

Nitrogen Metabolism in Plants

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NITROGEN METABOLISM IN PLANTS

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CHAPTER I

THE SOURCES OF NITROGEN FOR PLANTS

A. General

The atmosphere and the soil are possible sources of nitrogen for plants. The atmosphere has vast reserves of elemental nitrogen, with traces of ammonia and other gaseous nitrogen compounds. Soils contain nitrate, ammonium, and usually organic nitrogen compounds.

It has not always been recognized that nitrogen is essential for plant growth. Van Helmont (1577–1644) published posthumously in 1648 data believed to show that it requires only water. His experiment, carried out at Brussels and famous as an early quantitative study in plant physiology, was described as follows: 'I took an earthen vessel in which I put 200 pounds of soil dried in an oven, then I moistened the soil with rain water and pressed into it a willow shoot weighing 5 pounds. After exactly 5 years there had grown a tree weighing 169 pounds and about 3 ounces. The vessel received only rain water or distilled water to moisten the soil as needed, and it was still full of tightly packed soil. To keep out dust it was covered with an iron sheet coated with tin and perforated with many holes. I did not weigh the leaves that fell in autumn. At the end I dried the soil again. It weighed 200 pounds as when I started, less about 2 ounces. Therefore, 164 pounds of wood, bark and roots arose from water alone.' This experiment was proposed, but apparently not made, by Nicholas of Cusa (Cardinal N. Khrypffs, 1401–64), who predicted correctly that the soil would lose very little weight. Boyle (1661), repeating the experiment with 'squash, a kind of Indian pompion', got similar results and drew similar conclusions. The conclusions were wrong, though based on sound experimental work, because important factors were neglected in interpreting the results. It was not known that the air supplies much of the material accumulated in growing plants, or that rain water contains dissolved matter used in plant growth.

Woodward (1699) criticized the conclusions of Van Helmont and Boyle in a paper remarkable for sound experimental design, quantitative expression of results, and intelligent interpretation of data. He pointed out that they ignored dissolved matter in the water supplied

TABLE I (from Woodward, 1899)

<i>Distinction of the viols</i>	<i>The several sorts of water</i>	<i>Weight of the plant when put in</i>	<i>Weight of the plant when taken out</i>	<i>Weight gained in 56 days</i>	<i>Expense of water</i>	<i>Proportion of the growth of the plant to the expense of water</i>
H	Hyde Park conduit water	127	255	128	14190	1 to 110 $\frac{1}{4}$
I	Hyde Park conduit water	110	249	139	13140	1 to 94 $\frac{3}{4}$
K	Hyde Park conduit water in which dissolved 1 $\frac{1}{2}$ ounces of common garden earth	76	244	168	10731	1 to 63 $\frac{1}{4}$
L	Hyde Park conduit water with the same quantity of garden mould as the former	92	376	284	14950	1 to 52 $\frac{1}{2}$

All weights in grains; 'expense of water' is the amount transpired during the growth of each plant; experiment carried out during summer of 1892.

to their experimental plants, and showed it to be present in the clearest natural water. He grew spearmint (*Mentha* sp.) and *Solanum nigrum* in water culture in glass 'viols', and so avoided separating the roots from surrounding soil. The difficulty of quantitative separation must have reduced precision in the earlier work. Rain water, spring water, river water and water from the Hyde Park conduit were used; in some experiments a known amount of rich garden soil was added. Some experiments were run in duplicate. Control viols contained water but a dead stick closed the hole by which the stem passed through the cover. Water in the viols was replenished as necessary, all amounts added being measured. Woodward recorded his results in tables, shown here in part as Table 1.

This table would not be out of place in a modern paper, except perhaps for the peculiar fractions. They imply higher precision than the experimental work can have attained, but this fault is common today. Woodward's clear presentation of quantitative data contrasts greatly with the abstract and general arguments of his contemporaries. He noted that if water only was required, plant growth should be proportional to the water absorbed. The observed requirement per unit of plant growth varied from one water to another, those with more dissolved matter being effective in smaller volumes. In his own words 'Vegetables are not formed of water, but of a certain peculiar terrestrial matter. It has been shown that there is a considerable quantity of this matter contained in rain, spring, and river water, that the greatest part of the fluid mass that ascends up into plants does not settle there but passes through their pores and exhales up into the atmosphere: that a great part of the terrestrial matter, mixed with the water, passes up into the plant along with it, and that the plant is more or less augmented in proportion as the water contains a greater or less quantity of that matter; from all of which we may reasonably infer, that earth, and not water, is the matter that constitutes vegetables. Water is not the matter that composes vegetable bodies but 'tis the agent that conveys the matter to them, that introduces, and distributes it to the several parts for their nourishment.' In conclusion he adds: 'There's a procedure in every part of nature that is perfectly regular and geometrical, if we can but find it out.'

Woodward used his results to explain the necessity to fallow or fertilize agricultural land if yields of crops are to be maintained. He tried nitre as a fertilizer in water culture experiments, but the plants died, perhaps because the culture solution was too concentrated.

Somewhat earlier the importance of nitre in plant nutrition was stressed by Glauber (1656) and Mayow (1674). Davy (1836) quoted a statement by Sir Kenelm Digby in 1661 that barley grew very vigorously after being watered with a weak solution of nitre, but dismissed the observation as that of a 'speculative writer'. Glauber found accumulations of nitre in soil impregnated with the excreta of cattle, and concluded that it originated in plants eaten by them. On finding that nitre greatly increased the yield of crops, he proposed it as the 'principle' (chief or sole nutrient) of vegetation. Mayow showed nitre to be present in soils in the spring at the beginning of plant growth, but found none in soils which had supported abundant plant growth. This change he attributed to removal of nitre from the soil by growing plants.

Lemery (1693) attributed to 'a salt resembling saltpetre' the value of manure and other materials used to increase the fertility of soil; he added that such a salt could be extracted from some plants but not from others. Evelyn (1674) stressed the value of saltpetre in the following words: 'I firmly believe that were saltpetre (I mean factitious nitre) to be obtained in plenty, we should have need of but few other composts to meliorate our ground.' Stubbs (1667, 1668), noting that tobacco grown in some parts of Jamaica flashed when smoked, concluded that the ground was full of saltpetre. Sugar cane cultivated in such ground grew bigger and faster than elsewhere, and potatoes (whether *Solanum tuberosum* or *Ipomoea batatas* is not indicated, but the latter seems more likely) matured earlier. Both the sugar cane and the potatoes kept badly and the cane juice did not boil well to sugar. It is interesting that the adverse effects of excessive supplies of nitrate on sugar production were recognized so early; they were confirmed, both with cane and beet, by many later workers, e.g. Barral (1878). The importance of nitre as a plant nutrient was also recognized by Wolff (1723). Stahl (1747) detected nitrate in the green parts of *Fumaria*, *Parietaria*, and *Nicotiana tabacum*.

By 1800 the work of Priestley, Ingenhousz, Sénéquier, and De Saussure established that plants obtained their carbon from atmospheric carbon dioxide. De Saussure (1804) recognized nitrogen as an essential plant constituent, and showed that his experimental plants obtained it from the soil, not from the air. His work marked a great advance in technique, but had little immediate effect on general opinion in agricultural science. Davy (1836) remarked that the nitrogen of plants 'may be suspected to be derived from the atmosphere; but no experiments have been made which prove this; this might easily be instituted upon mushrooms and

funguses'. At that time it seemed natural to suppose that plants obtained nitrogen from the air, of which gaseous nitrogen forms almost 80 per cent, if they used carbon dioxide, forming a mere 0.03 per cent of the atmosphere, as a source of carbon. Some plants with symbiotic bacteria do use atmospheric nitrogen, but the majority cannot. This difference in behaviour caused considerable theoretical and practical difficulties, resolved (see Chapter 3) late in the nineteenth century. At its beginning opinion on how plants obtained nitrogen, and in what form, was very varied. Nitrates, ammonia, organic compounds in the soil, and atmospheric nitrogen were all proposed as their chief source of nitrogen. Burnett (1829) clearly appreciated the value of ammonia to plants; in discussing the digestion of insects in leaf-pitchers of *Sarracenia* he wrote: 'Nor must the nitrogen thus afforded to the prehensile plants be overlooked in the account, when we know how potent an excitant ammonia is to the vegetable frame.'

The first adequate data on nutrient requirements of crops came from experiments made by Boussingault (1838*a*, *b*, *c*, 1841) on his farm at Bechelbronn (Alsace). He was a young man when this work began in 1834, but already well known for scientific observations in South America, where he travelled first as a mining engineer and later as a Staff officer in Bolivar's army. Returning to France he spent the rest of a long life in chemical work, especially the application of chemistry to the study of agriculture, including animal nutrition. His farm was the first agricultural experimental station but unfortunately did not become a permanent centre of research like that founded by Lawes a little later on his estate at Rothamsted. Boussingault founded modern agricultural research by applying in field experiments the quantitative methods introduced on a laboratory scale by De Saussure thirty years earlier. He studied crop rotation over several years, weighing and analysing crops taken from the soil and manures added to it, and drew up balance sheets (expressed in kg/ha) for changes in carbon, hydrogen, oxygen, nitrogen, total dry matter, and total mineral matter.

Interest in such problems was intensified by a famous textbook (Liebig, 1843) in which the great German chemist expressed forcibly his views on plant nutrition and ridiculed all those who disagreed with them. He finally killed the theory then current, though exploded by De Saussure, in practical writings on agriculture, that plants obtain carbon from humus in the soil rather than from the air. His main contribution was probably the clear statement that plants require only water, carbon dioxide, ammonia, and mineral (ash) constituents, these

substances being liberated by decay of plant material and so passing in a continuous cycle between the plant and its environment. This was valuable exposition of sound though not new ideas; unfortunately Liebig also used his great prestige to support the erroneous theory that atmospheric ammonia was the main source of nitrogen for plants. He postulated a formal analogy between their uptake of carbon and of nitrogen, each being assimilated in gaseous form, carbon as carbon dioxide and nitrogen as ammonia. He held that nitrogen nutrition was identical in all plants, casting quite unjustified doubts on the analytical methods by which Boussingault established the special position of legumes.

Gaseous ammonia at low concentrations is assimilated by nitrogen-deficient plants, their pale yellow-green leaves soon turning dark green (Ville, 1850, 1852; Meyer & Koch, 1873; Schloesing, 1874). Normal air, however, contains insignificant amounts of ammonia (Mulder, 1844; Ville, 1855). Plants derive nitrogen mainly from inorganic compounds in the soil or, by bacterial symbiosis, from the free gas. The need of non-legumes for combined nitrogen in the soil was clearly shown at Rothamsted (Lawes, 1847; Lawes & Gilbert, 1851, 1855), and by Salm-Horstmar (1851) who grew oats in calcined sand with ammonium nitrate as nitrogen source. He also confirmed the observation (Gris, 1844) that plants require iron for healthy growth, becoming chlorotic in its absence. This demonstration requires good pot-culture technique, the small requirement for iron being easily masked by its absorption from experimental vessels or from salts used to supply other elements.

The assumption that either atmospheric ammonia or organic materials in the soil provided the main source of nitrogen for plants was gradually abandoned during the first half of the nineteenth century. Since that time attention has been focussed on nitrates and ammonium salts as available forms of nitrogen. The absorption of nitrogen is more complicated than that of other essential elements because it is available both as a cation (ammonium) and as an anion (nitrate). The first volume of the *Journal of the Royal Agricultural Society of England* shows the interest of progressive farmers and landowners in artificial nitrogenous fertilizers. Several papers (Barclay, 1840; Daore, 1840; Everitt, 1840; Kimberley, 1840; Zetland, 1840) reported increased yields, usually exceeding in value the cost of the fertilizer and its application, from nitrates in field trials with wheat, oats, turnips, and pastures. 'Gas-water', the washing produced in purifying coal gas, also gave good

results, correctly attributed to the ammonia content (Paynter & Handley, 1840).

Kuhlmann (1843) manured hay with ammonium chloride, ammonium sulphate, sodium nitrate at two levels of application, three types of organic manure, and 'gas-water'. The value of increased production was compared with the cost of each manure. Manuring was profitable only for organic manures and 'gas-water' at the prices then ruling for hay and nitrogenous materials. Duty-free nitrate would have been profitable, and Kuhlmann accordingly urged abolition of import duties on nitrates entering France; this was done in 1846. Schattenmann (1843) and Chatterley (1843) studied the effects of ammonium salts on several crops at various levels of application. Kuhlmann (1846) stressed that nitrates and ammonia were chemically interconvertible, suggesting that the nitrogen of organic materials and of nitrates was used by plants only after conversion to ammonia. This view, though untenable, took into account that nitrogen compounds may be transformed in the soil. Boussingault (1855a, 1856) showed clearly the value of nitrate for sunflower (*Helianthus*) and cress (*Lepidium*), and detected it in the sap of banana (*Musa*), beech (*Fagus*), hornbeam (*Carpinus*), *Hura crepitans*, vine (*Vitis*), and walnut (*Juglans*). Ville (1856) found potassium nitrate a better nitrogen source for some plants than ammonium salts.

Bineau (1856) showed that fresh-water algae used ammonium and nitrate. Schoenbein (1868) found that they rapidly reduced nitrate to nitrite. The reaction required living organisms; it did not occur with boiled algae and was inhibited by traces of hydrocyanic acid. Production of nitrite by green algae assimilating nitrate has been confirmed by many later workers, e.g. Mayer (1952), Kessler (1952), and Omura (1954). Pasteur (1860) showed that yeast could use ammonia for protein synthesis. Yeasts using nitrate include *Saccharomyces acetoethylicus* (Beijerinck, 1892) and *Hansenula anomala* (Sakamura & Maeda, 1950). Raulin (1879) showed zinc to be essential for *Aspergillus niger* and noted that it used both nitrate and ammonium.

The early field and pot experiments showed that plants used nitrogen supplied as nitrate, ammonium salts, or organic manures. The action of bacteria on substances added to soil made it doubtful, however, in what form the nitrogen actually entered the plant. Experiments in which sterility of the medium was claimed also showed that plants used both nitrates and ammonium salts as sources of nitrogen, though either might be superior for particular species.

Müntz (1889), using soil extracted to remove nitrates and then sterilized, showed that beans (*Vicia*, *Phaseolus*), maize, barley, and hemp (*Cannabis*) assimilated the nitrogen of ammonium salts. No nitrate was found at the end of the experiment in the experimental pots or in controls containing solutions of ammonium salts but no plants. This almost completely excludes the possibility, inherent in earlier work on assimilation of ammonium, that bacteria converted it to nitrate assimilated as fast as it was formed. Good agreement was found between the total nitrogen in mature plants (less the amount in the seeds), and that taken up as ammonia. Treboux (1904) reported similar results with mosses, diatoms, green algae, and *Lemna minor*. Griffiths (1891) and Pitsch (1896) showed that beans absorbed ammonium salts directly in sterile water culture. Mazé (1898a) found ammonium and nitrate equally satisfactory for maize in water culture. Hutchinson & Miller (1909) reviewed much early work on the utilization of ammonium, and demonstrated its direct assimilation in sterile water and sand cultures. Peas grew well with either nitrates or ammonium salts, but wheat did better with nitrates.

More recent work has shown that absorption and assimilation of nitrate and ammonium are sensitive to many environmental factors. Interpretation and comparison of results are thus difficult even in well-controlled experiments. Sterile cultures avoid bacterial activity, but the experimental plants are grown in highly abnormal conditions. In water and sand cultures the volume of nutrient solution is usually small enough for the action of plant roots to change the composition of the medium quite quickly. Concentrations of different ions and their relative abundance at the root surface are thus unstable unless the nutrient solution is replaced continuously or at least changed frequently. Finally, growth of the experimental plants may be limited by some factor other than that under study. In sterile cultures for instance, illumination rather than the nutrients supplied may limit growth. Even in experiments with unicellular algae, where conditions are more readily controlled than for higher plants, effects of pH, illumination, and aeration may obscure comparisons of different sources of nitrogen (Syrett, 1954). As a result of these complicating factors, most conclusions on the availability of different sources of nitrogen, and on their interaction with environmental factors, must be regarded as tentative.

B. Effects of various factors on the uptake of ammonium and nitrate

(a) THE SPECIES OF PLANT

The uptake of nitrate and ammonium has been studied in only a few species, but the known types of behaviour give some idea of the range that occurs. The main difficulty in studying differences between species is the sensitivity, already mentioned, of nitrogen uptake to environmental conditions. The degree to which these conditions are controlled or even recorded varies widely in different investigations and their results are therefore not strictly comparable. Behaviour attributed to a particular species may in fact be due to environmental factors. With these reservations in mind we may consider the available information on uptake of nitrate and ammonium.

Several workers (Hutchinson & Miller, 1909; Arnon, 1937; Arenz, 1941; Kappen & Wienhues, 1942; Burström, 1945) reported that cereals (barley, oats, rye, wheat) grew better with nitrates than with ammonia. Similar results are recorded for *Urtica dioica* and *Rumex obtusifolius* (Marthaler, 1937), and for sugar-beet (Krüger, 1905; Vlasjuk, 1940a, b; Okanenko & Ostrovskaya, 1951), which Tombesi, Fortini, Cervigni, Baroccio, Venezian, & Tarantola (1952) claim does better with ammonium salts. Dikussar (1934) got better growth of sugar-beet with ammonium than with nitrate at pH 7, the position being reversed at pH 5. Said & El Shishiny (1949) found radish root slices to absorb and assimilate nitrate better than ammonium.

Plants stated to grow better with ammonium than with nitrate include potato (Krüger, 1905; Street, Kenyon, & Watson, 1946a), pineapple (*Ananas comosus*) (Sideris, Krauss, & Young, 1938), *Pandanus veitchii* (Sideris, Krauss, & Young, 1938) and rice seedlings (Kellner & Sawano, 1884; Kelley, 1911; Willis & Carrero, 1923; Dastur & Malkani, 1933; Pardo, 1935; Bonner, 1946). *Chenopodium album* seems to use ammonium only, but accumulates nitrate which is not utilized (Marthaler, 1937). Other members of the Chenopodiaceae also store nitrate and show little ability to reduce it (Dittrich, 1930). Pirschle (1929-31) got better growth of *Chenopodium capitatum* with nitrate than with ammonium at several levels of pH. Many species are reported to grow better with nitrate and ammonium together than with either alone. The benefit is probably indirect. No plant is known to require both ions separately, but their simultaneous use avoids changes in acidity due to preferential absorption of a single ion.

Vauquelin (1809a, b) found much nitrate in leaves of *Nicotiana tabacum* and *Atropa belladonna*, and Braconnot (1827b) in those of sugar-beet. Berthelot (1884) detected it in a wide variety of plants, including a moss (*Hypnum triquetrum*), a horsetail (*Equisetum telmateia*), and a fern (*Pteridium aquilinum*). Molisch (1887) also found nitrate in many species, noting that it was commoner in herbs than in woody plants. The nitrate content of plants varies greatly; very high values are recorded for some species when growing in conditions of ample supply and slow utilization. Boutin (1873, 1874) found up to 22.8 per cent (calculated as potassium nitrate) of the dry weight in *Amarantus atropurpureus*, *A. blitum*, and *A. ruber*. *A. retroflexus* also accumulates nitrate (Woo, 1919); the percentage of total nitrogen occurring as nitrate varies from 1.2 in leaves and 1.8 in seeds to 32.8 in roots, 51.8 in stems, and 56.4 in branches. Berthelot (1884) found the stem to contain most of the nitrate in the plant in *Amarantus*, *Avena sativa*, *Borago officinalis*, and *Triticum sativum* (Table 2). This occurs also in buckwheat (*Fagopyrum esculentum*) and *Bryophyllum calycinum* (Pucher, Wakeman, & Vickery, 1939; Pucher, Leavenworth, Ginter, & Vickery, 1947a, b) and in pineapple (*Ananas comosus*) (Nightingale, 1942a).

TABLE 2

Percentage of total nitrate of plant found in various organs.
(Calculated from data of Berthelot, 1884.)

Species	Stem	Root	Leaves
<i>Amarantus</i> sp.	79	16	5
<i>Avena sativa</i>	76	22	2
<i>Borago officinalis</i>	76	8	16
<i>Triticum sativum</i>	76	10	14

Nitrate accumulation is reported in sunflower (*Helianthus annuus*) (Nedokuchayev, 1903), celery (*Apium graveolens*) (Platerius, 1931), rye grass (*Lolium perenne*) (Chibnall & Miller, 1931), oats (Sessions & Shive, 1933; Bradley, Eppson, & Beath, 1940; Whitehead, Olson, & Moxon, 1944), wheat (McCalla, 1933), tobacco (Eisenmenger, 1933), and *Salvia reflexa* (Williams & Hines, 1939). Fodder rich in nitrate may poison livestock; the toxic agent is nitrite (Rimington & Quin, 1933; Williams & Hines, 1939) produced by an enzyme of plant origin.

Angiosperms capable of growing either submerged in fresh water or in the air accumulate much more nitrate in the former situation (Combes, Brunel, & Chabert, 1942b; Combes, 1957; Combes, Gertrude, & Lévigne, 1950). Nitrate accumulated in under-water organs of partly submerged plants. This was not due to reduced protein synthesis; aquatic plants of *Veronica anagallis* contained more protein, both absolutely and in relation to soluble organic nitrogen, than those growing in air. The effect was more marked with *V. anagallis* and *Scirpus lacustris* than with *Phragmites communis* or *Oenanthe phellandrium*.

In marine algae Suneson (1932, 1933) reported large differences between species in nitrate accumulation. Species of *Laminaria* were the only brown algae found to accumulate nitrate; it was present in many red algae but absent from all ten *Polysiphonia* species tested. These variations are particularly striking as seaweeds grow in a medium where the nitrate supply is more constant than in different soils. The unicellular marine red alga *Rhodospirillum rubrum*, cultivated in chemically defined media, used nitrate well but urea and ammonium salts were toxic (Giraud, 1958). Chu (1942) found that many planktonic algae (*Pediastrum boryanum*, *Staurastrum paradoxum*, *Nitzschia acicularis*, *N. palea*, *Fragilaria crotonensis*, *Asterionella gracillima*, *Tabellaria flocculosa*, *T. fenestrata*, *Oscillatoria tenuis*, *O. rubescens*, *Pandorina morum*, *Euglena deses*, *Leptoclinis steinii*, *Cryptomonas ovata* and *Botryococcus braunii*) used both nitrate and ammonium. The only species showing a definite preference for either form of nitrogen was *Botryococcus braunii*, which grew better with nitrate. Most species grew equally well with either nitrate or ammonium at optimum levels of supply, but better with nitrate when the nitrogen supply was restricted. *Chlorella* is, however, reported to use ammonium exclusively when nitrate is also available (Cramer & Myers, 1948).

Morton & MacMillan (1954) found with *Scopulariopsis brevicaulis*, *Myrothecium verrucaria*, and some other fungi that ammonium, even in very low concentration, inhibited the uptake of nitrate. Cultures grown with ammonium nitrate did not utilize nitrate until almost all the ammonium had disappeared from the medium. Mycelia adapted to ammonium used nitrate readily in the absence of ammonium. Nitrite was taken up at the same time as ammonium. Other fungi, however, used nitrate and ammonium together, and some used only nitrate (Morton, 1956). *Sclerotinia sclerotiorum* is stated (Tanrikut & Vaughan, 1951) to grow well on ammonium salts, but Démétriades

(1953) found them much less favourable for this species than nitrate or some amino-acids.

(b) EFFECTS OF pH AND OF NON-NITROGENOUS NUTRIENTS

Many workers found that the pH of the medium affected absorption of both nitrate and ammonium. Plants grown with either nitrate or ammonium change the pH of the medium, solutions with nitrate becoming more alkaline and those with ammonium more acid. The excessive acidity produced by plants supplied with ammonium salts of strong acids was recognized and explained by Rautenberg & Kuhn (1864). A steady pH during the course of an experiment is best obtained by a continuous flow of culture solution, as used by Shive & Stahl (1927) and various later workers (e.g. Street & Roberts, 1952).

The effects of pH on the uptake of nitrate and ammonium have been attributed to changes in the ionic or molecular species present in the medium. This explanation is unlikely to be correct. Nitrate is present as the ion over a wider range of pH than is tolerated by most plant roots. Free nitric acid occurs in significant amounts only at pH levels below 3.0. Ammonium hydroxide molecules, present in neutral and alkaline solutions, have been considered to be the preferentially absorbed form of ammonium. This suggestion, however, fails to explain the high rates of absorption of ammonium observed at pH levels well below neutrality where little ammonium can exist as the hydroxide molecule. Tomato plants, for instance, absorb appreciable amounts of ammonium at pH 4.0 (Clark & Shive, 1934; Arrington & Shive, 1936).

Many workers have concluded that plants use ammonium best at a neutral or alkaline reaction and nitrates in acid media. Results supporting this view are reported for sugar-beet (Prianishnikov, 1929; Dikussar, 1930, 1934), tomato (Tiedjens & Robbins, 1931), and apple trees (Tiedjens & Blake, 1932). Weissman (1951) found that wheat seedlings in the dark gave maximum protein synthesis with equal amounts of nitrogen as nitrate and as ammonium at pH 5.3 and pH 6.3; at pH 4.3 the optimum ratio was one part of nitrogen as ammonium to nine parts as nitrate. Others, however, consider that both nitrate and ammonium can be effectively assimilated over a wide range of pH (Burström, 1940; Arnon, Fratzke, & Johnson, 1942; Arnon & Johnson, 1942; Nightingale, 1948). This difference of opinion is due, in part at least, to effects of the total ionic composition of the medium on the assimilation of nitrate and ammonium at different levels of pH.

Prianishnikov (1929), using maize (*Zea mays*), vetch (*Vicia sativa*), and oats (*Avena sativa*), showed that plants supplied with ammonium salts had a lower calcium and magnesium content than those with nitrate. The optimum calcium level in the nutrient solution was higher with ammonium than with nitrate. This effect was particularly noticeable at low levels of pH, a high calcium supply widening the range of pH at which the plants made good growth with ammonium. Similar results were reported for cotton (Holley, Pickett, & Dulin, 1931), maize (Wadleigh & Shive, 1939), barley (Arnon, 1939), and citrus trees (Van der Merwe, 1953). Cotton plants with adequate calcium in the nutrient medium utilized ammonium at pH 3.0; magnesium decreased the uptake of ammonium (Ivanova, 1934). A good supply of potassium tends to increase the uptake of nitrate; a high nitrate supply also favours potassium uptake (Dikussar, 1934; McCalla & Woodford, 1938; Arnon, 1939; Nightingale, 1942b). The accumulation of nitrate and carbohydrate in potassium-deficient plants suggests that potassium is required at some stage of nitrate assimilation (Nightingale, Schermerhorn, & Robins, 1930).

Interactions have often been noted between the uptake of phosphate and of nitrate. Barley plants grown with ammonium contained more phosphate than those grown with nitrate (Arenz, 1938). High concentrations of phosphate impeded the absorption of nitrate in peanuts (*Arachis hypogaea*) (Moore, 1937), apple and peach (Cullinan & Batjer, 1943), tomato (Breon & Gillam, 1944), and pineapple (Nightingale, 1942b; Sideris & Young, 1946a, b); with the last crop an increased nitrate supply impeded the absorption of phosphate. Absorption of phosphate by the marine diatom *Nitzschia closterium* increased with the nitrate content of the medium, but nitrate absorption was independent of phosphate level (Ketchum, 1939).

A high chloride content in the nutrient medium depressed the uptake of nitrate by citrus trees (Chapman & Liebig, 1940) and by tobacco (Shear, 1941). This effect, though stated to occur at non-toxic levels of chloride, apparently requires a high content of this ion. Kretschmer, Toth, & Bear (1953) studied the effect of chloride level on nitrogen uptake in eleven species. Differences in nitrogen content were mostly small and inconsistent in direction, indicating no marked effect of chloride level under the conditions of this experiment. Changes in sulphate level had no effect on nitrate uptake. Vladimirov (1934) found that chloride, as compared with sulphate, favoured uptake of nitrogen by sugar beet. The reported effects of chloride on nitrogen

nutrition are thus variable, and may depend on the species studied.

Among the micronutrient elements whose requirements are affected by the form of nitrogen supplied, molybdenum has been intensively studied; it is associated with enzymatic reduction of nitrate in the mould *Neurospora crassa* and in higher plants (Evans & Nason, 1952, 1953). Tomato and barley (Mulder, 1948), cauliflower (Agarwala, 1952), *Aspergillus niger* (Steinberg, 1937, 1939), and *Anabaena cylindrica* (Wolfe, 1954) all require more molybdenum with nitrate than with ammonium as the source of nitrogen. The importance of manganese in plant nutrition was pointed out earlier (Aso, 1903; Nagaoka, 1904; Loew & Honda, 1904); its association with reduction of nitrate to nitrite and ammonia by plants was stressed by Dony-Hénault (1911, 1912) and by McHargue (1919). A beneficial effect of manganese on nitrate utilization also appears in the results of Plate (1914). Manganese is now known to be essential for assimilation of nitrate in isolated wheat roots (Burström, 1939a, b) and in *Chlorella* (Noack & Pirson, 1939; Alberts-Dietert, 1941). Nitrates accumulate in manganese deficiency in oats (Leeper, 1941; Whitehead & Olson, 1941) and in *Phalaris minor* (Leeper, 1941), suggesting that manganese is required at an early stage in utilization of nitrate. In cauliflower, however, manganese deficiency leads (Hewitt, Jones, & Williams, 1949) to an accumulation of amino-acids, manganese appearing to act at a later stage of the reaction sequence leading from nitrate to protein.

Jones, Shepardson, & Peters (1949) found that manganese prevented an accumulation of nitrite in soybeans grown with nitrate in conditions of inadequate aeration; this recalls the formation of toxic materials from nitrate in pea seedlings grown anaerobically (Godlewski & Polzeniusz, 1901), and suggests an effect of manganese on the reduction of nitrite. The green alga *Uva lactuca* responds to manganese with nitrate but not with ammonium (Kylin, A., 1943; Kylin, H., 1943). Manganese stimulates a purified enzyme system from soybean leaves which reduces nitrite to ammonia (Nason, Abraham, & Averbach, 1954). Manganese thus seems essential in the utilization of nitrate; whether it acts at one or more stages remains uncertain. Deficiencies of other elements, e.g. sulphur (Eaton, 1942; Anderson & Spencer, 1950), also lead to an accumulation of nitrate. This probably indicates a general depression of protein synthesis, owing to a deficiency of essential sulphur-containing amino-acids, rather than a direct participation of sulphur or its simple compounds in nitrate reduction.