FACTORS AFFECTING ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN, PARTICULARLY IN TOMATO AND APPLE

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(WITH SIX FIGURES)

Introduction

Plants absorb ammonium and nitrate nitrogen in varying quantities, depending partly on the rate of assimilation[2] of either ion in the plant. That certain specific conditions are necessary for the most efficient assimilation of either form of nitrogen has been pointed out (**40**). Different species vary in the efficiency with which they utilize the nitrate or ammonium ion under a given set of conditions (**29**). The hypothesis that both ions probably are finally utilized as ammonium, even though external requirements may be different, involves an interesting study, a preliminary report of which has been published (**40**). A continuation of these studies has established certain relationships affecting the assimilation of nitrate and ammonium nitrogen. Such factors as hydrogen-ion concentration and available carbohydrates have a direct bearing on the assimilatory processes. That these relationships vary with the source of nitrogen, and that the location of the initial assimilatory processes varies with the type of plant, will be pointed out.

Experimental methods

Tomato and cotton plants, and small 1-year-old Delicious, Stayman, and Baldwin apple trees, all root-grafted, were grown in sand cultures. The nutrient solutions were modifications of one of the ammonium sulphate series recommended by JONES and SHIVE (**12**), the compositions of which are shown in table I.

Calcium and sodium salts were used as the source of nitrate, whereas ammonium sulphate, ammonium carbonate, and ammonium hydroxide were used as the sources of ammonium. Details of growing the plants for some of the experiments have been discussed elsewhere (**39, 40**).

Experiments were conducted with the tomato (*Lycopersicon esculentum* Mill.) cotton, and apple (*Pyrus malus* L.) in which the pH of the solutions percolating through the sand was maintained within 0.2 of the initial pH.

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[2] Assimilation in this report refers to the reduction and elaboration of the nitrate and ammonium ions after they have been absorbed by the plant.

TABLE I

PARTIAL VOLUME MOLECULAR CONCENTRATION OF COMPOUNDS IN NUTRIENT SOLUTIONS. INITIAL pH OF NUTRIENT SOLUTIONS WAS MODIFIED TO SATISFY REQUIREMENTS OF EXPERIMENTS WITH 0.1N SULPHURIC ACID OR POTASSIUM HYDROXIDE UNLESS OTHERWISE STATED

SERIES	pH*	KH_2PO_4	$CaCl_2$	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	$NaNO_3$	$MgSO_4 \cdot 7H_2O$
A	3–9 incl.	0.00633	0.00292	....	0.00584	....	0.00237
B	3–9 incl.	0.00633	0.00292	....	....	0.01168	0.00237
C	4–9 incl.	0.00633	0.00292	0.00560	....	....	0.00237
D*	6.4–9 incl.	0.00633	0.00292	....	....	....	0.00237
E	8.0	0.00530	0.00146	0.00840	....	....	0.00118
F	8.0	0.00633	0.00584	0.00140	....	....	0.00237
G	5.1	0.00633	0.00584	....	0.00146	....	0.00237

* Number of cc. of $\frac{M}{2}$ ammonium hydroxide per liter for each pH as indicated: pH 6.4 = 1, 6.8 = 2, 7.3 = 3, 7.8 = 4, 8.4 = 6, and 8.8 = 8 cc. The pH 8.4 cultures did not receive quite so much nitrogen as the C cultures supplied with ammonium sulphate.

In these cultures from 10 to 24 liters of nutrient solution, varying with the size of the plants, were supplied to the sand cultures every 24 hours. This was accomplished by running 5 to 12 liters of solution through in 12 hours. The solution after dripping from the cultures was adjusted to the desired pH and used several times. The solutions were changed every third day. Solution lost by evaporation and transpiration was replaced by fresh nutrient solution. The osmotic concentrations varied slightly during the 3-day period that the solutions were used. Because of the large amount of nutrient solution required, the experiments necessarily were limited in scope, but were sufficiently comprehensive to establish the constant pH at which the plants would grow with nitrate and ammonium nitrogen, and supplemented those experiments from which the data have been published (**39**, **40**).

Extraction of plant tissues by chemical methods.—Two methods were employed in obtaining an extract of ionizable substances from the plant tissue, namely, aqueous extraction of the fresh tissue, and extraction of the fresh tissue by electrodialysis, which separated the anions from the cations into two separate solutions. When time did not permit immediate extraction of the fresh tissue, it was weighed into quart Mason fruit jars, sealed, and stored at -6 to $-20°$ C. until analyzed. The freezing method employed has been found to be satisfactory for this purpose (**24**). Handling the tissue in this manner made it possible to harvest an entire series of twenty or more samples and analyze them as time permitted.

Extraction of plant tissue by electrodialysis.—The three-compartment electrodialyzer as employed and described by Mattson (**16**) was found satisfactory for obtaining extracts of plant tissue. Cations were removed by a copper cathode and anions were removed by means of a platinum anode. Most of the amino acids, amides, and polypeptide groups were collected in the cathode chamber. An advantage of this method of extraction was the separation of nitrate nitrogen from the other nitrogenous fractions, thus eliminating nitric acid, which apparently interferes with the analysis of other nitrogenous fractions (**39**). Electrodialysis also left the more complex proteins in the central chamber. The solution containing the cations was usually clear, but in some cases contained a yellow pigment. The solution containing the anions was clear and usually colorless. Ammonium and other monovalent cations were extracted from the tissue in a very short time, whereas the anions were removed much more slowly. This has been shown by Moore, Reeves, and Hixon (**19**). In the present studies it was found that ammonium ions were almost completely removed in 15 minutes.

The protein content in the tissue remaining in the central chamber was usually slightly lower in the electrodialyzed sample, and the soluble organic and inorganic nitrogen was higher than in the residue of sample extracted with water and coagulated with dilute acetic acid. This was accounted for by difference in the efficiency of the two methods in extracting nitrates and ammonium ions, which will be shown by data to be published later. Amide nitrogen was entirely removed only after carefully spacing the electrodes and removing the dialysate from the cathode chamber at frequent intervals. Amides and amino acids will take on a positive or negative charge, depending on the pH of the solution in which they are dissolved, and will then tend to move to the pole of opposite charge. Asparagine has

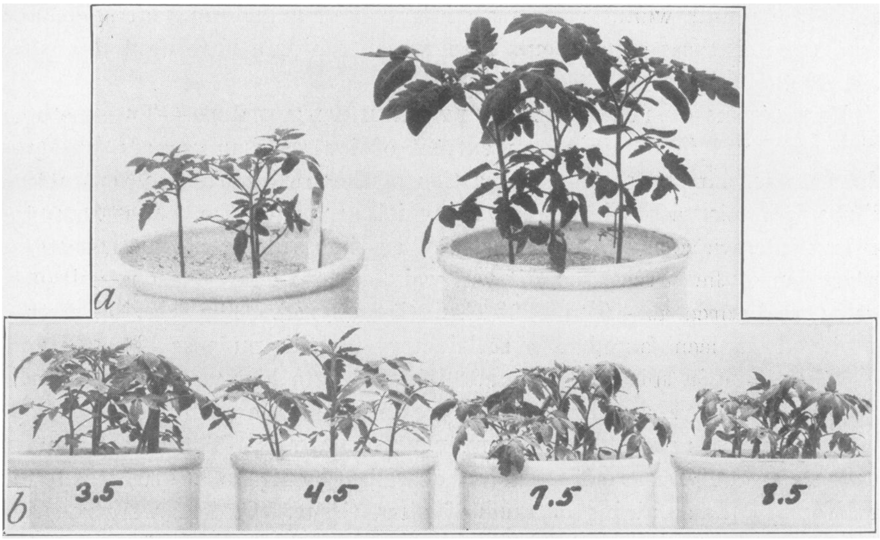

FIG. 1. *a*, tomato plants of experiment I in sand cultures, initially extremely high in carbohydrates, after 3 weeks with a complete nutrient solution containing sodium nitrate at initial pH 5.4 (left) and ammonium sulphate at initial pH 8.0 (right). *b*, tomato plants grown with sodium nitrate at initial pH 3.5 and 4.5 (left) and ammonium sulphate at initial pH 7.5 and 8.5 (right). These plants received the nutrient solution as soon as the first true leaf appeared.

an isoelectric point of pH 4.0 to 4.2. Asparagine in tomato sap at pH 5.4 probably has a negative charge; but when placed in the electrodialyzer, because of the rapid extraction of the monovalent cations, the material containing the asparagine in the central cell becomes very acid, causing the asparagine to take on a positive charge. It then tends to move in the direction of the cathode chamber, which by this time is definitely alkaline (above pH 8.0). Unless the solution from the cathode chamber is immediately removed, the charge on the asparagine is reversed and it will travel back to the central chamber. Amino acids with higher isoelectric points will tend to remain in the cathode chamber longer, and are therefore more easily removed. Details regarding the application of the electrodialyzer for complete fractionation of the ionizable nitrogen will be presented in a separate paper. There are numerous advantages in extracting tissue in this manner, including the possibility of using colorimetric methods for estimating certain fractions.

AQUEOUS EXTRACTION OF PLANT TISSUE.—The fresh or frozen tissue was extracted with water and freed of protein by coagulation with dilute acetic acid after the method of CHIBNALL (**4, 5**).

The methods used for analysis of extracts have already been described (**39**).

Presentation of results

EXPERIMENT I.—Tomato plants were washed free of soil and set in sand when 4 inches high. They were given a minus-nitrogen solution until the stems were stiff and woody and the leaves yellowish green. Starch was abundant in all parenchymatous tissue. The plants were then divided into two series of twenty cultures each. One series was supplied with ammonium sulphate in a complete nutrient solution at pH 8.0, the other sodium nitrate at pH 5.1. The solution was not held at a constant pH (**38**). The roots, stems, and leaves were analyzed for nitrogenous fractions. Figure 1*a* shows these plants 3 weeks after being given nutrient solutions B and C (table I). Three weeks later, on October 15, when the plants of both series were about 10 inches high, they were harvested and analyzed. The chemical data are shown in table II and in columns 1 and 2 of figure 2.

The ammonium-supplied plants were definitely succulent, and had in the upper stem a very active cambium and starch (only in the region near the phloem). In the base of the stem there was still some starch in the pith. The tap root contained starch only in the endodermis. The plants which received nitrate had not become so succulent as the ammonium-supplied series and the cambium was not so active. Starch was distributed more generally throughout the plants with the exception of the tip. The dry matter of the roots and leaves, however, was only slightly higher. The plants of the ammonium series, as compared with those receiving nitrate, were more spreading, owing to softer petioles and slightly larger leaflets which had a tendency to droop more. The internodes, however, were slightly longer in the plants receiving nitrate.

EXPERIMENT II.—One lot of twelve tomato plants was grown to the fruiting stage with an abundant supply of ammonium sulphate, to determine how much organic nitrogen would accumulate, and whether it would be possible so completely to utilize carbohydrates that the plant might be injured as a result. A complete nutrient solution of which one-half of the

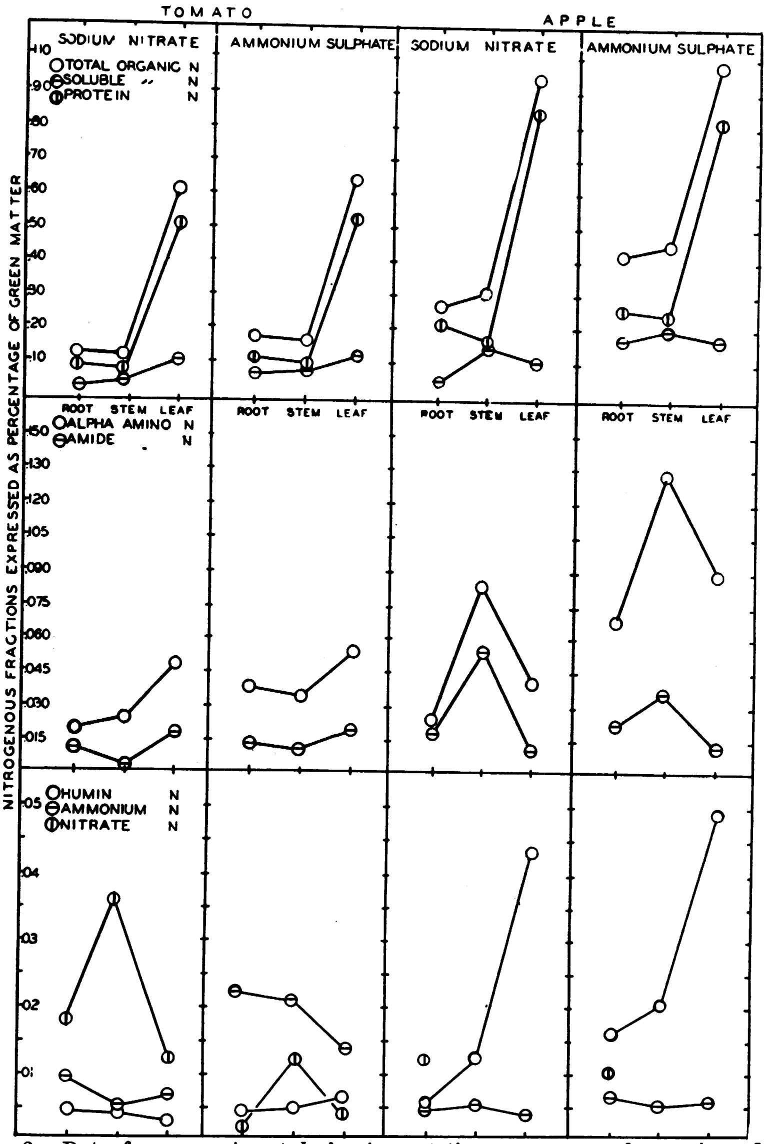

FIG. 2. Data from experiment 1 showing relative percentage of organic and inorganic nitrogen in ammonium and nitrate-supplied tomato. The comparable data on apple (experiment I) have been presented elsewhere (39) but are presented here for comparative purposes.

TABLE II

EXPERIMENT I

NITROGENOUS FRACTIONS IN CARBOHYDRATE-HIGH TOMATO PLANTS SIX WEEKS AFTER BEING SUPPLIED WITH SODIUM NITRATE AT pH 5.1 OR AMMONIUM HYDROXIDE AT pH 8.0

	Nitrogen as percentage of green matter						Percentage of total organic nitrogen					
	Roots		Stems		Leaves		Roots		Stems		Leaves	
	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3
	%	%	%	%	%	%	%	%	%	%	%	%
Percentage dry matter	8.0	6.0	7.0	8.0	13.0	12.0	......	......	......	......	......	......
Total organic N	0.1217	0.1754	0.1187	0.1631	0.6040	0.6420	100.00	100.00	100.00	100.00	100.00	100.00
Protein N	0.0856	0.1095	0.0728	0.0919	0.5055	0.5255	70.34	62.43	61.33	56.35	83.69	81.85
Soluble organic N	0.0361	0.0659	0.0459	0.0712	0.0985	0.1165	29.66	37.57	38.67	43.65	16.31	18.15
Humin N	0.0045	0.0040	0.0037	0.0048	0.0025	0.0060	3.70	2.28	3.12	2.94	0.41	0.93
α Amino N	0.0162	0.0387	0.0221	0.0375	0.0443	0.0544	13.31	22.06	18.62	22.99	7.33	8.47
Amide N	0.0083	0.0130	0.0017	0.0118	0.0110	0.0194	6.82	7.41	1.43	7.23	1.82	3.02
Ammonium N*	0.0092	0.0221	0.0059	0.0218	0.0075	0.0135	6.32	11.07	3.68	11.03	1.20	2.05
Nitrate N†	0.0147	0.0022	0.0357	0.0127	0.0125	0.0045	10.10	1.10	21.60	6.43	2.00	0.68
Other N	0.0071	0.0102	0.0184	0.0171	0.0407	0.0367	5.83	5.82	15.50	10.49	6.75	5.72

* Percentage of total nitrogen.

† Percentage of total nitrogen (includes nitrate determined in extract from electrodialyzed tissue; none was recovered from ammonium plants by ordinary extraction).

TABLE III

EXPERIMENT II

NITROGENOUS FRACTIONS AS PERCENTAGE OF GREEN MATTER IN DIFFERENT PARTS OF TOMATO PLANTS WHICH RECEIVED AMMONIUM SULPHATE AS THE ONLY SOURCE OF NITROGEN IN A COMPLETE NUTRIENT SOLUTION SUPPLIED AT INITIAL pH 8.0 AND FINAL pH 3.8

	PLANTS HARVESTED DECEMBER 11											
	FIBROUS ROOTS		STEMS		OLD BLADES		YOUNG BLADES		OLD PETIOLES		YOUNG PETIOLES	
	%	%	%	%	%	%	%	%	%	%	%	%
Percentage dry matter	9.68	....	10.13	....	9.2	....	10.4	....	7.03	....	6.4	....
Total organic N	0.4800	100.00	0.3765	100.00	0.4230	100.00	0.6641	100.00	0.2171	100.00	0.2162	100.00
Protein N	0.2490	51.88	0.1443	38.33	0.2818	66.62	0.5003	75.34	0.0978	45.05	0.1045	48.34
Soluble organic N	0.2310	48.12	0.2322	61.67	0.1412	33.38	0.1638	24.66	0.1193	54.95	0.1117	51.66
Basic N	0.0296	6.17	0.0200	5.31	0.0405	9.57	0.0390	5.87	0.0195	8.98	0.0146	6.75
α Amino N	0.1211	25.23	0.1220	32.40	0.0561	13.26	0.0665	10.01	0.0617	28.42	0.0561	25.95
Amide N	0.0416	8.67	0.0275	7.30	0.0175	4.14	0.0190	2.86	0.0240	11.05	0.0346	16.00
Ammonium N*	0.0690	12.34	0.0450	10.55	0.0730	14.46	0.0550	7.58	0.0670	22.90	0.0430	16.21
Nitrate N†	0.0103	1.84	0.0050	1.17	0.0090	1.78	0.0065	0.90	0.0085	2.90	0.0061	2.30
Other N	0.0387	8.06	0.0627	16.65	0.0271	6.45	0.0393	5.92	0.0141	6.49	0.0064	2.96

* A large amount of this ammonia is combined ammonia (40).

† Ammonia and nitrate expressed as percentage of total nitrogen.

concentration was ammonium sulphate (E, table I) was supplied at pH 8. The ammonium sulphate was gradually increased from solution A to E as the plants grew larger. The plants were harvested December 11, at which time the days were short and relatively cloudy. The night temperature of the greenhouse during this period was kept between 56° and 62° F.

The plants were extremely succulent, although they grew slowly, and the leaves contained only 10 per cent. dry matter and practically no starch. The stems were more or less hollow, owing to disintegration of pith cells, and were comparatively soft. The plants were unfruitful, although they produced occasional flowers. There was no external indication of the high percentage of ammonium present. A trace of nitrate was detected throughout the plant. The nitrogenous fractions in the different plant parts are shown in table III.

Experiment III.—From April 20 to August 15, two series each consisting of twenty 1-year-old Delicious apple trees were grown in sand cultures. They were supplied with sodium nitrate at initial pH 5.1, and ammonium hydroxide at initial pH 8 (B and D respectively, table I). The chemical data and observations are reported in detail elsewhere (**39**), but for comparative purposes are summarized graphically in figure 2, columns 3 and 4.

Experiment IV.—One-year-old Delicious apple trees were set in nitrogen-free sand and grown with minus-nitrogen solution until all the inorganic nitrogen in the plants had been assimilated. They made from 12 to 15 inches of stem growth, the leaves became yellow and eventually abscissed, and the trees become dormant. These dormant trees were divided into two series of twenty each, were supplied daily with nitrate or ammonium solutions (B and D, table I), and were analyzed at intervals to determine the relative rates of assimilation of the two forms of nitrogen in dormant trees. The results have been published (**39**) but will be referred to in the discussion.

Experiment V.—One-year-old Delicious apple trees were placed in sand cultures and supplied with nutrient solutions containing ammonium sulphate or sodium nitrate at initial pH values ranging from 3.5 to 8.5 (B and C, table I). Ten trees were grown at each pH value. Detailed results have been reported (**39**), but are summarized graphically in figure 3.

Experiment VI.—In order to determine how the results obtained with the tomato in sand cultures would apply to soil conditions, a sandy loam soil was selected with a reaction of pH 4.0. Lime was added to two lots of this soil, so that three series were established; the original, pH 4.0, and the two limed series, 6.0 and 7.4. Tomato plants high in carbohydrates, with stiff woody stems and small yellowish leaves, were planted in each soil series (twenty plants at each pH) and supplied with nutrient solutions containing sodium nitrate, ammonium sulphate, and ammonium hydroxide re-

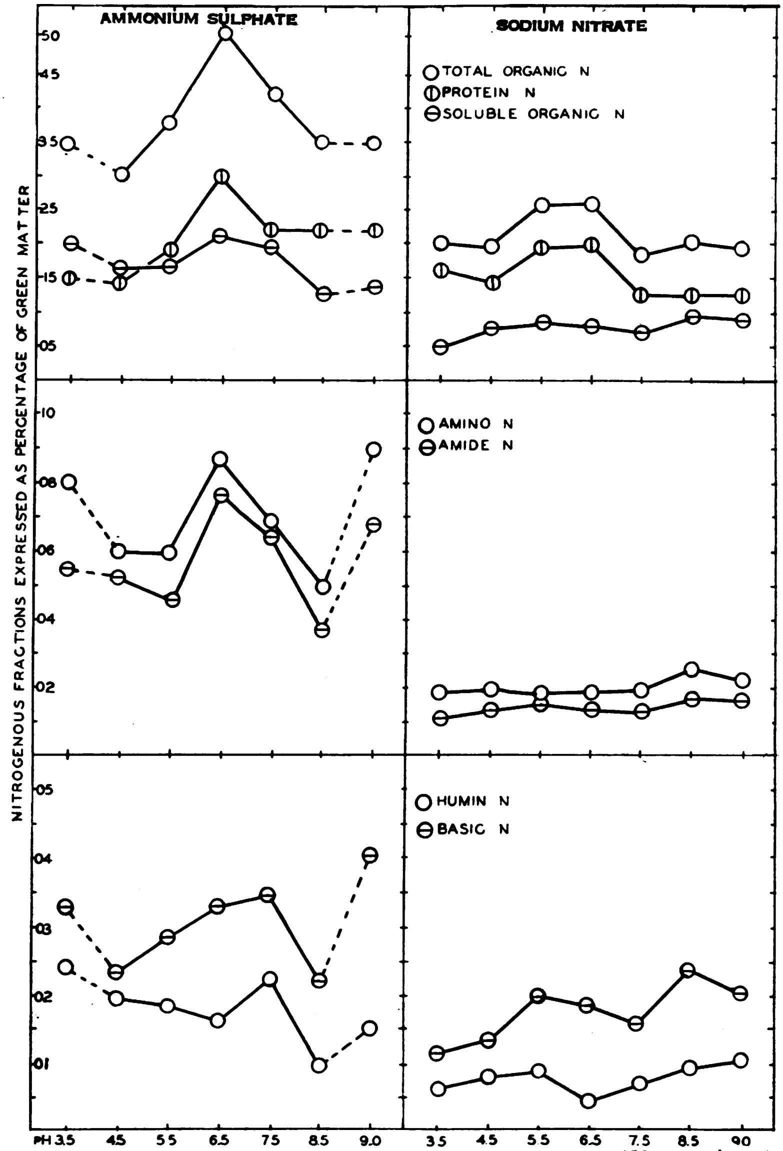

FIG. 3. Data from material previously published in detail (39 experiment III) showing relationships of H-ion concentration of nutrient medium to elaboration of nitrogenous fractions in roots of apple trees supplied with sodium nitrate or ammonium sulphate.

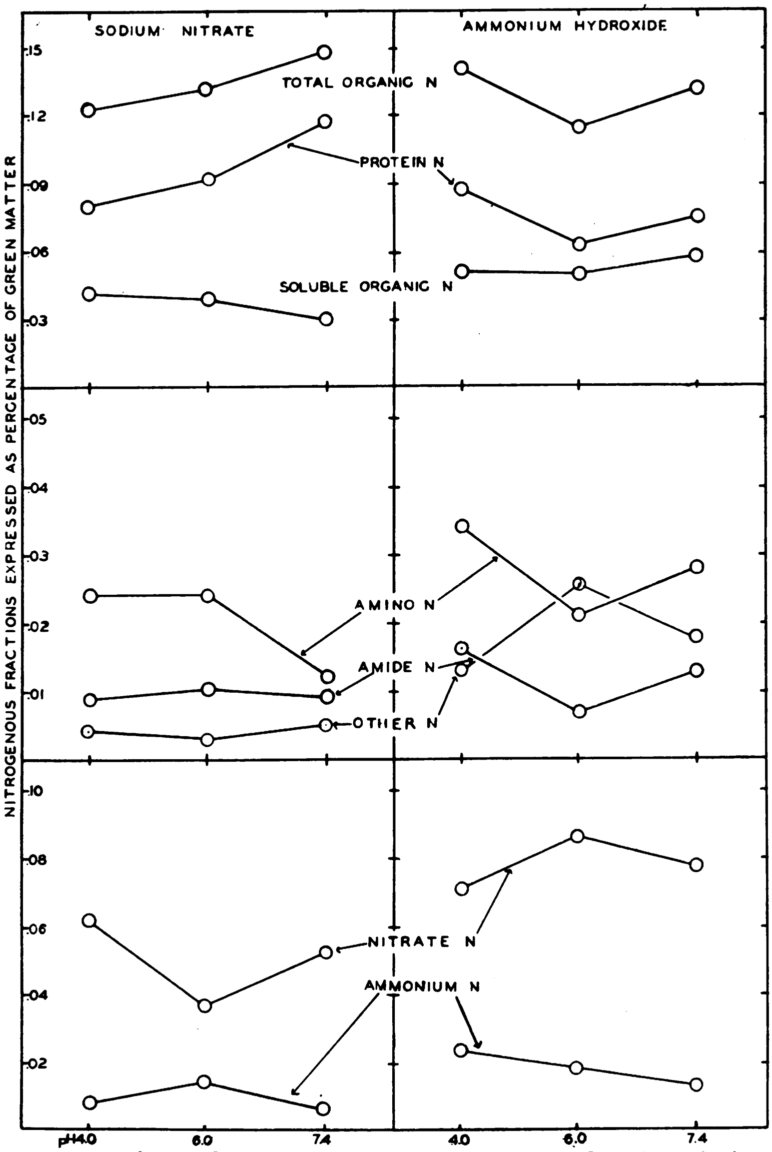

FIG. 4. Data from table IV, experiment VI, showing elaboration of nitrogenous fractions in stems of tomato plants grown on a soil adjusted to pH 4, 6.0, and 7.4 with hydrated lime and supplied with nutrient solution containing sodium nitrate or ammonium hydroxide.

TABLE IV

EXPERIMENT VI

TOMATO PLANTS GROWN IN SOIL ADJUSTED WITH HYDRATED LIME TO THE H-ION CONCENTRATION INDICATED AND SUPPLIED WITH NITRATE OF SODA OR AMMONIUM HYDROXIDE

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

Source of nitrogen	$NaNO_3$	$NaNO_3$	$NaNO_3$	NH_4OH	NH_4OH	NH_4OH	None
Dry matter (%)	6.5	6.8	6.5	5.6	6.0	6.3	7.7
pH of soil	4.0	6.0	7.4	4.0	6.0	7.4	4.0
Organic N	0.1226	0.1330	0.1473	0.1396	0.1137	0.1317	0.1214
Protein N	0.0800	0.0917	0.1193	0.0876	0.0618	0.0753	0.0671
Soluble organic N	0.0426	0.0413	0.0300	0.0520	0.0519	0.0564	0.0543
α Amino N	0.0239	0.0241	0.0125	0.0338	0.0211	0.0280	0.0324
Amide N	0.0098	0.0113	0.0093	0.0152	0.0071	0.0129	0.0235
Ammonium N	0.0082	0.0141	0.0066	0.0232	0.0178	0.0133	0.0000
Nitrate N	0.0615	0.0352	0.0525	0.0710	0.0851	0.0775	0.0000
Other N	0.0089	0.0059	0.0092	0.0130	0.0237	0.0165	0.0016

spectively. Plants when harvested (for analysis, table IV, figure 4) were 12–14 inches high; the remainder were grown to maturity. Here again the ammonium-supplied plants on the soil having a pH value of 7.4 were largest. At pH 4.0, plants supplied with ammonium sulphate made very poor growth. Microchemical tests for nitrate in the plants of the ammonium-supplied cultures showed only a trace as compared with abundant nitrates in the plants which received sodium nitrate at the same pH. Those in the cultures supplied with ammonium hydroxide at pH 4.0 did not make much growth until nitrification occurred in the soil. The plants in the nitrate cultures, in contrast to the ammonium group, made the most rapid growth at pH 4. Growth of the plants in the pH 6.0 and 7.4 cultures receiving ammonium nitrogen made a greater volume of growth than those receiving nitrate nitrogen at the same pH. The organic nitrogenous fractions are similar to those found in tomato plants grown in sand cultures with single sources of nitrogen (**40**).

EXPERIMENT VII.—During November and December, accompanied by almost continuously cloudy conditions, two series of tomato plants were grown in sand cultures at a night temperature of 58°–62° F. On occasional bright days the temperature was 5°–10° higher. One group received sodium nitrate in complete nutrient solution at an initial pH of 5.1, while the other received ammonium sulphate plus ammonium hydroxide in a complete nutrient solution at an initial pH of 8. Nutrient solutions with one-half of their concentrations made up with the nitrogenous salts (B and C, table I) were supplied to different groups of nine plants each at concentrations of approximately 1.50, 1.25, 1.00, 0.75, 0.50, and 0.25 atmospheres. Another lot of nutrient solutions (F and G, table I) were made up so that only one-fifth of their concentration was made up by the nitrogenous salts. These nutrient solutions were supplied to different groups of nine plants each at concentrations of approximately 1.00 0.75, 0.50, and 0.25 atmospheres. Thus different groups of plants had available concentrations of nitrogen varying from extremely high to extremely low.

The plants growing with sodium nitrate made their greatest volume of growth with the highest concentration of nitrate, and the volume of growth decreased as the concentration of nitrate in the nutrient solution and in the tissue decreased. Microchemical observations showed that starch increased in the stems as the nitrate concentration decreased, but reducing sugars did not show a definite correlation with growth.

The plants of the ammonium cultures made the greatest volume of growth with the 0.50-atmosphere concentration nutrient solution containing a low concentration of ammonium salt. The two cultures receiving the higher concentrations of ammonium salt, at 1.5 and 1.25 atmospheres, made very little growth and remained grayish green, although the plants including the roots appeared uninjured. Only the stems of the ammonium-supplied plants which were growing rapidly contained starch. As the ammonium concentration in the nutrient solution increased, growth was retarded, accompanied by a deficiency of carbohydrates. Only a trace of starch could be detected in the plants supplied with the high concentration of ammonium, and that was in the endodermis. The stems of the plants tended to be hollow, and the cell walls of the xylem tissue were thinner than any of the nitrate-supplied plants.

Experiment VIII.—An experiment with Delicious apple trees similar to experiment VII with tomatoes, but conducted from January 1 to April 1, resulted in observations of a comparable nature. The trees with different concentrations of nitrate nitrogen grew fairly well (fig. 5), but growth was most rapid at one atmosphere concentration. The trees receiving the extremely low concentration of ammonium nitrogen were equal to the nitrate trees, but very little growth occurred with the high concentration of ammonium.

Experiment IX.—Tomato and cotton plants, sweet clover seedlings, and Delicious, Stayman, and Baldwin apple trees were grown in sand cultures in which the pH of the nutrient solution dripping through the sand did not increase nor decrease more than 0.2 pH from that initially supplied. Solution B (table I) was used for the nitrate cultures and solution F (table I) for the ammonium series. From 4 to 12 liters of solution, depending on the size of the plants, were dripped through sand of the individual crocks every 12 hours. Sufficient cultures were set up to give cultures from pH 3.5 to 6.5 at intervals of 0.5 pH.

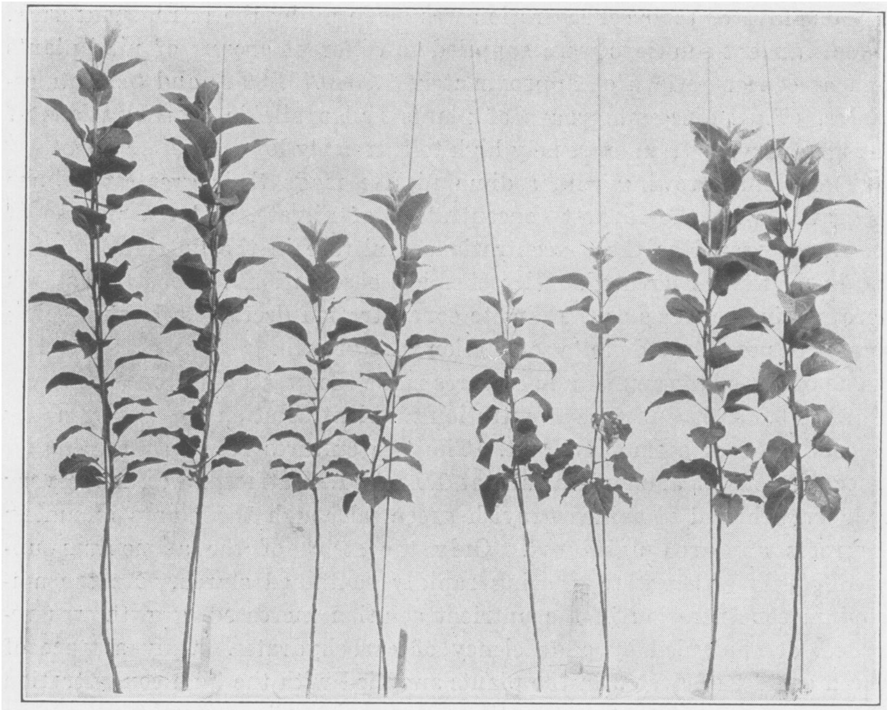

FIG. 5. Delicious apple trees of experiment VIII grown in sand cultures with equivalent concentrations of nitrate and ammonium nitrogen. From left to right, culture no. 1 received a high and no. 2 a low concentration of sodium nitrate, and no. 3 a high and no. 4 a low concentration of ammonium sulphate in the nutrient solution.

The tomato and cotton plants and sweet clover seedlings assimilated ammonium most satisfactorily (made the greatest volume of green growth) at pH 6.0 and nitrate at pH 4.0. The apple varieties assimilated ammonium nitrogen most satisfactorily at pH 6.5 and nitrate at pH 4.5. The Baldwin and Stayman trees made considerable top growth and fair root growth with ammonium at pH 4.0. The Delicious trees, however, made no root growth at 4.0 and only a fair growth at pH 5.0 with ammonium nitrogen. In a later experiment it was found that if the Delicious trees were supplied with a low concentration of ammonium (0.0014 p.v.m.) the roots grew slowly although the trees did not grow. A high concentration of ammonium retarded root growth more in the Delicious than in the other two varieties.

These results are interesting because they were corroborated in the field on Rome trees growing on sandy soils of New Jersey having a reaction of pH 3.7. Calcium nitrate stimulated root growth and top growth but ammonium nitrogen did not stimulate growth. When some of this same soil was taken into the greenhouse it was found that the trees did not respond to ammonium sulphate unless at least the equivalent of two tons of hydrated lime to the acre had been added. Sodium nitrate did not give a response unless calcium was supplied. It seemed fully as important to supply calcium in the field as in the sand cultures in order that good root growth and nitrate assimilation would take place. It was not necessary to change the reaction of the soil; however, the addition of calcium was not sufficient to cause ammonium to be assimilated. The reaction of the soil had to be increased to a higher pH value before the trees were stimulated by the ammonium. Incidentally, nitrification of the ammonium became more rapid as the pH of the soil was increased.

Discussion

The pH value of nutrient solution

Results from sand-culture experiments have a broader application to the growth of plants in their natural habitats if the pH of the culture can be maintained at a constant value. The pH value of a soil changes only slightly from year to year. Previous results on the growth of the tomato (**40**) and the apple (**39**) suggest that the pH value of the nutrient solution must be comparatively high for satisfactory assimilation of ammonium nitrogen. This may be misleading unless the pH values of the solutions, before and after dripping through the sand, are kept in mind.

When a nutrient solution containing nitrogen only as ammonium is supplied to a mature tomato plant at pH 8.0 and comes out of the culture at pH 4.0, as in the work already reported (**39, 40**), the roots of necessity are bathed in a nutrient solution of considerable variation, although the growth of the plant is very luxuriant, indicating rapid assimilation of the ammonium ion. If the initial pH of the solution is lower, growth is less luxuriant. In experiment IX it was shown that if the nutrient solution containing nitrogen only as ammonium was supplied at pH 6.0, and added in sufficient quantity to prevent a drop greater than 0.2 pH in 24 hours, growth was luxuriant. The growth at a practically constant pH of 6.0 was comparable with that produced by plants grown in a solution changing from pH 8.0 to 4.0 (**40**). This seems reasonable because the composite pH value of the variable culture was about 6.0. Similar responses to pH values were observed with cotton (**38**) and three varieties of apples (**39**). In sand culture, rapid renewal of the nutrient solution is necessary to prevent the pH from dropping materially. Results of hydrogen-ion concentration experiments previously published (**38, 39, 40**), therefore, should be interpreted on the basis of intermediate values between the initial pH of the nutrient solution and the pH after passing through the sand. The results would then be essentially comparable with the experiments in which the nutrient solution was maintained at an approximately constant pH.

Tomato, cotton, and apple with ammonium made very slow growth at a constant pH value of 8.0, more rapid at 7.0, and most rapid at 6.0 Nutrient solutions containing nitrogen as ammonium only gave unsatisfactory results unless a constant pH of 5.0 to 6.0 was maintained. Whether trees can assimilate ammonium and make rapid growth depends on the ammonium concentration of the nutrient solution and the carbohydrate reserves in the stem. Perhaps because of the carbohydrate reserves in woody plants, ammonium may be assimilated more rapidly over a wider range of pH values than would be true of herbaceous plants. Fluctuating concentrations of ammonium are also less noticeable in the resultant growth of woody plants because of the greater supply of carbohydrates. Plants supplied with complete nutrient solutions containing nitrogen only as nitrate tended to absorb both cations and anions, so that a residual pH of 6.0 was approximated (**40**). If the nitrate was substituted by ammonium, however, the residual pH did not come to equilibrium at 6.0, but decreased to pH values as low as 3.8 (**40**). These residual changes in the nutrient solutions occurred only when plants were assimilating nitrate or ammonium nitrogen rapidly.

The absorption of ions from a solution containing nitrate apparently depends on the effect of the pH of the nutrient solution on the rate of assimilation of the nitrate or ammonium ion, or on the growth rate of the plants (**40**). The absorption of the nitrate ion decreases anions more rapidly than cations at pH 4.0, and equilibrium is maintained at approximately pH 6.0. If the pH is high enough to bring about assimilation, solutions containing ammonium nitrogen do not come to equilibrium but become increasingly more acid, owing to the residual anions and the rapidity with which ammonium is assimilated. The ammonium ion is absorbed more rapidly than the anions. This increase in acidity depends on the rate of absorption of ammonium, which in turn depends on whether the ammonium ion is assimilated as rapidly as it is absorbed from the nutrient solution. If the ammonium ion accumulates in the cell and absorption ceases, the acidity of the nutrient solution does not increase as rapidly because the residual anions do not accumulate. This may cause the residual solution to come to equilibrium at a pH value comparable with that when the nitrate ion is present. Figure 6 shows that accumulation of the ammonium ion in the plant is directly or indirectly a function of the pH of the nutrient solution. Thus it is only those plants that are assimilating ammonium rapidly which maintain a rapid absorption of ammonium ions. If the cells become saturated with ammonium ions, they can no longer absorb more.

The best growth is made with nitrate nitrogen at pH 4.0, whether the

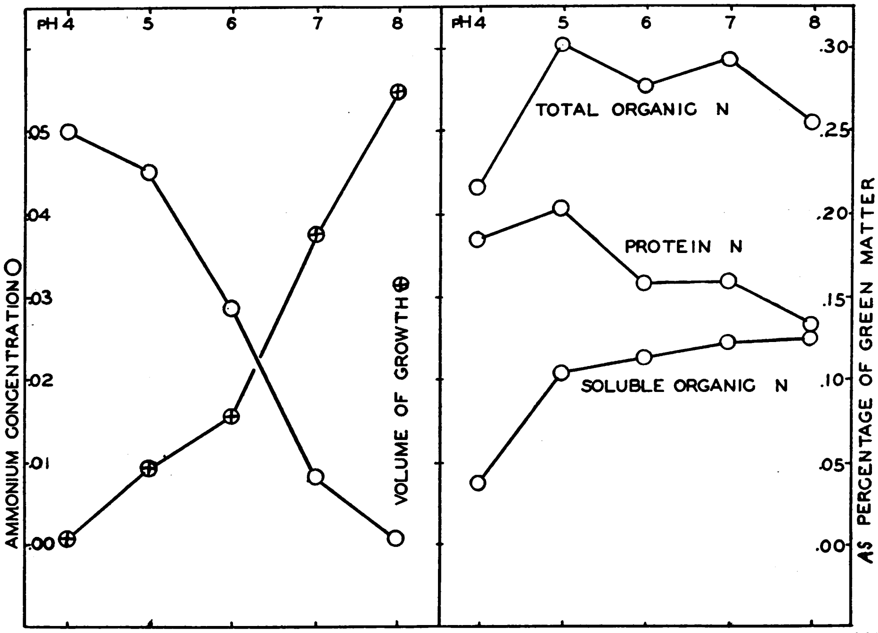

FIG. 6. Taken from data of experiments previously published in detail (table IV, 40) showing relationships between volume of growth (size of green plants in gm.) of tomato plants when supplied with nutrient solutions having different initial pH values and accumulation of ammonium in stems. Rate of assimilation of ammonia is directly correlated with growth.

pH of the residual solution remains constant or tends to come to equilibrium (**39, 40**). The pH values at which nitrate or ammonium nitrogen are satisfactorily assimilated are probably characteristic for the variety or species under observation, and are not particularly associated with the neutral point.

That plants will assimilate ammonium nitrogen satisfactorily has been demonstrated (**40**). PIRSCHLE (**29**) listed a great number of economic plants that make a rapid growth with ammonium nitrogen, and demonstrated that the pH of the nutrient solution had a controlling influence on its assimilation. He found that tobacco produced twice as much total dry matter with ammonium sulphate as with sodium nitrate at pH 6.0, although BEAUMONT *et al.* (**2**) state that tobacco does not assimilate ammonium satisfactorily. THOMAS (**36**) also states that ammonium is not satisfactorily assimilated in tobacco, and DAVIS (**7**) states that it is not assimilated in the apple; but in all instances the pH of the nutrient solutions may have been at fault, as has been demonstrated (**21, 29, 39, 40**). The pH of the nutrient solution, therefore, has directly or indirectly a controlling influence on the assimilation, particularly of the ammonium ion, and somewhat less pronounced of the nitrate ion.

Assimilation of ammonium and nitrate nitrogen

Eckerson (**8**) and others (**25, 26**) have found that plants, through the action of a nitrate-reducing material (reducase), oxidize carbohydrates or organic acids, and reduce nitrates to nitrites and ammonium, following which there are synthesized amino acids and other organic nitrogenous material. Reducase activity, however, may be affected by length of day, light intensity, or other external factors (**9, 11**), all of which will tend to modify the rate of assimilation of nitrate nitrogen (**11**). The utilization of absorbed ammonium by the tomato plants and apple trees in the experiments reported here was apparently rapid, and did not seem to be checked by lack of reducase as may possibly have been the case in the nitrate-supplied plants. This assumption would seem to be supported by the fact that amino acids increased more rapidly and in greater quantities in plants supplied with ammonium than in those supplied with nitrate nitrogen. This is shown by the data in tables II and IV for tomato and in figures 2 and 3 for apple. Likewise in 3 days the ratio of protein to non-protein nitrogen (**39**) in dormant apple trees changed from 73 : 27 to 61 : 39 in those supplied with nitrate, and to 56 : 44 in the trees of the ammonium series. Accompanying this change there was an 18 per cent. increase in total organic nitrogen in the roots of the trees receiving ammonium. Furthermore the maximum increase in total organic nitrogen in the trees supplied with ammonium occurred in 35 days, as compared with 56 days for the trees supplied with nitrate nitrogen. That a similar condition exists in the tomato is shown in table II and figure 6. Thus the general idea that ammonium is more slowly available than nitrate is not borne out by these experiments, but supports the contention (**37**) that ammonium may be as quickly available as nitrate nitrogen.

Eckerson (**9, 10, 11**) finds that some plants, including the tomato, but especially soy bean, have comparatively weak reducase activity during the short days of winter, and that carbohydrates may accumulate in the presence of abundant nitrate. It would seem, therefore, that with synthesis of organic nitrogen from ammonium there should be more rapid decrease in carbohydrates than with nitrate nitrogen under similar conditions. This is apparently borne out, at least for tomato, by figure 1 *a, b*. The relatively rapid decrease of starch and reducing sugars in apple roots supplied with ammonium as compared with nitrate nitrogen further emphasizes this assumption (**39**). Benecke (**3**) has suggested a similar relationship.

Observations and microchemical tests of the tomato plants of experiment VII showed definitely that during short days carbohydrates decreased as ammonium concentration increased. Nitrate-supplied plants showed considerable starch, however, even at the highest concentration of nitrate nitrogen. Thus it would seem that, in order to obtain a comparable growth with ammonium and nitrate nitrogen, it is necessary to supply only as much ammonium to a culture as is equal to that made available by the reduction of nitrate in the cells of a plant growing in a nitrate supplied culture. This is supported by data presented by PIRSCHLE[3] (**29**). He shows that much less ammonium than nitrate nitrogen is necessary for the production of a given amount of growth.

Data by NIGHTINGALE *et al.* (**27**) showed that during comparatively long days in April the proportion of protein to soluble organic nitrogen was 52 to 48 per cent. in stems of highly vegetative non-fruitful tomato plants, under conditions which were favorable for both reducase (**10, 11**) and photosynthetic activity. This may be compared with the data on the ammonium-supplied tomato plants shown in table III, which were grown during the winter months. Here the ratio of protein to soluble organic nitrogen in the stems was 38 to 62 per cent. Thus ammonium nitrogen increased the soluble nitrogenous fraction in the tomato almost as rapidly during short winter days, when reducase activity was somewhat low, as nitrate nitrogen did under conditions favorable for abundant reducase activity. The result was a rapid utilization of carbohydrates in amino acid synthesis and extremely soft succulent growth. The stems of the tomato plants grown by NIGHTINGALE (**27**) contained 11–14 per cent. dry matter and were described as soft and succulent, yet plants of these experiments had only 10 per cent. of dry material in the stems. Under favorable environmental conditions, there seems to be no way to prevent extremely rapid utilization of carbohydrates in ammonium-supplied tomato plants or apple trees, except by limiting the external supply of ammonium.

Lack of carbohydrates does not favor condensation of amino acid to proteins (**27, 33**). It has been shown that in plants supplied with high concentrations of ammonium nitrogen (**13, 39, 40**) the proportion of complex protein nitrogen decreased.

The data on reducase show a paucity of activity in apple trees supplied with ammonium nitrogen (**39**). Apparently the synthesis of reducase did not occur, or it was inactive in the presence of appreciable quantities of ammonium. In nitrate-supplied tomato plants the rate of reducase activity seemed to prevent excessively rapid utilization of carbohydrates. In am-

[3] In the H-ion concentration studies by PIRSCHLE, it should be pointed out that many data were obtained from young plants, which have a more alkaline composite pH than mature plants. Also the concentration of ammonium nitrogen was comparatively high, so that sugars were used more rapidly than in the nitrate-supplied plants. Many of the plants which received ammonium might have synthesized as much dry matter as the plants grown with nitrate if the concentration of ammonium had been less.

monium-supplied apple trees, however, it is possible to deplete carbohydrates to the point where growth and carbohydrate storage cannot keep pace with elaboration of nitrogenous compounds. Even trees supplied with nitrate nitrogen may show signs of carbohydrate deficiency, but in a much less marked degree. This has been observed, but only during dull cloudy weather of midwinter when day length was at a minimum, whereas with ammonium nutrition it may occur in midsummer. These results are in indirect agreement with those obtained by MEVIUS and DIKUSSAR (**18**). They found that nitrites properly supplied were more rapidly assimilated than nitrates.

ORGANS CONCERNED IN ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN IN TOMATO AND APPLE

NIGHTINGALE (**27**) showed that nitrate may be found throughout the entire tomato plant. It has likewise been shown (**40**) that ammonium may be traced in quantity even to the leaves of the tomato plant. The data in table III show that ammonium constitutes 7.58 per cent. of the total nitrogen in the young blades and 14.46 per cent. in the old blades. Thus it seems reasonable to suppose that the assimilation of nitrate and ammonium nitrogen occurs at least to some extent in all parts of the tomato.

NIGHTINGALE (**22**) suggests that the fine roots of pear seedlings and blackberry bushes reduce nitrates and synthesize amino acids in the roots. KRAYBILL (**14**) and THOMAS (**35**) state that a similar condition exists in the apple. The chemical data on nitrate reduction previously presented (**39**), and the fact that there were no nitrates found in the stems and leaves of the apple trees, substantiate these observations. ECKERSON, however, found a trace of nitrate in the terminal buds of the 1-year-old apple trees of experiment V. The initial pH of the nutrient solution in which these trees were grown was 8 to 9, a value which was much too high for efficient assimilation of nitrate in tomato (**40**) and the apple (**39**). Nitrate may likewise be found in the tops of other plants which usually contain nitrates in the roots only, if growth conditions are unfavorable (**25, 26**).

An appreciable concentration of ammonium was recovered in the stems and leaves of the apple (**39**). This may indicate that some ammonium is assimilated in all parts of the tree. This ammonium in the upper part of the tree was probably the result of proteolysis (**27**), however, rather than of absorption and translocation. The distribution of ammonium is therefore no proof that ammonium assimilation and the reduction of nitrates are not chiefly root processes except in unusual cases, as has been pointed out. More ammonium was found in the woody stems and leaves of the apple (**39**) than in the stems and leaves of the tomato (**40**) when these plants were grown during the summer with ammonium nitrogen.

The data of experiment IV (**39**) show amino acids to be 0.132 per cent. on a green-weight basis in the stem of the ammonium-supplied trees, as compared with only 0.078 in the nitrate series. A similar comparison may be made in the leaves and both may be largely accounted for by translocation of amino acids from the roots (**39**). There occurred also an increase in dry matter in the roots, resulting from translocation of carbohydrates from the stems; and in view of the fact (**39**) that ammonium ions are almost immediately synthesized to amino acids in the presence of carbohydrates (3 days), it is probable that the larger part of the elaboration of amino acids from both ammonium and nitrate takes place in the roots. That this also is at least partly true for relatively complex organic nitro-fractions is indicated by the increase in humin and "other" nitrogen.

That the nitrate ion does not go beyond the fine roots in the apple may be due to its combination with the protoplasm (ampholytes) in the root hairs. Adsorption is particularly favored when the nutrient solution has a low pH (4.5) value, and less favored in a nutrient solution at a high pH (7.5) value. This may account for the fact that ECKERSON found nitrate in the tops of trees grown in the more alkaline nutrient solution (**39**).

EFFECTS OF PH OF NUTRIENT MEDIUM ON ELABORATION OF NITROGENOUS FRACTIONS

In figure 3 there are recorded nitrogenous fractions in the roots of apple trees which were supplied with ammonium sulphate or sodium nitrate. These data are taken from previously published tables (**39**). The greater total amount of elaborated nitrogen in the ammonium as compared with the nitrate-supplied trees is evident. This is probably due to more rapid elaboration because of the greater amount of ammonium available when directly supplied than to that obtained through nitrate reduction. The greatest difference in organic nitrogen occurred when the nutrient solution of both lots of trees was supplied with an initial pH of 6.5. The greatest volume of growth of the ammonium trees, however, occurred at pH 6.5 to 8.5. This increase in total elaborated nitrogen is accounted for by a greater proportion of protein, as well as by an increase in soluble organic nitrogen. Where the volume of growth was largest (at initial pH 7.5), the protein and soluble organic nitrogen fractions are almost equal. The greatest volume of growth in the nitrate plants occurred at pH 4.5 to 5.5, whereas total elaborated nitrogen was highest at pH 5.5 to 6.5. Although the protein fractions in the ammonium and nitrate-grown trees were more or less the same in total amount, the various determined soluble nitrogenous fractions were much higher in the ammonium-supplied plants. These data are more or less in agreement with those previously presented for tomato and soy bean (**40**).

In the nitrate-supplied trees the effect of pH of the nutrient solution on the soluble organic nitrogen fraction of the plant is negligible except at the extreme pH values. At pH 3.5 and 9.0, at which the plants made comparatively poor growth and slight root injury was apparent, there was an increase in the soluble nitrogen fraction, possibly proteolytic (**23**). The amount of growth made by the ammonium-supplied trees follows rather generally the curves for soluble organic fractions of nitrogen, providing the data from the trees grown at pH 3.5 and 9.0 are omitted for the reasons indicated.

PRIANISCHNIKOW (**30**) emphasizes the amide nitrogen fraction in plants, and concludes that asparagine probably is a readily available reserve. He further concludes that it is elaborated when an abundance of ammonium nitrogen becomes suddenly available. MOTHES (**20**) and NIGHTINGALE (**23**), however, show that asparagine likewise increases rapidly in the dark, owing to proteolysis, and decreases in the light. This fraction is very low in the tomato (**40**), except at certain pH values, and it is probable that these exceptions in the tomato are due to "combined" ammonium (**40**) nitrogen rather than to nitrogen in the amide form. Further evidence of this will be given in a later report.

EFFECTS OF SOIL pH ON ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN IN TOMATO

Data in figure 4 show that on pH 6.0 and 7.4 sandy loam soils where ammonium nitrogen may be nitrified, the tomato absorbed rather large quantities of ammonium nitrogen, and that at these pH values the plants grew similarly to tomatoes in sand culture supplied with ammonium nitrogen at pH 7.5 to 8.5. It must be remembered that the soil cultures maintained a constant pH as compared with a variable one for most of the sand cultures. There was a greater percentage of protein at pH 4 than at 6 or 7.4, but soluble organic nitrogen increased as the pH increased. Amino nitrogen was low and amide nitrogen was high at pH 6, but was reversed at pH 7.4.

The nitrate-supplied plants made their greatest volume of growth at pH 4, which was accompanied by the largest percentage of soluble organic nitrogen in their tissues but the lowest percentage of protein. Amide nitrogen was low and amino nitrogen was high in the pH 6.0 and 7.4 cultures. Nitrate nitrogen was high in the pH 4.0 cultures, probably because of increased adsorption (**40**). The ammonium content in the nitrate-supplied plants at all pH values was low because conditions were favorable for nitrate assimilation. At pH 6 protein increased slightly in the nitrate cultures and the plants showed a slightly less succulent condition, which was particularly emphasized at pH 7.4, where protein nitrogen was high and soluble organic nitrogen was low.

In spite of the fact that the ammonium-supplied plants were comparable in growth with plants grown in sand cultures with only ammonium nitrogen, they contained rather high fractions of nitrate nitrogen; in fact, much higher than the sand-culture plants supplied with nitrate at any pH value. The lower percentage of nitrate at pH 4.0 and high concentration of ammonium and organic nitrogen suggest nitrate assimilation. Furthermore these plants contained the least dry matter of any of the soil-grown plants, 5.6 per cent.

In the plants of the ammonium-supplied cultures at pH 6.0 and 7.4, however, ammonium was low and organic nitrogen high, as for tomato in sand cultures (**40**), indicating at these pH values efficient assimilation of ammonium and less efficient assimilation of the nitrate ion. In general the fractions of soluble organic nitrogen also follow closely in trend those previously pointed out for tomato plants in sand culture under comparable pH values of the nutrient medium. There would seem to be little doubt that the ammonium-supplied plants grown in soil produced a large part of their growth with ammonium nitrogen in spite of the absorbed nitrate nitrogen which tended to accumulate.

Summary

1. It is necessary that the limitations of the use of nitrogenous salts be determined before a fair comparison of their relative merits as plant nutrients can be made. Any of the following comparisons made between ammonium and nitrate nitrogen are on the assumption that the respective salts are supplied to the plants at the pH values of the nutrient medium or soil which are optimum for the assimilation of the particular ion under consideration.

2. The hydrogen-ion concentration of the nutrient medium directly or indirectly had a controlling influence on the assimilation, particularly of the ammonium ion. The nitrate ion was assimilated most satisfactorily in tomato and apple when absorbed from an acid nutrient solution of approximately pH 4.0. The ammonium ion was assimilated most satisfactorily when absorbed from a nutrient solution having a constant pH value of 5.0 to 6.5, varying somewhat for the variety.

3. Ammonium ions were immediately absorbed by plants without further change, and were assimilated (synthesized to amino acids and other organic nitrogenous materials) directly and more rapidly than the nitrate ion.

4. Nitrate ions were apparently absorbed, reduced to nitrite, and finally to ammonium ions by the action of reducase (nitrate-reducing material in

the plant). The ammonium ion was assimilated directly and as rapidly as it was absorbed, however, whereas the nitrate ion usually tended to accumulate, at least partly, because of limited reducase activity under certain external and internal conditions (**11**). The ammonium ion was, therefore, more quickly available for the synthesis of amino acids. The ammonium ion did not accumulate in plant tissue unless the plant could not assimilate this form of nitrogen.

5. The volume of growth obtained from nitrate and ammonium depended on the concentration of the nitrogenous salt in the nutrient solution and available carbohydrates.

6. There was a direct correlation between the concentration of nitrate nitrogen and the volume of growth produced if the plant was actively reducing nitrate. Plants required a much lower concentration of ammonium than nitrate nitrogen in the nutrient solution to produce an equal volume of growth.

7. Plants containing a large amount of available carbohydrates assimilated ammonium much more rapidly than those containing a comparatively small supply of available carbohydrates. Tomato and apple grown with ammonium contained a much higher concentration of soluble organic nitrogen than those supplied with an equal quantity of nitrate. There was a direct correlation between the concentration of ammonium in the nutrient solution and the amount of soluble organic nitrogen elaborated by the tomato and apple.

8. Tomato and apple growing on soil containing ammonium and nitrate ions absorbed both ions. Whether they assimilated both ions depended partly on the pH of the soil. On acid soils having a pH of 4.0, plants tended to accumulate ammonium and assimilate nitrate ions. On neutral or slightly alkaline soils, plants tended to accumulate nitrate ions and assimilate ammonium. When ammonium salts were applied to soil cultures, some of the ammonium ions were oxidized to nitric acid and were absorbed by plants as nitrate ions.

9. In general, ammonium and nitrate salts produced equally good growth, provided their limitations were recognized. Whether nitrates were reduced in the plant to ammonium nitrogen or the nitrogenous nutrient was supplied directly as ammonium, the quality of organic nitrogen in plants grown with salts from either group was similar when the total concentration of elaborated nitrogen was the same.

10. In field experiments where comparisons are made between salts of either group, comparative data may be of little value unless the condition of the soil and the quantity of nitrogenous ions absorbed and assimilated by the plants supplied with salts from either group are specifically evaluated. If salts from the two groups are used under optimum conditions for

each, the only advantage one salt would have over the other would be brought about as a secondary effect by the ion with which the nitrate or ammonium ion was associated.

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LITERATURE CITED

1. Addoms, Ruth M., and Mounce, F. C. Further notes on the nutrient requirements and the histology of the cranberry, with special reference to the sources of nitrogen. Plant Physiol. **7**: 643–656. 1932.
2. Beaumont, A. B., Larsinos, G. J., Pickenbrock, P., and Nelson, P. R. The assimilation of nitrogen by tobacco. Jour. Agr. Res. **43**: 559. 1931.
3. Benecke, W. Beiträge zum Problem der Kohlensäureassimilation. Zeitschr. Bot. **13**: 417–460. 1921.
4. Chibnall, A. C. Investigations on the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 334–362. 1922.
5. ————. Investigations on the nitrogenous metabolism of the higher plants. III. The effect of low-temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 599–607. 1922.
6. ————. Synthesis of the i-hydroxy asparagines. Biochem. Jour. **24**: 945–953. 1930.
7. Davis, M. B. The influence of ammonium sulphate as a direct source of nitrogen for apple trees. Sci. Agr. **8**: 41–55. 1929.
8. Eckerson, Sophia H. Protein synthesis by plants. I. Nitrate reduction. Bot. Gaz. **77**: 377–391. 1924.
9. ————. Properties of the nitrate reducing substance in plants. Amer. Jour. Bot. **16**: 851–852. 1929.
10. ————. Seasonal distribution of reducase in the various organs of an apple tree. Contributions from Boyce Thompson Institute **3**: 405–412. 1931.
11. ————. Conditions affecting nitrate reduction by plants. Contributions from Boyce Thompson Institute **4**: 119–130. 1932.
12. Jones, L. H., and Shive, J. W. Influence of ammonium sulphate on plant growth in nutrient solutions and its effect on H-ion concentration and iron availability. Ann. Bot. **37**: 355–377. 1932.
13. Kraus, E. J., and Kraybill, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.

14. KRAYBILL, H. R. (A communication.) 1932.
15. LOO, TSUNG-LE. Further studies on the absorption of ammonia and nitrate by the root system of the higher plants. Bull. Dept. Biology. College of Sci., Sun Yatsen Univ., no. 10. 130 pages. (Reprint.) 1931.
16. MATTSON, SANTE. Electrodialysis of the colloidal soil material and the exchangeable bases. Jour. Agr. Res. **33**: 553–567. 1926.
17. MEVIUS, W. Die Wirkung der Ammonium Salze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. Planta **6**: 379–455. 1928.
18. ————, and DIKUSSAR, I. Nitrite als Stickstoffquellen für höhere Pflanzen. Ein Beitrag zur Frage nach der Assimilation des Nitrite stickstoffs. Jahrb. wiss. Bot. **72**: 633–703. 1931.
19. MOORE, J. C., REEVES, R. G., and HIXON, R. M. Electrodialysis as a means of studying biochemical differences in abnormal apple tissue. Plant Physiol. **2**: 313–324. 1927.
20. MOTHES, KURT. Physiologesche Untersuchungen über das Asparagin und das Arginin in Coniferen. Planta **7**: 585–649. 1928.
21. NAFTEL, J. A. F. The absorption of ammonium and nitrate nitrogen by various plants at different stages of growth. Jour. Amer. Soc. Agron. **23**: 142–158. 1931.
22. NIGHTINGALE, G. T. The growth responses of some horticultural plants and associated chemical changes in reserve foods. New Jersey Agr. Exp. Sta. Ann. Rpt. 168–170. 1927.
23. ————, ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
24. ————, ROBBINS, W. R., and SCHERMERHORN, L. G. Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. New Jersey Agr. Exp. Sta. Bull. 448. 1927.
25. ————, and ————. Some phases of nitrogen metabolism in polyanthus narcissus (*Narcissus tazetta*). New Jersey Agr. Exp. Sta. Bull. 472. 1928.
26. ————, and SCHERMERHORN, L. G. Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.
27. ————, ————, and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.

28. ——————, ——————, and ——————. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.

29. PIRSCHLE, K. Nitrate und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. Planta **14**: 583–676. 1931.

30. PRIANISCHNIKOW, D. N. Über den Aufbau und Abbau des Asparagins in den Pflanzen. Ber. deut. bot. Ges. **40**: 242–248. 1922.

31. ——————. Ammoniak, Nitrate und Nitrite als Stickstoffquellen für höhere Pflanzen. Erg. d. Biol. **1**: 407–446. 1926.

32. ——————, and IWANOWA, W. S. The influence of carbohydrate supply on the behavior of seedlings toward ammonium nitrate. Ann. Acad. Agron. Petroosk. 2 pp. 277–286. 1928. (Trans. title and German abst.)

33. SAPOSCHNIKOFF, W. Proteins and carbohydrates of the green leaf as products of assimilation. (In Russian.) 1894. (Abstr. in Bot. Centralbl. **63**: 246–251. 1895.)

34. THERON, J. J. Influence of reaction on interrelations between the plant and its culture medium. Univ. California Pub. Agr. Sci. **4**: 413–444. 1924.

35. THOMAS, WALTER. The seat of formation of amino acids in *Pyrus malus* L. Science **66**: 115–116. 1927.

36. THOMAS, R. P. The relation of nitrate nitrogen and nitrification to the growth of tobacco following timothy. Wisconsin Agr. Exp. Sta. Res. Bull. 105. 1930.

37. TIEDJENS, V. A. Several forms of nitrogen quickly available to trees. New Jersey State Hort. Soc. News **12**: 369. 1931.

38. ——————. Growing cotton and other crop plants with ammonium nitrogen. Science **75**: 648. 1932.

39. ——————, and BLAKE, M. A. Factors affecting the use of ammonium and nitrate nitrogen by the Delicious apple. New Jersey Agr. Exp. Sta. Bull. 547. 1932.

40. ——————, and ROBBINS, W. R. The use of ammonia and nitrate nitrogen by certain crop plants. New Jersey Agr. Exp. Sta. Bull. 526. 1931.