapid growth had been passed, on this particular field, before the time of the June 8 sampling.

As represented by the data in table 13 the application of sodium nitrate to a Hillsdale sandy loam soil increased the nitrate content of the sap from 189 to 277 parts per million during the early part of the growth period.

<u>Rye</u>

Rye grown on Miami loam, according to the data in table 14, gave results similar to those obtained from the other crops except that the differences were apparent later in the development of the plants. This may be explained on the ground that there was probably more available nitrogen in this soil.

On Hillsdale sandy loam soil, as shown by the results in table 15, the nitrate content of the sap of rye correlated with the yields but not with the nitrate applications to the soil. Because of a sag in the field, the plot receiving the heavy nitrogen application was rather poorly drained. This might have decreased the nitrate content of the soil and correspondingly would cause a decrease in the nitrate content of the sap.

Total Nitrogen

An examination of all these data on all crops studied on two soil types shows that in general the total nitrogen content of the dried tissue paralleled quite closely the nitrate content of the sap. There were very few exceptions to this at the first sampling, although during the later periods of growth the results were less consistent. In the case of wheat and rye it might even be said that there was no correlation between the total nitrogen in the dried tissue and the nitrate in the sap during the later part of the season, except that they were both at a low level during this period. This is not surprising, however, because the differences in nitrate in the sap during the later part of the season were very small and were less consistent than at early growth.

In oats and barley there was a close correlation between the nitrogen in the dried tissue and the nitrate content of the expressed sap at all stages of growth.

Since it was found that increased growth due to the addition of some element other than nitrogen tended to decrease the nitrate in the sap, as the above results indicate, the same thing should be true as regards total nitrogen. Increases in the concentration of potassium, phosphorus, or calcium in the crop, therefore, may be obtained by fertilizer applications at the expense of the protein content. As the nitrate plots have shown, this condition may be avoided by the addition of some form of soluble nitrogen.

Ratio of Leaves to Stems

The question came up quite early in the work as to whether or not some of the results found may have been due to differences existing in the nitrate content of the leaves and stems. Table 16 shows that the stems usually contain more nitrate and less total nitrogen than do the leaves.

This discovery led to a study of the proportion of leaves to stems on different sized plants. If this proportion were different where the growth was small, as on the check plots, from that where the plants were larger as in case of fertilized plots, it might explain some of the results found. The data given in table 17 reveal the fact that the proportion of leaves to stems did not vary consistently on check and fertilized plots. Oats plants had only one per cent more leaves on the check plot than on the fertilized plot. These samples were collected on Miami loam where the check plot gave 33.3 bushels per acre and the fertilized plot 60.4 bushels per acre.

Barley and wheat respectively gave four and seven per cent more leaves on the check plots than on the fertilized plots. The leaves of the fertilized plants had less moisture at the time of sampling than did those of the untreated plants, which was due to the nearness of the treated plants to maturity. Less moisture in the leaves would, of course, cut down the weight of leaves on the fertilized plants. Taking this into consideration it may be said that there were no appreciable differences in proportion of leaves to stems.

Rye had about two per cent less leaves on the untreated plants than on the treated plants. This is the reverse of the condition found in the other three crops, but the difference is too small to be significant.

Soil Type

The data presented in tables 1 to 15, inclusive, furnish a means of studying the effect of soil type on nitrate in the expressed sap and total nitrogen in the dried tissue of the crops. In three cases, Miami loam produced plants having higher nitrate and total nitrogen levels than did Hillsdale sandy loam. In two cases the condition was reversed.

Results were obtained from two fields of oats and barley on Miami loam. In both cases the field giving the higher average yield of grain gave the higher nitrate and total nitrogen levels.

A comparison of the state of fertility of each individual field, as indicated by the yield of grain, with the analytical results obtained during the investigations indicates that the state of fertility has more influence on the nitrate and total nitrogen content of the crop than does soil type. While natural fertility is largely dependent upon soil type it is a condition that is easily changed by agricultural methods.

Stages of Growth

The variations in total nitrogen and nitrate nitrogen at different stages of growth are rather interesting. Table 10 gives averages of the nitrate in the sap and total nitrogen in the tissue on all the general fertilizer plots. The total nitrogen in the dried tissue steadily decreased throughout the season. This was true of all crops on all soil types and corresponds with the results of Wilfarth, Roemer, and Wimmer (24) who worked with wheat, barley, and potatoes. It also corresponds with results obtained by Huley and Miller ([illegible]), Schweitzer ([illegible]1), and Imes (7) in their work on corn.

In all cases, with the exception of oats on Miami loam, the nitrate nitrogen in the sap dropped from a high level during early growth to a low level at the second sampling, then increased again at the last sampling. The explanation for this behavior lies in the ability of the plants to accumulate nitrate in the sap during periods of slow growth. At the first sampling the plants were getting their roots established and consequently the growth was slow. At the second period the plants were growing and using nitrate rapidly. Later, at the third stage, rapid growth had ceased and nitrate accumulation again began. The exception to this in the case of oats on Miami loam can be easily explained. From the moisture content at the third sampling it is apparent that the plants were less mature than were those from other fields at the same period. This fact would indicate that they were still growing rapidly and had not yet started to accumulate nitrate in the sap.

Table 10 gives the effect of period of growth on the nitrate content of the sap and the total nitrogen content of the tissue on all plots fertilized with sodium nitrate.

As was found on the general fertilizer plots, total nitrogen in the tissue steadily decreased throughout the season. The nitrate content of the sap at the third sampling was similar to that at the second sampling. In two cases there were slight increases while in five cases the decrease in amount of nitrate was continuous throughout the season. This circumstance is probably due to the presence of enough nitrate in the soil, due to addition as a fertilizer, to prevent the nitrate content of the sap from reaching the low level attained during rapid growth by plants on soil not treated with nitrogen.

Density

It does not appear to be difficult to change the density of the sap of plants. MacDougal (15), for example, in experiments on living cell masses of Opuntia, found that when the cells were surrounded by a salt solution there was a decrease in permeability of the cell wall and plasmatic layer surrounding the vacuole. According to him a decrease in permeability in living cells is always accompanied by an increase in water intake and he interpreted this to be the result of coagulation, by the salt ions, of the colloidal material in the cell wall and plasmatic layer.

The ions of fertilizer salts surrounding the root cells might be expected then to cause an increase in water intake and perhaps a decrease in the density of the sap.

The density of the expressed sap of plants grown on well fertilized and unfertilized plots was ascertained and the results obtained are given in table 16. The data which were obtained during the period of most rapid growth show that in all but four cases the sap from the high yielding plots was less dense than that from the low yielding plots. In two of these four cases there was no difference, and in the other two cases only a very small difference. Furthermore, these four exceptions were all from fields on which benefits from fertilizer have been small.

An examination of several of the tables reveals that in most cases greater differences in yield were accompanied by differences in sap density.

CONCLUSIONS

1. Any fertilizer, with the exception of nitrogen, that increased plant growth had a tendency to decrease the nitrate content of the sap. Nitrogen fertilizers tended to increase the nitrate content of the sap.

2. The total nitrogen content of the dried tissue varied directly with the amount of nitrate in the sap.

3. The stems, as a general rule, contained more nitrate nitrogen in the sap and less total nitrogen in the tissue than did the leaves of the same plants.

4. The size of the plants of the small grains seemed to make no difference in the proportion of stems to leaves.

5. The spring grains possessed greater ability to accumulate nitrate in the sap than did the winter grains.

6. The total nitrogen in the tissue, regardless of fertilizer treatments declined steadily throughout the growing season.

7. The state of fertility of the soil had more effect than soil type on the nitrate content of the sap and the total nitrogen content of the tissue of the small grains.

8. Plants treated with sodium nitrate showed a steady decrease in the nitrate content of the sap throughout the season.

9. Plants treated with all fertilizers showed an increase in nitrate in the sap during the period when the grain was forming as compared to the earlier rapid growth period.

10. Any fertilizer that increased plant growth caused

a decrease in sap density during the period of rapid growth.

[illegible]

1. [illegible], J.A. - "[illegible]". [illegible]. 19[illegible].

2. [illegible]ll, [illegible] - "[illegible]". [illegible]. 19[illegible].

3. [illegible], [illegible] - "[illegible]". [illegible].

4. [illegible], J.A. - "[illegible]". [illegible].

5. [illegible], T.L. and [illegible], [illegible] - "[illegible]". [illegible].

6. [illegible], [illegible] and [illegible], [illegible] - "[illegible]". [illegible] 55:1[illegible]-1[illegible]. 19[illegible].

7. [illegible], J.W. - "The composition of the maize plant". [illegible]. 1[illegible].

8. [illegible], L. - "[illegible]". Jour. [illegible] 1167-1173. 19[illegible].

9. [illegible], T.L. - "[illegible] of Agriculture".

10. Lyon, T.L. and [illegible]ll, J.A. - "[illegible]". Results for [illegible] 191[illegible] to 191[illegible] inclusive. [illegible]ell Univ. Agr. Exp. Sta. Mem. 1[illegible].

11. ------- ---- and ---------------- - "[illegible] of [illegible] to the formation of nitrates in soils". [illegible] Univ. Agr. Exp. Sta. [illegible] 1.

12. ------------ and ---------------- - "The influence of [illegible] on the production of nitrates in soils". Jour. [illegible] 37:101-107. 191[illegible].

13. ----------------, -------------- and Wilson, B.D. - "The formation of nitrates in a soil following the growth of red clover and of timothy". Soil Science 9:53-64. 1920.

14. ----------------, -------------- and ---------------- "Depressive influence of certain higher plants on the accumulation of nitrates in soils". Jour. Amer. Soc. Agron. 15:457-467. 1923.

15. MacDougal, D.T. - "Permeability and the increase of volume of contents of the living and of artificial cells". Proc. of the American Philosophical Society. 62:1-25. 1923.

16. McCool, M.M. - "Relation of soils to plant cell sap". Mich. Agr. Exp. Sta. Tech. Bul. 9. 60-64. 1926.

17. ---------------- - "The effect of soil fertilization on the moisture content, density, heat of wetting and phosphorus content of the cell sap of plants". Proc. 1st. International Soil Science Congress. 1927.

18. ---------------- and Weldon, M.D. - "The effect of soil type and fertilization on the composition of the expressed sap of plants". Jour. Amer. Soc. Agron. 20: 778-792. 1928.

19. Nightingale, G.T. - "The chemical composition of plants in relation to photoperiodic changes". Wis. Agr. Exp. Sta. Res. Bul. 74.

20. Rhode Island Agricultural Exp. Sta. Fortieth annual report of the director 1928.

21. Schweitzer, P. - "Study of the life history of corn at its different periods of growth". Missouri Agr. Exp. Sta. Bul. 9.

22. Smith, J.B. - "Distribution of nitrates in three layers of fallow soils". Soil Sci. 26:347-350. 1928.

23. Smith, J.B. - "The nitrate content of the Rhode Island experimental plots as influenced by fertilizers and crops". Jour. Amer. Soc. Agron. 18:888-896. 1926.

24. Wilfarth, H., Roemer, H., and Wimmer, G. - "Über die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums". Landw. Vers. Sta. 63:1-70 1906.

25. Woo, M.L. - "The chemical constituents of Amaranthus retroflexus". Bot. Gaz. 68:313-345. 1919.

Table 1. The effect of fertilization on the sap and tissue of oats at different stages of growth on Miami loam.

Treatment*	Nitrate Nitrogen in sap	Total N in dry Tissue	Density of sap	Water per gm dry wt.	Yield grain per A.
	ppm.	per cent		grams	bu
		June 1, 1928			
Lime	1470	5.48	1.036	6.46	46.0
" N	1620	5.98	1.038	6.35	47.3
" P	1070	5.44	1.033	6.58	55.8
" NP	1460	5.97	1.036	6.69	59.3
" PK	1040	———	1.031	———	61.9
" NPK	1390	5.72	1.034	6.82	79.2
		June 27, 1928			
Lime	267	3.38	1.031	6.58	
" N	125	2.80	1.030	6.68	
" P	94	2.65	1.029	6.81	
" NP	133	2.76	1.030	6.58	
" PK	102	2.25	1.030	6.93	
" NPK	136	2.40	1.030	6.94	
		July 13, 1928			
Lime	140	1.67	1.040	3.17	
" N	140	1.49	1.038	3.22	
" P	115	1.27	1.037	3.22	
" NP	175	1.58	1.039	2.96	
" PK	122	1.53	1.046	2.85	
" NPK	142	1.52	1.042	2.93	

* N, P, and K, were applied as urea, superphosphate, and potassium chloride respectively.

Table 2. The effect of fertilization on the sap and tissue of oats at different stages of growth on Miami loam.

June 15, 1928

Treatment*	Nitrate Nitrogen in sap	Total N. in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain Per A.
	ppm	per cent		grams	bu.
Check	1230	4.66	1.044	5.12	32.3
PK	942	4.22	1.039	5.48	51.5
NK	1170	4.82	1.044	5.40	32.8
N (P400)	1640	4.83	1.036	6.35	50.5
N (P200)K	870	4.54	1.041	5.95	47.6
N (P400)K	980	4.65	1.037	5.95	57.2
N (P600)K	760	4.86	1.036	5.83	60.4
July 6, 1928					
Check	547	3.03	1.033	5.28	
PK	156	2.16	1.023	6.69	
NK	150	2.06	1.026	6.81	
N (P400)	149	2.03	1.025	5.20	
N (P200) K	147	2.58	1.025	5.41	
N (P400)K	67	1.97	1.025	6.69	
N (P600)K	184	.85	1.020	6.94	
July 24, 1928					
Check	812	1.76	1.034	2.91	
N (P600) K	73	1.13	1.032	2.56	

*Fertilizers were applied for sugar beets the preceding year and were not repreated for the oats.

Table 3. Effect of Nitrate fertilization on the sap and tissue of oats at different stages of growth on Miami loam.

Sodium Nitrate pounds per A.	Nitrate Nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
June 4, 1928					
0	34	3.98	1.033	6.37	53.7
50	495	4.82	1.035	6.37	46.8
100	700	4.91	1.034	6.25	48.1
150	815	5.50	1.034	6.57	59.0
June 27, 1928					
0	97	1.72	1.030	6.04	
50	93	2.30	1.031	6.41	
100	89	1.84	1.031	6.13	
150	92	1.85	1.028	6.46	
July 17, 1928					
0	92	1.05	1.043	2.53	
50	95	1.04		2.46	
100	88	1.03		2.39	
150	92	1.15	1.044	2.56	

Table 4. The effect of fertilization on the sap and tissue of oats at different stages of growth on Hillsdale sandy loam.

May 31, 1928

Treatment*	Nitrate Nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	PPM	per cent		grams	bu.
Check	795-	6.05	1.043	5.25	46.5
Lime	810	5.40	1.036	5.95	47.4
" P	810	5.10	1.037	6.69	45.8
" NP	920	5.85	1.035	6.46	52.6
" PK	302	6.33	1.034	6.58	54.2
" NPK	535	5.67	1.032	7.63	54.1
June 22, 1928					
Check	89	3.26	1.030	6.47	
Lime	85	2.48	1.032	6.20	
" N	116	2.86	1.029	6.47	
" P	63	2.04	1.034	5.71	
" NP	99	2.42	1.031	6.23	
" PK	47	1.92	1.032	6.05	
" NPK	73	1.97	1.029	6.20	

Table 4 (continued)

July 12, 1928

Check	155	1.19	1.044	2.99	
Lime	150	1.09	1.044	2.72	
" N	192	1.50	1.048	2.70	
" P	144	1.23	1.045	2.37	
" NP	170	1.18	1.045	2.57	
" PK	51	0.86	1.045	2.41	
" NPK	105	1.09	1.044	2.28	

* N, P, and K were applied as urea, superphosphate and potassium chloride respectively.

Table 5. The effect of Nitrate fertilization on the sap and tissue of oats at different stages of growth on Hillsdale sandy loam.

Sodium Nitrate pounds per A.	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
June 11, 1928					
0	950	5.75	1.035	5.66-	31.8
50	1130	5.94	1.032	6.46	33.7
100	975	7.65	1.036	5.85	36.8
150	870	5.71	1.032	6.46	37.8
July 5, 1928					
0	147	1.90	1.033	4.62	
50	118	2.38	1.033	5.71	
100	112	2.32	1.031	5.67	
150	106	2.44	1.032	6.19	
July 19, 1928					
0	127	1.36	1.034	2.70	
50	171	1.49	1.036	2.75	
100	132	1.50	1.037	2.76	
150	117	1.46	1.034	2.98	

Table 6. The effect of fertilization on the sap and tissue of barley at different states of growth on Miami loam.

Treatment*	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
May 22, 1928					
Check	600	5.60	1.024	8.44-	17.7
P	450	4.57	1.032	6.82	18.7
PK	535	5.85	1.024	8.72	19.3
NPK	505	5.12	1.027	6.35	22.3
June 21, 1928					
Check	61	2.59	1.027	6.62	
P	72	2.38	1.025	6.62	
PK	42	1.79	1.028	5.53	
NPK	46	1.70	1.026	5.21	
July 13, 1928					
Check	100	1.13	1.052	2.73	
P	85	.96	1.048	2.56	
PK	88	.76	1.047		
NPK	96	.84	1.050	1.86	

* N, P, and K were applied as ammonium sulfate, superphosphate and potassium chloride respectively.

Table 7. The effect of fertilization on the sap and tissue of barley at different stages of growth on Miami loam.

May 22, 1928

Treatment*	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
Check	760	5.32		5.25	27.3
P	895	5.80	1.034	5.85	35.8
PK	655	5.71	1.032	6.14	27.0
NPK	1090	6.07	1.033	6.57	43.3
June 20, 1928					
Check	169	2.90	1.031	5.15	
P	107	2.42	1.036	4.95	
PK	70	1.90	1.034	4.46	
NPK	106	2.26	1.029	4.97	

* N, P, and K were applied as ammonium sulfate, superphosphate and potassium chloride respectively.

Table 8. The effect of Nitrate fertilization on the sap and tissue of barley at different stages of growth on Miami loam.

May 23, 1928

Sodium nitrate pounds per A.	Nitrate nitrogen in Sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
0	1390	6.14	1.036	5.76	32.2
50	1320	5.56	1.035	6.14	41.3
100	1450	6.87	1.035	6.82	35.6
150	1060	5.97	1.034	6.34	35.2
June 20, 1928					
0	151	2.82	1.033	5.33	
50	330	2.76	1.034	5.33	
100	284	2.59	1.031	4.95	
150	266	4.02	1.032	5.21	

Table 9. The effect of nitrate fertilization on the sap and tissue of barley at different stages of growth on Hillsdale sandy loam.

Sodium nitrate pounds per A	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water - per gm. dry wt.	Yield grain per A.
June 11, 1928					
	ppm	per cent		grams	bu.
0	560	5.67	1.037	5.15	11.6
50	705	5.72	1.034	5.95	18.1
100	645	5.71	1.036	5.55	19.3
150	850	5.88	1.035	6.40	22.9
July 5, 1928					
0	159	1.98	1.039	4.16	
50	157	2.10	1.035	4.16	
100	159	2.04	1.032	4.05	
150	121	1.90	1.028		
July 19, 1928					
0	83	1.43	1.030	2.95	
50	92	1.55	1.032	2.72	
100	76	1.28	1.034	2.18	
150	141	1.48	1.032	2.35	

Table 10. The effect of general fertilization on the sap and tissue of wheat at different stages of growth. Miami loam soil type.

November 26, 1927.

Treatment	Nitrate Nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm dry wt	
	ppm	per cent		grams	
Lime	188	4.66		3.14	
" N		5.58		4.38	
" P	220	3.88		2.93	
" NP	194	4.86		3.35	
" PK	203	4.80		4.52	
" NPK		4.96		4.08	

June 8, 1928

Treatment	Nitrate Nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm dry wt	
Lime	193	3.19	1.045	3.68	
" N	113	2.23	1.054	3.05	
" P	110	2.88	1.046	3.81	
" NP	145	2.50	1.047	3.21	
" PK	91	1.45	1.053	3.19	
" NPK	118	1.91	1.048	3.24	

Table 10 (continued)

June 28, 1928

Lime	215	1.26	1.058	2.23	
" N	198	1.10	1.053	2.67	
" P	114	1.25	1.045		
" NP			1.050	2.32	
" PK	151	1.06	1.063		
" NPK	184	1.07	1.052	2.21	

* N, P, and K were applied in the form of urea, superphosphate and potassium chloride respectively.

Table 11. The effect of fertilization on the sap and tissue of wheat at different stages of growth. Hillsdale sandy loam.

November 29, 1927

Treatment	Nitrate Nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.
	ppm	per cent		grams
Check -	115-	4.64		3.58
Lime	109	4.68		3.46
" N	161	5.14		3.94
" P	240	5.22		3.69
" NP	188	4.94		4.32
" K	74	4.60		4.10
" NK	118	4.64		3.81
" PK	76	4.14		3.61
" NPK	99	4.44		4.01

May 7, 1928

Treatment	Nitrate Nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.
Check	281-	4.20	1.064	3.10
Lime	292	3.72	1.064	2.90
" N	274	3.53	1.064	3.04
" P	288	3.80	1.065	2.97
" NP	270	4.14	———	3.24
" K	224	3.59	1.064	3.13
" NK-	270	3.93	1.064	2.73
" PK	267	3.06	1.063	3.24
" NPK	286	4.01	1.058	3.06

Table 11 (continued)

June 7, 1928

Treatment	Nitrate Nitrogen in sap	Total N in dry tissue	Specific gravity of sap	Water per gm. dry wt.
	ppm	per cent		grams
Check	249	1.31		3.28
Lime	245	1.58		3.33
" N	288	1.37		3.43
" P	267	1.31		3.00
" NP	309	1.30		3.44
" NK	213	1.42		3.64
" PK	236	1.44		3.19
" NPK	330	1.12		3.26

July 2, 1928

Lime	305	0.84	1.070	1.79
" N	300	1.02	1.069	———
" P	239	———	1.069	1.74
" NP	340	0.78	1.076	1.64
" K	268	0.80	1.068	———
" NK	345	0.78	1.074	———
" PK	221	0.86	1.070	1.95
" NPK	305	0.75	1.072	1.70

All plots were limed except the check. N, P, and K were applied as urea, superphosphate, and potassium chloride respectively.

Table 12. The effect of nitrate fertilization on the sap and tissue of wheat at different stages of growth. Miami loam soil type.

May 10, 1928

Sodium nitrate pounds per A	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
0	292	3.61	1.056	2.90	31.7
50	390	3.76	1.068	3.39	33.4
100	396	4.02	1.064	3.63	39.0
150	370	4.05	1.062	3.54	36.7

June 8, 1928

0	209	1.97	1.045	3.58	
50	202	2.01	1.044	3.74	
100	278	1.65	1.046	3.42	
150	174	1.86	1.048	3.54	

June 27, 1928

0	197	1.40	1.057	2.24	
50	220	1.26	1.058	2.21	
100	237	1.13	----	1.80	
150	203	1.02	1.057	1.98	

Table 13. The effect of nitrate fertilization on the sap and tissue of wheat at different stages of growth on Hillsdale sandy loam.

Sodium nitrate pounds per A	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
May 7, 1928					
0	189	3.17	1.064	2.97	27.4
50	215	3.16	1.064	2.94	27.9
100	277	3.19	1.062	3.35	31.9
150	274	3.59	1.056	3.38	33.1
May 31, 1928					
0	146	1.18	1.053	2.93	
50	164	1.56	1.052	3.55	
100	146	1.62	1.045	3.59	
150	154	1.48	1.050	3.27	
June 22, 1928					
0	151	.94	1.056	2.16	
50	159	1.01	1.051	2.38	
100	138	.94	1.048	2.39	
150	166	.93	1.053	2.02	

Table 14. The effect of nitrate fertilization on the sap and tissue of rye at different stages of growth on Miami loam.

May 9, 1928

Sodium nitrate pounds per A	Nitrate nitrogen in sap	Total N. in dry tissue	Density of sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent		grams	bu.
0	540	4.81		4.15	10.6
50	480	4.28		3.86	11.7
100		4.64		3.95	14.0
150	515	4.68		4.00	13.8
June 12, 1928					
0	167	2.06	1.040	3.85	
50	170	2.98	1.041	4.40	
100	306	2.57	1.037	4.52	
150	355		1.035	4.88	
July 3, 1928					
0	191	1.17	1.046	2.15	
50	274	1.64	1.046	2.38	
100	235	1.32	1.051	2.01	
150	268	1.50	1.049	2.32	

Table 15. The effect of nitrate fertilization on the sap and tissue of rye at different stages of growth on Hillsdale sandy loam.

May 9, 1928

Sodium nitrate pounds per A	Nitrate nitrogen in sap	Total N in dry tissue	Density pf sap	Water per gm. dry wt.	Yield grain per A.
	ppm	per cent	.	grams	bu.
50	276	2.93	1.050	4.0	40.0
100	330	3.30	1.048	4.21	52.2
150	228	2.93	1.049	3.8	42.2
June 12, 1928					
50	190	1.01	1.035	5.53	
100	228	.94	1.032	5.76	
150	170	1.01	1.032	6.14	

Table 17. A study of the sap and tissue of the leaves and stems.

June 9 - 10, 1928

Treatment material	Nitrate nitrogen in sap	Total N in dry tissue	Density of sap	Water per gm. dry wt.	Total wt. of green plants.
	ppm	per cent		grams	per cent
		Oats			
NPK leaves	63	1.97	1.025	4.44	33.28
" stems	75	.63	1.021	5.45	66.72
CK leaves	260	3.78	1.028	5.50	34.70
" stems	313	1.49	1.023	6.75	65.70
		Barley			
NPK leaves	103	1.37		1.53	26.00
" stems	92	1.49		1.90	74.00
CK leaves	116	1.73		1.94	30.30
" stems	168	.50		1.69	69.70
		Wheat			
NPK leaves	315	.72		.80	11.90
" stems	328	.60		1.38	88.10
Lime leaves	278	3.27		1.52	18.00
" stems	328	1.07		1.74	82.00
		Rye			
$NaNo_3$ Leaves		1.68		1.04	11.80
" stems		.82		1.58	88.20
CK leaves		1.76		1.12	9.00
" stems		.76		1.59	91.00

Table 18. The effect of stage of growth on the sap and tissue on different soil types. Averages of all the general fertilizer plots sampled.

Soil Type	Nitrate nitrogen in sap			Total nitrogen in tissue		
	1st Sampling	2nd Sampling	3rd Sampling	1st Sampling	2nd Sampling	3rd Sampling
	ppm	ppm	ppm	per cent	per cent	per cent
			Wheat			
Miami		127	181		2.36	1.24
Hillsdale	270	268	296	3.89	1.36	.83
			Oats			
Miami	1100	394	525	4.65	1.98	1.44
Miami	1350	143	141	5.72	2.71	1.51
Hillsdale	715	83	143	5.73	2.42	1.16
			Barley			
Miami	525	55	93	5.28	2.11	.92
Miami	845	118	225	5.72	2.37	
Brookston	1070	79	173	6.27	1.95	1.30

Table 19. The effect of stage of growth on the sap and tissue on different soil types. Averages of all the nitrate fertilizer plots sampled

Soil type	Nitrate Nitrogen in sap			Total Nitrogen in Tissue		
	1st Sampling	2nd Sampling	3rd Sampling	1st Sampling	2nd Sampling	3rd Sampling
	ppm	ppm	ppm	per cent	per cent	per cent
		Wheat				
Miami	360	220	212	3.36	1.87	1.23
Hillsdale	240	152	155	3.28	1.46	.95
		Rye				
Miami	460	258	39	4.60	2.53	1.41
Hillsdale	280	190		3.02	.98	
		Oats				
Miami	490	91	94	4.80	1.93	1.07
Hillsdale	990	118	133	6.26	2.26	1.45
		Barley				
Miami	1310	264	224	6.13	3.05	
Hillsdale	690	151	96	5.74	2.00	1.43

Table 20. The effect of fertilizers on the specific gravity of the sap of wheat, oats and barley.

Crop	Specific gravity of sap	
	From low yield plot	from high yield plot
Wheat	1.045	1.046
"	1.064	1.064
"	1.053	1.050
Rye	1.040	1.035
"	1.050	1.048
Oats	1.044	1.036
"	1.031	1.028
"	1.034	1.032
"	1.033	1.033
Barley	1.037	1.026
"	1.034	1.028
"	1.038	1.024
"	1.033	1.034
"	1.039	1.028