AN ^{15}N STUDY OF THE EFFECTS OF NITRATE, AMMONIUM, AND NITRATE + AMMONIUM NUTRITION ON NITROGEN ASSIMILATION IN ZEA MAYS L.

by

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THE BOOK-WORMS.

Through and through the inspired leaves,
Ye maggots, make your windings;
But oh! respect his lordship's taste,
And spare his golden bindings.

Robert Burns.

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ABBREVIATIONS AND SYMBOLS.

A%E	atom per cent excess
ATP	adenosine triphosphate
FeEDTA	ethylenediaminetetra-acetic acid (ferric monosodium salt)
GLN	glutamine
GLU	glutamic acid
GOGAT	glutamate synthase
GS	glutamine synthetase
GSA	glutamine synthetase activity
NADH	nicotinamide adenine dinucleotide (reduced)
NR	nitrate reductase
NRA	nitrate reductase activity
N-SERVE	2-chloro-6(trichloromethyl)pyridine

Abstract.

A brief review of the literature on the effects of nitrate and ammonium nitrogen sources on plant growth, and the assimilation of those nitrogen sources, has been presented.

A water culture technique for the growth of maize was developed. The use of a gravel rooting medium with nutrients recycling through the rooting medium, and an iron supplement in the form of ferric citrate, resulted in superior growth of nitrate-fed plants in comparison to that observed with a hydroponic growth technique and a standard Long Ashton nutrient solution.

The effects of nitrogen source on plant growth, organic nitrogen and inorganic nitrogen contents, and the rates of incorporation into nitrogenous compounds were studied. The observed differences were explained with reference to the effects of the various nitrogen sources on the physiology of the plants.

The experimental techniques included assays of the enzymes nitrate reductase and glutamine synthetase, whole plant growth studies, and the analysis of nitrogenous compounds of xylem exudate and those extracted from the leaf blade, leaf base, and root regions of maize plants after feeding with a nutrient solution containing nitrogen as ^{15}N.

After 48 days of growth ammonium- and nitrate + ammonium-fed maize had larger fresh weights than those fed with nitrate. Plants grown with ammonium as the sole nitrogen source had greater organic nitrogen contents than those grown with

nitrate + ammonium, while nitrate-fed plants contained considerably less organic nitrogen than plants grown with the two ammonium-containing nutrient solutions. In 20-day-old maize the highest rates of incorporation of nutrient nitrogen into organic forms was seen when ammonium was the only nitrogen source, while plants supplied nitrate + ammonium displayed greater incorporation rates than nitrate-fed maize. It was concluded that ammonium nutrition produces optimum growth, with nitrate + ammonium being a better nitrogen source than only nitrate.

Leaf blade nitrate reductase activity exceeded that of the root in nitrate-fed plants, suggesting that the shoot is the major region of nitrate assimilation. This is further supported by the results of xylem exudate analysis, where 93% of the newly-absorbed nitrogen exported by the roots was detected as nitrate. Evidence in support of this hypothesis was also obtained by studying the distribution of ^{15}N in the various nitrogenous compounds extracted from whole plants after feeding with labelled nitrate for 4h and 8h.

After feeding with ^{15}N ammonium for 4h and 8h, most of the ^{15}N in the xylem exudate of 20-day-old plants was found in organic forms, indicating that most of the assimilated nutrient ammonium exported to the shoot had been assimilated in the root. Analysis of extracts from whole plants fed with labelled ammonium for 4h and 8h also supported the belief that nutrient ammonium is assimilated into organic compounds predominantly in the root.

CHAPTER 1

INTRODUCTION.

With increasing nitrogen fertilizer costs new ways of improving crop yield in relation to nitrogen fertilizer application are constantly being sought. The application of nitrate fertilizers is widespread and generally results in increased yield in terms of mass and protein content. Much of this applied nitrogen is, however, not recovered in crop harvesting. Nitrate is rapidly denitrified under reducing conditions, resulting in heavy losses of gaseous nitrogen from heavy-textured soils with low permeability, and paddy soils. Even greater losses of applied nitrate fertilizer occur because of the leaching of the extremely water-soluble NO_3^- ion ; the net negative charge found on the colloids of most temperate soils accelerates this process by repelling the negatively-charged nitrate ion. It has been estimated that approximately 50% of the agriculturally applied nitrate fertilizer is washed into rivers and out to sea.

Nitrogen may also be supplied to crop plants as ammonium or urea. This does not necessarily overcome the problems of nitrogen loss, as ammonium can undergo nitrification by soil bacteria to produce nitrate. Further drawbacks to the use of ammoniacal fertilizers include the restricted availability of ammonium to the plant due to its binding to the soil particles, and a reduction in soil pH following the absorption of ammonium by the plant.

Recent reports have shown that the use of an ammonium fertilizer supplement to nitrate fertilizer regimes can result in a considerable increase in growth rate and yield in a number of plants, eg Cox & Reisenauer (1973), Schrader et al (1972). In South Africa maize is one of the most important crop plants, being one of the principal foods in the diet of a large section of the population. It is therefore extremely important discover if these observations apply to maize and, if so, to ascertain the physiological reasons for the improved productivity.

The aim of this project was to compare the productivity of maize grown under nitrate, ammonium, and mixed nitrate-ammonium feeding regimes, and to try to explain the observed differences in productivity in terms of the effect of these nitrogen sources on the nitrogen physiology of the plant.

CHAPTER 2

LITERATURE SURVEY

2.1. Nitrogen Assimilation.

2.1.1. Nitrate Reduction.

Absorbed nitrate has to be reduced to ammonium before being incorporated into nitrogenous organic compounds. Ammonium formed by nitrate reduction, or absorbed directly from the external medium, is then assimilated via the GS/GOGAT pathway (see Section 2.1.2.). The reduction of nitrate to ammonium is a two-step process involving firstly the reduction of nitrate to nitrite, then the reduction of nitrite to ammonium (Beevers & Hageman, 1972). The enzymes used by higher plants to catalyze these reactions are nitrate reductase (EC 1.6.6.1.) and nitrite reductase (EC 1.7.7.1.) respectively.

The nitrate reductase which is found in higher plants, as well as in green algae and fungi, is dependent on reduced pyridine nucleotides as electron donors, and catalyzes the reduction of nitrate to nitrite according to the equation :-

$$NO_3^- + NAD(P)H + H^+ \longrightarrow NO_2^- + NAD(P) + H_2O \qquad (1)$$

Three sub-classes of nitrate reductase have been distinguished according to specificity for NADH or NADPH, but the form found in higher plants is specific to NADH (Beevers & Hageman, 1969; Beevers & Hageman, 1972).

Nitrate reductase in higher plants is stimulated by light (Hageman & Flesher, 1960; Beevers et al, 1965) and is induced by nitrate (Wray & Filner, 1970), as well as being influenced by changes in temperature, oxygen tension, and other environmental factors. Nitrate reductase activity has also been shown to follow a diurnal periodicity in a wide variety of plant species (Schrader, 1978).

The reduction of nitrite to ammonium in photosynthetic organisms is catalyzed by a ferredoxin-dependent nitrite reductase (Hageman et al, 1962; Vega et al, 1980) and proceeds according to the reaction:-

$$NO_2^- + 6Fd_{RED} + 8H^+ \dashrightarrow NH_4^+ + 6Fd_{OX} + 2H_2O \qquad (2)$$

Nitrate reductase is normally present in plants at lower activity levels than nitrite reductase so nitrite seldom accumulates in living tissues. The reduction of nitrate to nitrite is therefore the rate-limiting step in the reduction of nitrate to ammonium, and it has been suggested by Beevers & Hageman (1969) that nitrate reductase would be the logical place for plants to effectively control the input of reduced nitrogen when utilizing nitrate-N. Some reports have been made of growth, yield, and protein content of plants being correlated with nitrate reductase activity (NRA) measurements (Hageman, 1979; Gašić et al, 1981; Srivastava, 1980), and NRA measurements have been proposed as a possible indicator of high-protein varieties (Dalling & Loyn, 1977).

Recent work has cast doubt on the accuracy of in vivo methods of nitrate reductase determination. In 1981, Yoneyama reported that his ^{15}N labelling studies with leaf discs from several species revealed the formation of amino acids and ammonium from both nitrate and nitrite, in the dark, under in vivo assay conditions - with some species the amount of labelled N in the amino acid fraction exceeded that in the nitrite fraction. He expressed the opinion that in vivo assays under aerobic conditions could greatly underestimate nitrate reduction due to the further reduction of product nitrite, and pointed out that performing these assays under anaerobic conditions would result in increased nitrite accumulation, although some incorporation of ^{15}N into ammonium and amino acids still occurred.

The work of Ben Shalom et al (1983) indicated that, over a 20min incubation period and under aerobic conditions, 98% or more of the nitrite produced in barley leaves was further assimilated in both light and darkness.

The standard practice of measurement of nitrite production under anaerobic conditions was also shown to be suspect by the work of Gray & Cresswell (1983), who demonstrated that a stoichiometric relationship between nitrate disappearance and nitrite accumulation does not exist. Nitrate utilization in maize roots was found to exceed nitrate accumulation by 80-100 fold, indicating that most of the nitrite produced by nitrate reduction had taken part in further reactions.

It would appear that nitrate utilization provides a more accurate reflection of nitrate reduction than does nitrite accumulation in the in vivo assay procedures, though those methods employing the incubation of plant tissues in a nitrate-containing incubation medium suffer the limitation that the nitrate supply to the enzyme may vary from that of the intact plant. No similar studies have been made on the in vitro method of NR assay. The latter method cannot be used in attempts to construct balance sheets for nitrogen assimilation as the results will not reflect the true rate of nitrate reduction in living tissues; they do however provide an indication of nitrate reductase potential.

Plants exhibit variety in the distribution of sites of nitrate reduction, and this is reflected in the composition of the xylem sap by the relative amounts of nitrate and organic nitrogen (ammonium rarely constitutes a significant portion of the nitrogen in the xylem). Amongst those which contain mostly nitrate in the xylem sap are *Xanthium* (Wallace & Pate, 1967), *Cucumis* (Olday *et al*, 1976), and *Gossypium* (Radin *et al*, 1975), where over 95% of the xylem-borne nitrogen is in the nitrate form, *Datura* (Probyn, 1978) with over 80% as nitrate, *Helianthus* (Kaiser & Lewis, 1980) with 77-94% as nitrate, and *Hordeum* (Lewis *et al*, 1982a) with 80-90% as nitrate. In such cases it is believed that root nitrate reductase activity is low, and there is little surplus reduced nitrogen for export, although root NRA may provide at least a part of the reduced nitrogen requirement of the root (Radin, 1977; 1978). Roots of *Xanthium* can synthesize and export amide-N if fed reduced nitrogen as ammonium or urea, suggesting that nitrate reduction is the limiting factor in the assimilation of nitrate-N (Wallace & Pate, 1967).

Other plants have shown a greater capacity to reduce nitrate in their roots, eg. *Lupinus* and *Raphanus* had almost no nitrate in the xylem stream when supplied with 10mM nitrate, only 7% of the N being carried as nitrate (Pate, 1973). A third group of plants export both nitrate and amino acids from the roots, the nitrate concentration increasing with increasing nitrate feeding levels (Pate, 1971; Pate, 1973; Schrader, 1978).

2.1.2. Incorporation of Ammonium.

It is now widely accepted that the incorporation of ammonium, whether produced as a result of nitrate reduction or absorbed directly from the nutrient medium, is via the Glutamine Synthetase/Glutamate Synthase (GS/GOGAT) pathway, as proposed by Lea & Miflin (1974).

The enzyme glutamine synthetase (EC 6.3.1.2.) catalyzes the amidation of glutamate as shown in equation (3):-

$$\text{Glu} + \text{NH}_3 + \text{ATP} \dashrightarrow \text{Gln} + \text{ADP} + \text{Pi} \qquad (3)$$

It has a high affinity for ammonia, K_m values being reported by O'Neal & Joy (1974) and Stewart & Rhodes (1977) in the range $1\text{-}2 \times 10^{-5}$M. This enzyme is also generally found to have much higher activity levels in plant tissues than nitrate reductase (NR) eg. Lewis et al (1982a). Glutamine synthetase is therefore able to incorporate any free NH_3 into glutamine provided sufficient energy (in the form of ATP) and carbon skeletons are available, and the ammonium levels within the tissues can be kept to levels which are not toxic.

The early evidence that ammonia was assimilated via the GS/GOGAT pathway was based on indirect evidence such as enzyme kinetics studies and enzyme activity levels. More substantial evidence for the importance of this pathway has been obtained using the GS analogue methionine sulfoximine (MSO) which acts as an inhibitor of GS, and by the use of labelled N to monitor nitrogen assimilation rates. Probyn (1978) found that pretreating Datura stramonium with MSO prior to ^{15}N feeding to the leaves resulted in the accumulation of labelled ammonium, and decreases in the free amino acid pools, suggesting that nitrogen assimilation had effectively been inhibited by the treatment. Fentem et al (1983a) noted that MSO almost completely blocked the incorporation of labelled nitrogen into amino acids in barley roots when nitrate was the nitrogen source, and the labelling patterns with ammonium feeding were consistent with a model in which ammonium was assimilated solely into glutamine. Fentem et al (1983b) also reported that, in a continuous culture system in which the root and shoot ammonium and amino acid levels remained constant over a 6h experimental period, MSO reduced root GS levels and they observed that root ammonium levels increased whilst glutamine levels decreased.

The enzyme GS has also been associated with the re-assimilation of ammonia produced as a result of photorespiration. The ammonia is released in conjunction with the production of carbon dioxide by the conversion of glycine to serine (Mahon et al, 1974). Using MSO to inhibit the GS enzyme, Martin et al (1983) found that this ammonia also appears to be incorporated into glutamine.

Two forms of the enzyme glutamate synthase (GOGAT) exist. One form utilizes NADH or NADPH (Dougall, 1974; Fowler et al 1974) and is found in non-green tissue (Miflin & Lea, 1975), catalyzing the formation of glutamic acid from glutamine as shown in equation (4):-

$$\text{Glutamine} + \text{2-oxoglutaric acid} + \text{NAD(P)H} + H^+ \text{ -----> } \text{2-L-glutamic acid} + \text{NAD(P)} + H_2O \qquad (4)$$

The second form exists in chlorophyllous tissue and non-green tissue (Lea & Miflin, 1974; Miflin & Lea, 1975), is dependent on reduced ferredoxin as the electron donor and catalyzes the transfer of the amido group of glutamine to form 2 molecules of glutamic acid as given in equation (5):-

$$\text{L-glutamine} + \text{2-oxoglutarate} + \text{FdH} \text{ ----> } \text{2-L-glutamic acid} + \text{Fd} \qquad (5)$$

The synthesis of other amino acids takes place by transaminations from glutamic acid to α-keto acids, and by further reactions on those.

2.2. Nitrate Nutrition.

The absorption of nitrate is considered to be an active process. Respiratory and oxidative phosphorylation inhibitors were found to decrease nitrate absorption (Rao & Rains, 1976), and other reports have indicated that nitrate acquisition was an energy-requiring process.

An increase in the external pH has been frequently seen to accompany nitrate absorption eg. Minotti et al (1968), Breteler (1973), Smith (1973). Wallace & Mueller (1963) suggested that this may result from the expulsion of a hydroxyl ion or a bicarbonate ion, and Smith (1973) said that it could be the result of NO_3^-/OH^- exchange.

Workers in Israel (Ben Zioni et al, 1970; 1971) proposed a model relating bicarbonate excretion to nitrate reduction which would explain how the plant is able to maintain electroneutrality. It was suggested that a recycling of K took place, with K and malate (synthesized concomitantly with nitrate reduction) being transported from the shoot to the root via the phloem. The malate would then be decarboxylated in the root and bicarbonate excreted, whilst K would be returned to the shoot via the xylem. A variation on this model was proposed in 1974 by Kirkby, differing in that the carbon would be retained after the decarboxylation of the malate, with OH^- being expelled into the nutrient medium. In situations where nitrate reduction occurred in the root, the hydroxyl ions generated could be excreted directly into the external medium.

Keltjens (1981) suggested that, in maize, the supply of K^+-malate via the phloem was insufficient to account for the total OH^- efflux from the root, and that most of the hydroxyl ions excreted were synthesized in association with nitrate reduction. Using tungstate to inhibit root nitrate reductase, Keltjens (1981) obtained evidence for nitrate reduction being the driving force behind OH^- efflux from the root in that the efflux was almost stopped when root NR was inhibited.

The use of the chlorate ion as an analogue for nitrate in absorption studies by Deane-Drummond (1982) in Canada has also provided evidence in support of the hypothesis that OH^- efflux is linked to nitrate reduction. Whilst working with barley seedlings, she observed that there was an apparent independence of nitrate uptake and OH^- efflux, raising doubts about NO_3/OH^- antiport as a model for nitrate absorption, and suggested that OH^- efflux was related to nitrate reduction.

Other than that it is an active process and that electrostatic balance is maintained by the excretion of a hydroxyl (or bicarbonate) ion, little is known of the nitrate uptake mechanism. The control of nitrate uptake may be related to a feedback mechanism (Deane-Drummond & Glass, 1983), but these workers proposed a short-term mechanism with (1) an influx term dependent on the external nitrate concentration and independent of the internal nitrate concentration, working in conjunction with (2) an efflux term which was directly related to the internal nitrate concentration. They pointed out, however, that the internal nitrate concentration is also influenced by nitrate reduction and by translocation to the shoot.

The uptake of other nutrients is affected by the nitrogen source. Plants grown with nitrate as the nitrogen source generally show increased cation uptake when compared with ammonium-fed plants. Cox & Reisenauer (1973) found that increasing rates of nitrate uptake resulted in increased absorption of K, Mg, and Ca, whilst ammonium absorption resulted in decreased absorption of Ca and Mg. Nitrate-fed plants have been shown to have higher concentrations of K, Mg, and Ca in a wide number of species by

Barker & Maynard (1972), Breteler (1973), Edwards & Horton (1982), Magalaes and Wilcox (1983a, 1983b), and many others. The reduction in cation uptake with ammonium feeding was explained by Cox & Reisenauer (1973) as ionic competetion at the site of intake either with ammonium ions _per se_, or with hydrogen ions expelled after ammonium absorption (Wallace & Mueller, 1963).

Ikeda & Osawa (1979) found that nitrate-fed plants contained higher concentrations of Ca, K, and Mg than those which were grown with ammonium, but this decrease in cation uptake occurred only slightly in maize and in strawberry. Krajina _et al_ (1973) stated that species which are ecologically adapted to high levels of ammonium nutrition tend to show no Ca or Mg deficiency symptoms, whereas other species develop Ca and Mg deficiency symptoms under those conditions. Little work has been done on the subject of differential nutrient absorption under the influence of the various nitrogen sources, and the physiological basis for the difference in cation absorption remains unknown.

The nitrogen source has been shown to influence the absorption of anions too, with phosphate and other anion absorption being increased in the presence of ammonium (Blair _et al_, 1970; Breteler, 1973; Cox & Reisenauer, 1973; Harada _et al_, 1968), and this is generally considered to be the result of stimulated anion uptake in response to increased cation (ie. NH_4^+) absorption. Barneix & Arnozis (1980) reported that wheat seedlings grown with ammonium had 30% higher P contents in tops, and 20% higher P contents in roots, than did nitrate-grown seedlings, and they suggested that this may relate to ammonium feeding resulting in a more rapid syntheses of a nitrogenous intermediate related to phosphate uptake.

Iron deficiency, and resulting chlorosis, may be a problem with nitrate nutrition, this being due to reduced uptake and/or chelation within the plant. One recent example of reduced iron uptake was reported by Smith et al (1983) where nitrate nutrition resulted in reduced iron content in several species, with maize being more severely affected than others. Hewitt (1966) quotes several examples of iron deficiency in nitrate-fed plants being overcome by the supply of ammonium, but it is not clear whether these deficinecies actually resulted from reduced absorption or utilization of iron. Iron is absorbed more readily at low pH (Hewitt, 1966; Mengel & Kirkby, 1978) and it is possible that the external pH rise accompanying nitrate absorption causes restricted Fe uptake.

The reduction of nitrate results in higher levels of free inorganic cations than free inorganic anions. Ionic balance is attained by the production of organic acid anions, the form accumulated depending on the plant species, but malate is frequently the first organic acid anion to be produced (Cram & Laties, 1974; Osmond & Laties, 1969; MacDonald & Laties, 1964). Organic acid synthesis associated with cation excess has been discovered to result from dark CO_2 fixation (Cram, 1974) but it is not known which of the carboxylation enzyme systems is involved. It should also be recalled that Ben Zioni et al (1970) suggested that malate synthesis takes place in association with nitrate reduction. Wallace (1971) proposed that the excess organic anions may combine with iron, thereby reducing the activity of the iron within the plant.

Nitrate accumulation can take place within plant tissues when nitrate is the nitrogen source. Spinach, beet, radish, eggplant, celery, lettuce and turnip greens are among the vegetables that contain high concentrations of nitrate (Ashton, 1970), and various factors have been shown to affect the free nitrate levels of plants including nitrate fertilizer levels (especially during the fruiting stage) (Griffith, 1960), plant nutrient deficiencies (Schütte, 1964; Hewitt & Jones, 1947), and water availability (Hanway & Englehorn, 1958). High levels of nitrate have also been detected in water supplies, particularly those from wells. Nitrates in themselves are fairly innocuous, being rapidly excreted by animals, but can undergo reduction under certain circumstances. Reduction may take place in the rumen of cattle (Bradley _et al_, 1939), during vegetable storage (Walker, 1975), and reduction in infant's stomachs can result in methemoglobinemia (Knotek & Schmidt, 1964). Nitrite as a free radical is toxic to animals (and man) in that it binds strongly to the iron of haemoglobin (Walker _et al_, 1957), and it also reacts with some amines to form nitrosamines which are carcinogenic (Magee & Barnes, 1956). Caution must therefore be exercised when nitrate fertilizers are being used to avoid the hazardous effects which can be caused by nitrate accumulation in food plant tissues and drinking waters.

2.3. _Ammonium Nutrition._

Epstein (1972) stated that, over a limited concentration range, the absorption of ammonium from a nutrient solution follows a classical enzyme kinetics pattern, although this has been interpreted differently by workers with differing opinions on the uptake mechanism. In 1964, Smith & Epstein performed a quantitative study on the absorption of ammonium, potassium, and rubidium, in which kinetic analyses showed ammonium absorption to be competitive with the absorption of K and Rb. This led to the conclusion that ammonium, potassium, and rubidium compete for the same carrier, although the affinity of NH_4^+ for the common transport mechanism was only one tenth of the affinity of K^+ and Rb^+.

Apart from this active uptake of ammonium, passive ammonia uptake can occur. Ellenburg (1958), whilst discussing ammonium absorption, stated that in sand and in water culture at pH 6.0, 0.2% of the ammoniacal nutrient nitrogen is in the NH_3 form, this being capable of rapid diffusion into the roots. Barr et al (1974) observed that when Nitella cells were transferred to a non-growth medium containing 1mM K^+, previously-accumulated ammonia was lost from the cells. They interpreted their results as indicating that NH_3 travelled from the vacuole, where NH_4^+ was stored, to the external medium. The permeability factor of ammonia was 4.4×10^{-4} cm s^{-1}, this probably applying to the movement of ammonia from the vacuole to the external medium, via the tonoplast, cytoplasm, and cell membrane. Other workers have also suggested that ammonia may be able to diffuse through biological membranes (Moore, 1974; Heber et al, 1974).

The absorption af ammonium causes a drop in nutrient medium pH due to an accompanying proton release (Wallace & Mueller, 1963; Wallace & Wallace, 1982), and it is depressed by a lowering of the nutrient pH (Naftel, 1931; Stahl & Shive, 1933).

Nitrogen from nutrient ammonium is incorporated into amino acids via the GS/GOGAT pathway as discussed in Section 2.1. This provides one of the advantages over nitrate nutrition, as ammonium-fed plants do not have any energy expenditure on the reduction of absorbed nitrogen. Ammonium-N absorbed directly from the external medium is incorporated into organic compounds more rapidly than is nitrate. This difference may relate to the reduction of nitrate, which is widely considered to be the rate-limiting factor in nitrate assimilation. The rapid incorporation of nutrient ammonium was demonstrated by Yoneyama et al (1977) who noted that labelled ammonium was rapidly assimilated by maize roots into glutamine, glutamic acid, and aspartic acid, in that order. Labelled nutrient nitrate was incorporated into the same compounds, but the assimilation rate was slower than that of the absorbed ammonium. Differences in the absorption rates of nitrate and ammonium did not appear to be responsible for the differing incorporation rates observed by Yoneyama and co-workers as the total nitrate and ammonium absorbed by the experimental material did not appear to be considerably influenced by the nitrogen source. This difference in the rate of incorporation into organic compounds is not a new revelation, although the GS/GOGAT pathway was only recently discovered. In experiments performed over short time-periods, Kinoshita (1897) ascertained that ammonium salts were rapidly incororated into asparagine by barley, but N from nitrate feeding was only slowly incorporated into asparagine. Kinoshita also remarked that no trace of ammonium could be found in his barley plants, and modern techniques succeed in detecting only small quantities of ammonium in the tissues of healthy plants.

Ammonium is normally absorbed more rapidly than nitrate (Trelease & Trelease, 1935; Wallace & Mueller, 1957; Lycklama, 1963; Clarkson & Warner, 1979, etc.) and this affects the nitrogen status of the plant. Plants grown with ammonium nutrition have higher levels of organic nitrogen than those grown with nitrate as the nitrogen source. This has been observed in maize (Schrader et al, 1972), in barley (Lewis & Chadwick, 1983), and in several other species.

The rapid absorption of nutrient ammonium has important effects on the use of carbon skeletons by the plant. Ammonium, unlike nitrate, has toxic effects and cannot be allowed to accumulate within the plant tissues, eg. ammonium levels of 2mM or greater are known to disrupt photosynthesis by uncoupling photosynthetic phosphorylation (Good, 1960). The rapid assimilation of ammonium into nitrogenous organic compounds requires a suitable supply of carbon skeletons, as demonstrated by Kinoshita (1897) who observed that in the absence of suitable carbon compounds, ammonium accumulated in the plant tissues. Ammonium assimilation is therefore directly linked to photosynthesis. Larsen et al (1983) established that ammonium decreased carbon fixation into carbohydrates and increased fixation into carboxylic acids (which are the source of carbon skeletons for amino acid synthesis) and amino acids, and this change in the direction of carbon fixation could serve as part of a detoxification mechanism. The diversion of carbon away from carbohydrates could have significant effects on plant growth, as shown by the diminutive plants obtained with ammonium feeding in comparison to nitrate feeding by Cox & Reisenauer (1973), Lewis & Chadwick (1983), and other researchers.

Ammonium nutrition has been reported to overcome the iron deficiency which is sometimes found in nitrate-fed plants in association with reduced leaf chlorophyll content. Some examples of ammonium supply relieving symptoms of iron deficiency are listed by Hewitt (1966). This difference between plants supplied with ammonium and those supplied with nitrate could arise in either of 2 ways. Firstly, the absorption of ammonium causes the nutrient pH to decrease, which results in improved Fe absorption. Secondly, the rapid assimilation of ammonium results in stimulation af amino acid production and less organic acid accumulation than occurs with nitrate feeding, thus reducing the internal chelation of iron by organic acid anions which Wallace (1971) suggested was a cause of iron deficiency in nitrate-fed plants.

The effect of ammonium nutrition on the upkake of other nutrients has been discussed in Section 2.2.

2.4. Effect of Nitrogen Source on Yield.

2.4.1. Enhancement of Growth by Ammonium.

Increased yields on the addition of ammonium to nitrate-containing nutrient solutions have been observed by many workers, and in a variety of plant species eg. Naftel (1931), Johnson et al (1957), Hewitt (1966), Maynard & Barker (1969), and Blair et al (1970). Cox & Reisenauer (1973) found that the addition of ammonium to nitrate-containing solutions produced increased growth in wheat, but that the maximum growth rate in ammonium-containing nutrient solutions was limited by ammonium toxicity at high levels of nutrient ammonium. Working with maize, Warncke & Barber (1973) recorded that the maximum nitrogen uptake occurred at a nitrate:ammonium ratio of 1:2.5, but no direct correlation was found between nitrate:ammonium ratio and plant dry mass. The results of Edwards & Horton (1982) indicated that the best growth, in terms of dry mass, occurred when nitrate and ammonium were present in the nutrient medium in proportions of 1:1 and 1:3, the total nutrient N being the same in each nitrogen treatment. The total nitrogen absorption rate increased with increasing levels of nutrient ammonium. In chrysanthemum, the relative growth rate was increased by adding ammonium to nitrate-containing nutrient solutions (Elliott & Nelson, 1983). As the nutrient ammonium concentration rose, the ammonium and total nitrogen absorbed by the plants increased, but the absorption of nitrate decreased.

The effects of nitrogen source on plant growth were found to vary between crop plant species by Ikeda & Osawa (1979, 1980, 1981). In a number of solution culture experiments many of the plant species exhibited the greatest top dry weight when nitrate + ammonium was the nitrogen source. The growth of several species was inhibited when ammonium alone was the nitrogen source, in comparison with the growth obtained in the plants which were fed only nitrate-N. In maize and strawberry, however, the greatest dry masses were obtained when ammonium was the sole nitrogen form supplied.

Different nitrogen sources may have beneficial effects on some aspects of plant growth, but detrimental effects on others. One example of this is seen in the work of Schrader et al (1972). Fresh and dry weights were greater when nitrate + ammonium was supplied to maize than when either inorganic form was supplied individually. In the same specimens, however, the total nitrogen present in the shoot and in the root was greatest in plants grown on ammonium-N only, even though the nitrate which accumulated in the tissues during nitrate and nitrate + ammonium feeding was included in the total plant nitrogen figures. A second example of discordant results produced by different nitrogen sources is seen in the work of Lewis & Chadwick (1983), who studied nitrogen assimilation in barley. In 20-day-old plants the largest fresh weights and highest ^{15}N incorporation rates were obtained when nitrate + ammonium was supplied, with nitrate-fed plants having the lowest incorporation rate, and ammonium-fed plants having the lowest fresh weight. Most organic N was detected in the ammonium-fed barley, with mixed-feed plants having intermediate quantities of organic nitrogen and nitrate-fed barley the least.

If the nitrogen source is to be controlled to maximise crop plant yield, it is therefore vital to be specific about which factors are in fact important in determining the final yield. With cereals, grain yield is normally more important than the actual plant size. Eighty to ninety percent of the N accumulated in the leaf is normally exported to the grain (Williams, 1955: Dalling et al, 1976), and this may constitute 56-80% of the total grain nitrogen (Perez et al, 1983) so it may be that the amount of nitrogen accumulated during the vegetative stage is more important in some cases than the actual plant size.

2.4.2. Ammonium Toxicity.

Toxic effects produced by ammonium nutrition have been widely documented. Cox & Reisenauer (1973) documented detrimental effects of high levels of ammonium absorption in wheat. The leaves of affected individuals were extremely dark, with minute chlorotic spots, whilst roots (which were in pH controlled nutrient solutions, pH 5.8 ± 0.2 for ammonium and pH 5.6 ± 0.2 for nitrate + ammonioum) were short and thick. Plant mass was smaller in ammonium-fed wheat than in those supplied with nitrate or ammonium + nitrate. The authors believed that the growth of tops and roots was affected equally as the top:root mass ratios were not significantly altered.

Ulrich & Mostafa (1980) reported that their experiments on sugarbeets resulted in ammonium nutrition producing much smaller plants than nitrate nutrition, and that the former had lower sucrose concentrations. In their experiments calcium carbonate was added to the nutrient medium in an attempt to overcome damage to root systems resulting from very low nutrient pH produced by ammonium absorption.

Ikeda & Osawa (1979, 1980, 1981a) reported toxic effects of ammonium in a variety of crop species, and found that injured specimens contained higher internal ammonium levels than did healthy ones. In a subsequent paper, also published in 1981, the same authors suggested that contrary to the widely-held view of ammonium being preferentially absorbed when it is present with nitrate in equal concentrations, only certain plants showed this preference. Those which displayed a marked preference for nitrate included spinach, Chinese cabbage, and turnip. They concluded from their results that species which preferentially absorbed nitrate were the most sensitive to NH_4^+ toxicity.

Ammonium nutrition and nitrate : ammonium ratios of 1:1 and 1:3 caused chlorosis in mature leaves of peach seedlings, according to Edwards & Horton (1982).

Radish plants are extremely sensitive to ammonium (Goyal et al, 1982a), with ammonium nutrition resulting in leaf chlorosis and cessation of growth. Plants grown in distilled water exhibited N deficiency, which was quite different to ammonium toxicity. Those plants which were supplied with ammoniacal nitrogen only accumulated more ammonium in shoots and roots than plants given nitrate or nitrate + ammonium. Goyal et al (1982b) suggested that this toxicity was related to inhibited photosynthesis in ammonium-fed radish.

Tomato plants grown in sand culture displayed signs of ammonium toxicity with ammonium nutrition, this being more prominent under high light intensity than under low light intensity (Magalhaes & Wilcox, 1983a). These symptoms included stunting, leaf roll, wilting, interveinal chlorosis of older leaves, and reduced dry weight when compared with nitrate-fed plants.

The stimulatory effect of ammonium addition to nitrate-containing nutrient solutions is therefore dependent on plant species, and increasing the ammonium concentration does not necessarily bring increased returns in terms of plant growth. For different plant species there may be an optimal nitrate : ammonium ratio for maximum yield.

2.5. Ammonium and Nitrate in the Soil.

An excellent review of the literature on soil nitrogen has been compiled by Bartholomew & Clark (1965) which, despite its age, is still the most comprehensive treatise on the subject. The object of this section is to survey briefly the literature on some characteristics of ammonium and nitrate which affect their availability to the plant.

2.5.1. Release of Organic Nitrogen.

Most organic nitrogen present in the soil is found in organic forms which are relatively unavailable to the plant.

The release of nitrogen from organic forms in the soil of most ecosystems is dependent on microbial activity. The first step in this process is the decomposition of organic matter by heterotrophic micro-organisms to release ammonium (ammonification). The second step is the microbial oxidation of ammonium by soil bacteria, firstly to nitrite and then to nitrate (nitrification). The conversion of ammonium to nitrate is performed by autotrophic organisms, *Nitrosomonas* spp. and *Nitrobacter* spp., and provides the organisms with an energy source. The combined action of decomposers and nitrifiers is referred to as mineralization.

Nitrification is considerably influenced by physical and chemical factors. These include soil pH, with a marked reduction in the activity of nitrifying bacteria occurring below pH 6 (Dancer _et al_, 1973). This can affect the balance of the inorganic nitrogen forms in the soil, with ammonium sometimes accumulating in the soil due to the comparative insensitivity of the ammonifying organisms to environmental factors. Temperature also affects nitrification, with 5°C and 40°C being the upper and lower limits for profuse oxidation of ammonium.

A complex interaction of several environmental factors influences nitrification, including soil moisture content, oxygen availability, and those factors mentioned above. The dominant factor will be determined by the specific environment and is not predictable, but generally nitrate formation in temperate zones is most rapid in spring and autumn (Alexander, 1977).

2.5.2. Behaviour of Ammonium in the Soil.

Ammonium which has been added to soils may become unavailable to the plant because of binding to soil particles. Binding may occur when ammonium (and indeed other cations) replace the cations in the expanded lattice of certain clay minerals. Other mechanisms have been suggested and these have been detailed by Mortland & Wolcott (1965). Most absorption of ammonium occurs when large amounts of micaceous clay minerals are present, eg in vermiculite, montmorillonite, and illite (Nommik, 1957; Nielsen, 1972; Raju & Mukhopadhyay, 1974). Evidence that recently-bound ammonium is relatively unavailable to the plant comes from Black & Waring (1972) who found that 15% of the newly-applied ammonium was bound and only 50% of this was recovered in a single crop. Further evidence lies in the work of Chaminade (1971) who reported that recently-bound ammonium made no contribution to the nitrogen status of ryegrass.

Ammonium and potassium are bound to the soil by the same mechanism (Nommik, 1957; Raju & Mukhopadhyay, 1973, 1974, 1976). Pre-treating the soil with potassium can considerably reduce the retention of applied ammonium (Raju & Mukhopadhyay, 1974, 1976), allowing greater uptake by the plant and greater yield (Raju & Mukhopadhyay, 1974).

2.5.3. Behaviour of Nitrate in the Soil.

The most important aspects of the behaviour of nitrate in the soil relate to possible losses of nitrogen through leaching and denitrification.

Denitrification occurs under reducing conditions, such as are prevalent in waterlogged soils (eg paddy fields). The presence of plants may help provide conditions favouring denitrification. Stefanson (1972) discovered that the root rhizosphere contained 90x more denitrifying bacteria than the surrounding soil, this being attributed to root growth through the soil reducing the number of pores for oxygen diffusion, and to exudates providing a source of energy to denitrifying bacteria.

The anaerobic respiration carried out by denitrifying bacteria may be expressed as shown in equation (6), taken from Broadbent & Clark (1965):-

$$C_6H_{12}O_6 + 4NO_3^- \text{--->} 6CO_2 + 6H_2O + 2N_2 \qquad (6)$$

Intermediates between nitrate and gaseous nitrogen are known to include nitrite and nitrous oxide (Cady & Bartholomew, 1960).

Nitrates are extremely water-soluble and considerable losses can occur as a result of leaching. The amount of nitrate lost is dependent on the actual amount of water which passes through the soil, and is not directly related to short-term rainfall (Gardner, 1965), but heavy rainfall will increase the amount of nitrate which is lost, this being particularly important if nitrate fertilizer is applied.

2.5.4. Nitrification of Ammonium and Nitrification Inhibitors.

The nitrification of ammonium is mainly performed by 2 groups of autotrophs, *Nitrosomonas* spp. and *Nitrobacter* spp. These bacteria are respectively responsible for the oxidation of ammonium to nitrite, and nitrite to nitrate (Alexander, 1965).

It appears that under climax vegetation many plant species inhibit nitrification. This has been observed in oak-pine forest, post oak-blackjack forest, and tall grass prarie by Rice & Pancholy (1972), and in savanna and forest soils by Berlier *et al* (1956). Lodhi (1979) reported that badly disturbed soils showed more nitrification than was detected in undisturbed areas, and noticed a general trend of decreasing nitrification through successional stages, the degree of nitrification inhibition being highest in climax communities.

Tannins from several species were shown to inhibit nitrification by Rice & Pancholy in 1973, and other naturally-released compounds were shown by Rice and Pancholy (1974) to be strongly inhibitory to nitrification at low concentrations (10^{-6} to 10^{-8}M). Chemical inhibitors of nitrification extracted from ponderosa pine needles, bark, and A horizon soil by Lodhi & Killingbeck (1980) included caffeic acid, chlorogenic acid, quercitin, and tannins. Foliar leachates and leaf extracts from balsam fir and balsam poplar were found to inhibit the oxidation of ammonium under laboratory conditions by Thibault et al (1982).

In agricultural situations nitrification is also very important. Apart from influencing the form of inorganic nitrogen which is available to the plant (the effects of which were discussed in Sections 2.2., 2.3. and 2.4.), it increases nitrogen losses due to leaching and denitrification.

Considerable interest is now being displayed in nitrification inhibitors. These may be used under field conditions to prevent or limit the microbial oxidation of ammonium to nitrate, the likely effects of this having been discussed in Sections 2.2.-2.4.

The nitrification inhibitor which has been most widely researched is nitrapyrin, or 2-chloro-6(trichloromethyl)pyridine (named N-Serve by the manufacturer). Other nitrification inhibitors may be as effective in reducing the action of nitrifying bacteria, for example Rogers & Ashworth (1982) reported that nitrapyrin, etridiazole (5-ethoxy-3-trichloromethyl-1,2,4-thiadizole), and dicyandiamide all reduced nitrification by 40% in field trials conducted at the Rothamsted Experimental Station. Dow Chemical Co., who manufacture N-Serve, appear to have a more effective marketing strategy than either BDH Ltd. or Midox Ltd., who produce etridiazole and dicyandiamide respectively!

Nitrapyrin effectively inhibits nitrification in many soils (Goring, 1962; Bunday & Bremner, 1974; Mills et al, 1976; Mills & Pokorny, 1978). It is claimed to be specific in its action, having inhibitory effects on Nitrosomonas spp. (Goring, 1962), and neither it nor its principal metabolite, 6-chloropicolinic acid, are phytotoxic at recommended application rates (Geronimo et al, 1973; Mills et al, 1973). It has a half-life of 4-21 days and therefore does not accumulate in soils or plant tissues (Goring, 1962; Redeman _et al_, 1965).

The use of nitrapyrin has been reported to result in increased yields of numerous crop plants including cotton and sugarbeet (Swezey & Turner, 1962), The effects of nitrapyrin on maize yields have been variable, with Swezey & Turner (1962), McElhannon & Mills (1981), and Warren _et al_ (1975) reporting yield increases, while Hendrickson _et al_ (1978) and Touchton _et al_ (1979) reported no significant yield increases. Where increased yields were obtained, nitrification was found to have been reduced by the nitrapyrin application; where no increase was noted, there was either a lack of nitrification inhibition or N losses from the root region were low. For example, in the field-trials of Chancy & Kamprath (1982) the application of nitrapyrin with ammoniacal fertilizers gave higher yields of maize than nitrate fertilization only in years when leaching conditions occurred.

Nitrapyrin has now been credited with reducing denitrification under field conditions by McElhannan & Mills (1981), both with nitrate and ammonium fertilization.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Material.

Seeds of Zea mays L. var. K64R and R201 (Starke-Ayres [Pty.] Ltd., Evans Ave., Epping 1, Box 304, Epping Ind. Est., Cape Town) were germinated on trays of moist vermiculite in a Conviron Model G30 growth chamber (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) under constant conditions of 100% Relative Humidity, a temperature of 25°C, and dim light. The seedlings were transplanted into nutrient solutions 7 days after sowing, which allowed the utilization of seed reserves. The final nutrient solution composition was a modified Long Ashton formula (Hewitt, 1966), as shown in Tables 1&2. It contained nitrogen as 2mM NO_3^-, or 2mM NH_4^+, or 1mM NO_3^- + 1mM NH_4^+.

3.2 Plant Culture.

Plants for ^{15}N-feeding, and for enzyme studies, were grown in a Conviron Model E15 growth chamber under conditions of 75% R.H., day- and night-temperatures of 27°C and 18°C respectively, and a photoperiod of 14h. Irradiance was supplied by Sylvania (GTE Sylvania Canada Ltd., Drummondville, Quebec, Canada) cool-white fluorescent lamps supplemented with 60W incandescent lamps, adjusted to provide a photon flux of $450 \mu E\ m^{-2} s^{-1}$. Plant material for long-term growth studies was grown under greenhouse conditions during the summer months November-February.

Extensive experimentation was required to obtain a growing method and nutrient composition which would provide satisfactory growth of maize plants, and a brief report of the problems which were encountered and efforts to overcome these problems is given below.

The ion-exchange capacity of vermiculite makes it impractical for use in ^{15}N studies because of the difficulty of changing rapidly from a ^{14}N nutrient source to a ^{15}N nutrient source. Attempts were therefore made to grow maize hydroponically in 8ℓ asbestos pots filled with an aerated standard 2mM N Long Ashton nutrient solution, with each pot containing 2 plants. This proved to be unsuccesful because the nitrate-fed plants became chlorotic, with new leaves being striated, after 3-5 days in the hydroponic solution. Attempts to remedy this included making the following changes in the culture conditions:-

(1) The macronutrient concentration was doubled.

(2) The micronutrient concentration was doubled, and tripled.

(3) The nitrogen concentration in nitrate-containing nutrient solutions was increased to 10mM NO_3^-.

(4) The iron content, as FeEDTA, was increased two- and three-fold. Iron deficiency in nitrate-fed plants has been reported by Nelson & Selby (1974) with *Picea sitchensis* and *Pinus sylvestris*, Colgrove & Roberts (1956) with *Rhododendron*, and in young leaves of cranberry by Ingestad (1973).

(5) Seedlings were planted into Hoagland's No. 1 solution (Hoagland & Arnon, 1938), to ascertain whether nutrient imbalance was occurring with the standard and modified Long Ashton nutrient solutions. Nitrate levels of 7mM and 14mM were used.

None of the above-mentioned modifications brought any improvement in the growth of nitrate-fed plants. Further alterations in the culture procedure were made, each one bringing about a decrease in the leaf striation:-

(6) The variety of maize used was changed from K64R, a slow-growing variety, to the fast-growing R201 variety.

(7) Iron availability was increased by augmenting the recommended quantity of FeEDTA with 5 times as much iron in the form of ferric citrate. The pH of the nutrient solution was lowered to pH5 (Hewitt, 1966). Smith _et al_ (1983) found that a ferric citrate supplement gave increased yields in nitrate-fed maize grown in nutrient solutions.

(8) The growth technique was changed from the aerated hydroponic system to a gravel culture system. Aeration of the roots was improved by planting seedlings into pots filled with 9mm diameter builder's stone, with nutrients being continuously cycled through the rooting medium from a 8ℓ reservoir, which was located below the plant pot, using an air lift. The flow rate of the nutrient solution through the rooting medium was approximately 100ml min^{-1}.

The final methods used for the growth of maize plants were as follows:-

Material for ^{15}N feeding and enzyme studies was potted in 2ℓ pots filled with 9mm diameter builder's stone, with each 8ℓ nutrient reservoir supplying 2 plants, except in one $^{15}NO_3^-$ feeding experiment where the aerated hydroponic culture method was used. These plants were grown in a Conviron growth chamber, under the environmental conditions specified at the beginning of this Section.

Plants for long-term growth experiments were potted in 8ℓ pots filled with builder's stone, with each 8ℓ nutrient reservoir supplying one plant (as illustrated in Plate 1), and were grown under greenhouse conditions.

Table 1.

Amounts of macronutrients used to make 10ℓ of nutrient solution (g), for nitrate, ammonium, and mixed N feeding.

Macronutrient	2mM nitrate	2mM ammonium	1mM nitrate + 1mM ammonium
$Na_2HPO_4.12H_2O$	1.42	1.42	1.42
$MgSO_4.7H_2O$	2.48	2.48	2.48
$CaCl_2.2H_2O$	3.36	3.36	3.36
K_2SO_4	1.58	1.58	1.58
KNO_3	2.02	1.01	----
NH_4Cl	----	0.53	1.07

Table 2.

Amounts of micronutrients used in 10ℓ of nutrient solution (mg).

Micronutrient	Mass
HBO_3	85.8
$MnSO_4.4H_2O$	46.4
$ZnSO_4.7H_2O$	6.6
$CuSO_4.5H_2O$	2.4
$Na_2MoO_4.2H_2O$	0.6
$[CH_2N(CH_2COO)_2]_2FeNa_2$ (FeEDTA)	330
$C_6H_5O_7Fe.5H_2O$ (FeCitrate)	1650

Both Tables from Hewitt, 1966, but Table 2 includes a ferric citrate supplement.

Plate 1.

Illustration of the technique used for the cultivation of plant material. An air-lift was used to re-cycle the nutrient solution through the 9mm diameter builder's stone, serving as the rooting medium, from the 8ℓ nutrient reservoir below.

3.3. Prevention of Nitrification.

In order that any benefits obtainable by field application of ammonium fertilizers can be realised it is essential to prevent or retard the bacterial nitrification of ammonium, this subject having been discussed in Section 2.5.4. It is also vital to control nitrification when conducting laboratory experiments aimed at investigating the effects of ammonium and nitrate nutrition on plant growth, and the experiments conducted in this context will be discussed here.

Preliminary experiments were performed with maize grown under greenhouse conditions to test the suitability of the nitrification inhibitor nitrapyrin for use in ^{15}N feeding experiments and in growth studies. After 3 days in nutrient solutions containing 5ppm N-Serve (supplied as an emulsifiable concentrate at 240g ℓ^{-1} by Dow Chemical Company Ltd., King's Lynn, Norfolk PE30 2JD, U.K.), there were signs of toxicity. This manifested itself as a bleeching of the developing leaves, beginning at the leaf veins and spreading throughout the leaf so that within a few days the entire leaf was white, with no visible trace of natural pigmentation. The effect was independent of the nitrogen source, plants fed with nitrate, or ammonium, or nitrate + ammonium suffering equally. Nitrapyrin was therefore ruled out as a nitrification inhibitor for N feeding studies as its physiological effects are unknown; even if lower N-Serve concentrations did not produce visible signs of toxicity the effects on plant metabolism would remain unknown. Nitrapyrin toxicity has been reported elsewhere with other species, eg. soybeans (Morris _et al_, 1980) and sunflower (Maftoun _et al_, 1982).

When the nutrient solutions were changed every second day, no nitrate could be detected in the external medium, and only trace amounts were found in the xylem exudate and plant extracts with ammonium feeding. Analysis of the nutrient solutions showed that they did not become devoid of nitrogen before being renewed. This practice was therefore adopted for the cultivation of all experimental plant material.

3.4. Feeding with ^{15}N-Containing Nutrient Solutions.

The day before ^{15}N was administered to the maize plants, the nutrient solution in each reservoir was renewed. On the day of the ^{15}N feed four robust 20-day-old plants were selected and the nutrient reservoirs were replaced with ^{15}N nutrient solutions, after first flushing the gravel-filled plant pots with deionized water to wash out the ^{14}N nutrient solutions. The ^{15}N solutions were the modified Long Ashton nutrient solutions of Tables 1&2 with nitrogen as 2mM 99A%E ^{15}N KNO_3, or 2mM 99A%E ^{15}N NH_4Cl (BOC-Prochem, Deer Park Road, London, U.K.), or a 2mM 1:1 mixture of both nitrogen sources. The changeover from ^{14}N feeding to ^{15}N feeding took under 30s for each plant.

Feeding with ^{15}N nutrient solutions was always commenced 2h after the beginning of the light period.

3.5. Harvesting of ^{15}N-Fed Plants.

The ^{15}N-fed plants were harvested 4h and 8h after the commencement of ^{15}N feeding. Duplicate plants were separated into root, leaf base + stem (mainly leaf base as the stem of young maize is very small), and leaf blade fractions. Each fraction was immediately weighed, the roots first being washed with distilled water and blotted dry.

3.6. Separation of Nitrogen Fractions and Preparation for ^{15}N Determinations.

After weighing, the plant material fractions were immediately killed in liquid nitrogen. The material was then homogenized with an Ultra-Turrax homogenizer in 80% ethanol at 0°C (1g fresh weight plant material : 25ml ethanol). Extraction was continued at 0°C for 24h with occasional gentle agitation of the extract. The homogenate was filtered through Whatman No.4 filter paper and the alcohol-soluble fraction evaporated under an air stream to a volume of 25ml. This was filtered again and the precipitate added to the original precipitate to form the bound N fraction. The soluble fraction was again reduced to 25ml under an air stream and aliquots taken for amino acid analysis, free nitrate, and free ammonium determinations, as described in Section 3.7.

The filtrate from each extract was divided into 2 equal portions. Each portion was steam distilled in a Markham semi-micro steam distillation unit with MgO (previously heated to 600°C for 4h to drive off CO_2), to remove the free ammonium, which was collected in 0.02M HCl (Barker, 1974). The amount of ammonia collected was estimated by back-titration with Tashiro's indicator (0.125g methyl red + 0.083g methylene blue, in 100ml 100% ethanol). This was then re-acidified with 2 drops of 0.02M HCl (to prevent NH_3 loss) before volume reduction using an air stream and heating, and final ^{15}N determination. Tests showed that the amides, glutamine and asparagine, did not undergo alkaline hydrolysis and release of ammonia if the distillation period was no longer than 3 minutes.

The separate distillates from each extract were combined to save labour before further processing. The remainder of the soluble nitrogen fractions was filtered through Whatman No.4 filter paper to remove the MgO precipitate and neutralised with 2M HCl before being concentrated under an air stream to a volume of ±10ml. The nitrate and organic nitrogen fractions were separated on 3cm x 1cm ion exchange columns of Dowex 50W-X8 standard H^+ resin, 100-200 mesh particle size (BDH, Poole, Dorset, England, U.K.), using a modification of the method of Atkins & Canvin (1971). The nitrate fraction was eluted from the columns with 50ml distilled water and the soluble organic N fraction with 100ml 2M HCl. Both fractions were reduced to a suitable volume (20ml for the nitrate fraction and ±5ml for the soluble organic fraction) by warming under an air stream.

Duplicate aliquots of the nitrate fraction (5ml or 10ml, depending on the amount of nitrate present) were distilled in a Markham semi-micro steam distillation unit with 0.3g Devarda's alloy (to reduce the nitrate to ammonium) and 0.2g MgO which had been heated as previously described. The released ammonia was collected in 0.05M HCl, quantified, and prepared for ^{15}N determination as described for the free ammonium fraction.

The soluble organic N fraction was digested in a micro-Kjeldahl flask for 2h (after clearing) with 3ml N-free concentrated sulphuric acid and a catalyst tablet containing 1g Na_2SO_4 and the equivalent of 0.1g Hg (BDH). The volumes were then made up to 50ml with distilled water, and 5ml or 10ml duplicate aliquots (depending on the amount of nitrogen present) were steam distilled in a Markham semi-micro distillation unit. Alkaline conditions were obtained by the addition of 10ml 50% w/v NaOH containing 2.5% sodium thiosulphate, to prevent loss of ammonia through its binding to the mercuric oxide precipitate which formed on the addition of the NaOH. The liberated ammonia was collected and prepared for ^{15}N analysis as described for the free ammonium fraction.

The bound N fraction was dried at 80°C for 24h and weighed. Aliquots of 0.1g were digested with 3ml N-free conc. sulphuric acid and a mercury catalyst tablet. The digestion was carried out for 2h (after clearing) in 80ml Pyrex tubes in an aluminium heating block at 375°C, as described by Nelson & Sommers (1972). Samples were then prepared for ^{15}N determination by the distillation method described for the soluble organic fraction.

The methods of separation and processing of plant extracts from ^{15}N feeding experiments are summarised in Figure 1.

3.7. Quantitative Determination of Nitrogen in Plant Extracts.

3.7.1. Amino Acid Analysis.

Amino acid analyses on plant extracts were performed on a Beckman 120C automatic amino acid analyser (Beckman Instruments, Inc., Fullerton, CA 92634, U.S.A.) using the method of Kedenburg (1971). Samples were loaded onto a 22cm x 9mm column of Beckman UR-30 spherical ion exchange resin. Eluting sequentially with 3 lithium citrate buffers permitted separation and quantification of the amino acids and the amides, glutamine and asparagine. The sequence of elution was:-

Buffer	A	pH 2.83	pumping time	84min.	flow rate 1ml min^{-1}
"	B	pH 3.70	" "	31 "	" " " "
"	C	pH 3.75	" "	175 "	" " " "

All buffers were supplied by Beckman Instruments Inc.

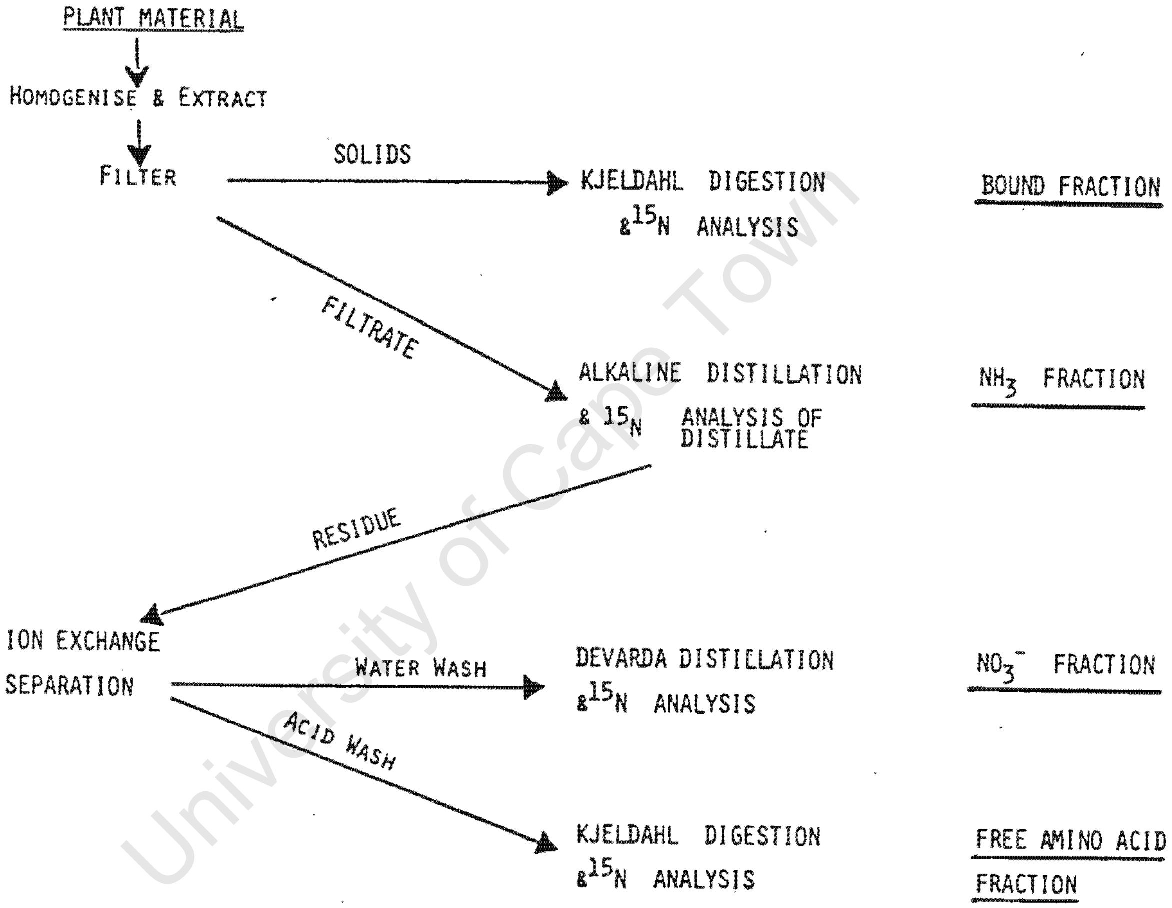


Figure 1.

Separation and processing of plant extracts for ^{15}N determinations after feeding with a nutrient solution containing labelled nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM 1:1 mixture of both.

The separated components were mixed automatically with triketohydrindene hydrate (ninhydrin) and passed through a hot water bath (100°C) to promote the amino acid-ninhydrin reaction. The mixture was then passed through 2 photocells. The first of these measured the optical density at 450nm to detect proline, and the second at 540nm to estimate the development of the colour produced by the other amino acid-ninhydrin reaction products. The optical densities were recorded by a Beckman 125 digital integrator and its logarithmic recorder. The readings from the integrator were used with a specific conversion constant, obtained for each amino acid by calibration runs, to calculate quantitative values for each amino acid. Internal standards were always used to compensate for the decay of ninhydrin with time.

3.7.2. Determination of Free Nitrate.

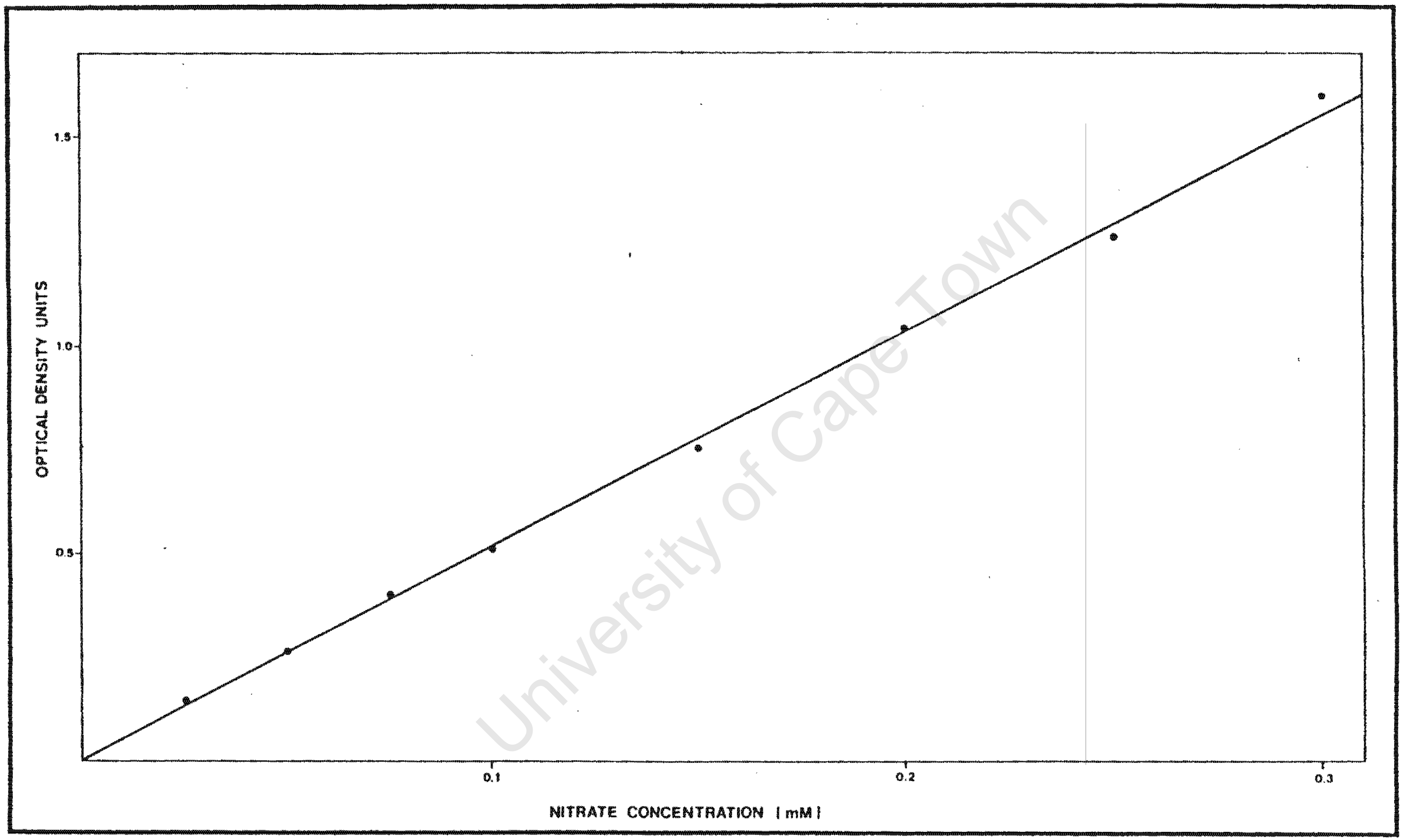
Free nitrate in plant tissues, xylem sap, and nutrient solutions was determined colorimetrically using Szechrome NAS reagent (diphenylamine sulphonic acid chromogene, supplied by Yedetek Ltd., Rimon 10, Omer 84065, Israel) in a 1:1 conc. sulphuric acid:orthophosphoric acid mixture. To 0.5ml of diluted sample was added 2.5ml reagent. After thorough mixing and 30min. of colour development the absorbance at 570nm was measured on a Beckman Model 42 Spectrophotometer. Each sample was tested in triplicate, the readings being averaged before calculating the nitrate concentration. Because of variation between batches of the reagent, and deterioration of the reagent with age, standards were run on each occasion the reagent was used. A typical standard curve is shown in Figure 2.

The large dilution required to perform nitrate determinations on samples from plant extracts, xylem sap collections, and nutrient solutions helped to eliminate possible interference effects from chloride and other ions.

3.7.3. Measurement of Free Ammonium.

Free ammonium in nutrient solutions was measured with Nessler Reagent (AOAC, 1965), using 0.5ml diluted sample and 2.5ml reagent. A Beckman Model 42 Spectrophotometer was used to measure absorbance at 420nm exactly 1min. after the sample and reagent were mixed. The triplicate results for each sample were averaged, and compared against standards run each time the reagent was used, to obtain the ammonium concentrations. A typical standard curve is shown in Figure 3.

The free ammonium concentrations of xylem sap and plant extracts were obtained from the automatic amino acid analyser readout, as described in Section 3.7.1.



<u>Figure 2.</u> Standard curve for the colorimetric determination of nitrate concentration by the use of Szechrome NAS Reagent.

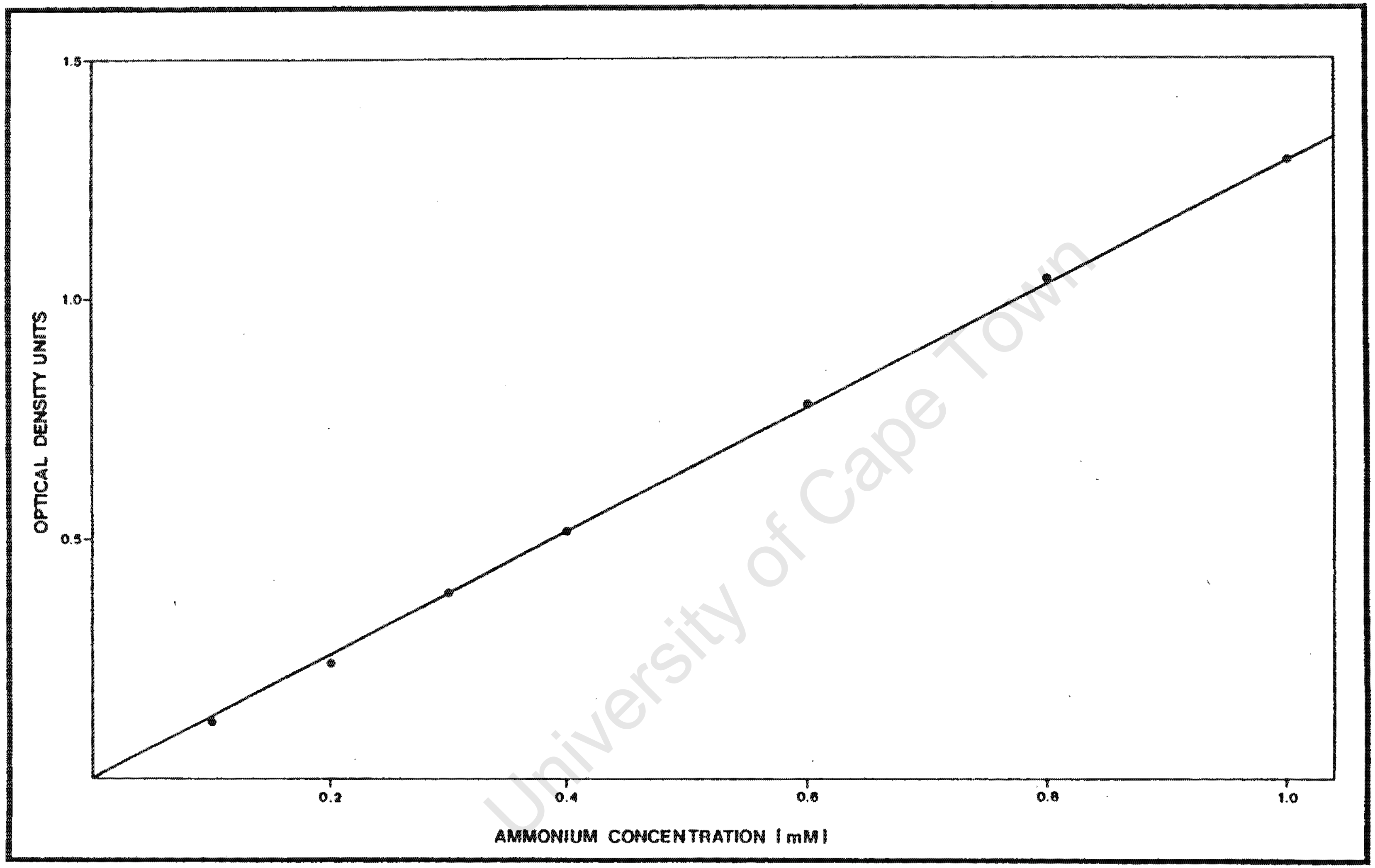


Figure 3. Standard curve for the colorimetric determination of ammonium concentration by the use of Nessler Reagent.

3.8. Determination of ^{15}N Enrichments and Calculation of Results.

3.8.1. Sample Preparation.

The procedure was based on the method of Faust (1967) with an alkaline sodium hypobromite solution being used as the oxidant. By a process of freezing and thawing under vacuum, dissolved nitrogen was excluded from the sample, then dinitrogen was released from the sample according to the reaction:-

$$2NH_3 + 3NaOBr \longrightarrow N_2 + 3H_2O + 3NaBr \quad (7)$$

Before the reaction occurred, adsorbed carbon dioxide and surface water were driven from the glass discharge tube with a hot flame. On reacting, the liberated nitrogen gas filled the discharge tube, which was then sealed with a hot flame.

The vacuum system comprised an Edwards high vacuum pump (Edwards High Vacuum, Crawley, England, U.K.) in series with a mercury diffusion pump, resulting in a final pressure of 1.0Pa. Two liquid nitrogen cold fingers were used to reduce the vapour pressure of the system.

Between 15µg and 30ug of nitrogen needed to be reacted to obtain a final pressure of $0.4-0.7 \times 10^2$ Pa in the discharge tube, this being the pressure required for an accurate determination of the ^{15}N enrichment of the sample.

The apparatus used for the final sample preparation is shown in Figure 4.

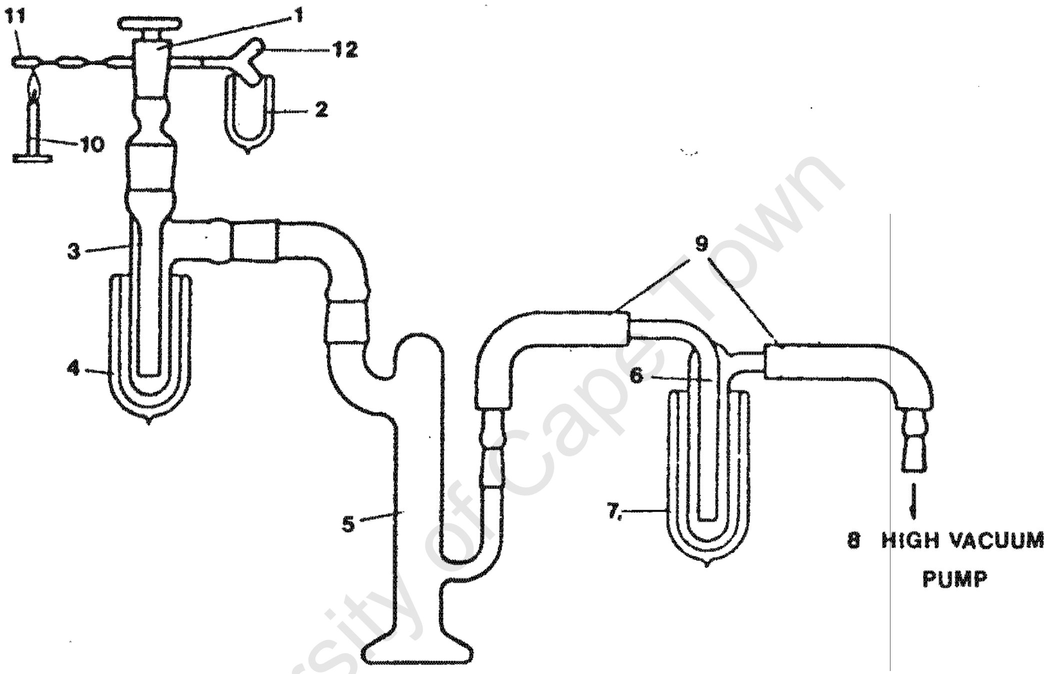


Figure 4.

Apparatus used for the preparation of N$_2$ gas for ^{15}N-determination (from Faust, 1967). (1) Rotaflo valve (2) Liquid nitrogen container (3) Cold finger (4) Liquid nitrogen container (5) Mercury diffusion pump (6) Cold finger (7) Liquid nitrogen container (8) Electric high vacuum pump (9) Rubber tubing (10) Bunsen burner (11) Discharge tube (12) Reaction vessel containing sample and sodium hypobromite in separate arms.

3.8.2. ^{15}N Determination and Calculation of Results.

A Statron NOI-4 molecular emission spectrometer (Packard Instruments, S.A., Zurich, Switzerland) was used to determine ^{15}N enrichments. The discharge tubes were fixed between the terminals of a Hg-4 oscillator which generated a frequency of 27.12 MHz. This excited the N_2 gas in the tube and caused it to emit light, the wavelengths being characteristic of the $^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$ molecules. The outgoing light was chopped at 333.3 Hz and focused onto the entrance slit of a SPM 1 monochromator. It was spectrally dispersed by a NaCl prism, then passed into a photomultiplier, which produced an a.c. voltage proportional to the intensity of the chopped light. This a.c. signal was amplified and rectified before being pen-recorded on a Unicorder U-125M chart recorder (Nippon Denshi Kagaku Co. Ltd., Japan). Figure 5 shows a typical trace with good separation of the peaks associated with the various dinitrogen molecules.

Enrichments were calculated using the formula:-

$$\%E = \frac{1}{2(A/B \times V_b/V_a) + 1} \qquad (8)$$

where A and B represent the headbands of the $^{14}N^{14}N$ and $^{14}N^{15}N$ molecules respectively (Figure 4), and V_a and V_b are the amplifier gain settings at which the headbands were recorded. When the enrichment was over 50A%E the formula used was:-

$$\%E = \frac{1}{1/2(B/C) + 1} \qquad (9)$$

where B and C are the headbands of the $^{14}N^{15}N$ and $^{15}N^{15}N$ molecules respectively.

A standard curve was drawn up and used to correct the enrichments (Figure 6). The percentage enrichment above the natural occurrence of ^{15} (A%E) was obtained by subtracting the natural abundance of the ^{15}N isotope (0.37%) from the corrected percentage enrichment.

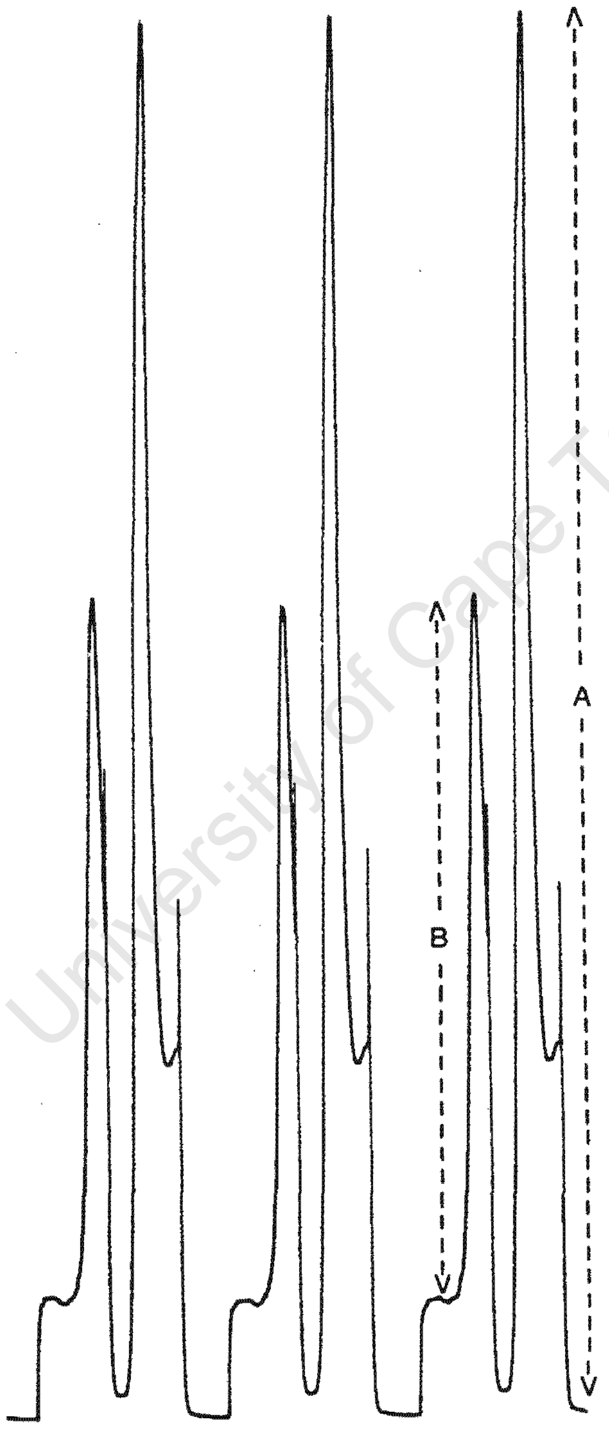


Figure 5. A typical trace showing good separation of the two nitrogen molecules : $^{14}N^{15}N$ and $^{14}N^{14}N$, where A and B represent the peak heights of the $^{14}N^{15}N$ and $^{14}N^{14}N$ bandheads respectively. The peak corresponding to the $^{15}N^{15}N$ molecule is very small, due to the low A%E of the particular sample used to make this trace.

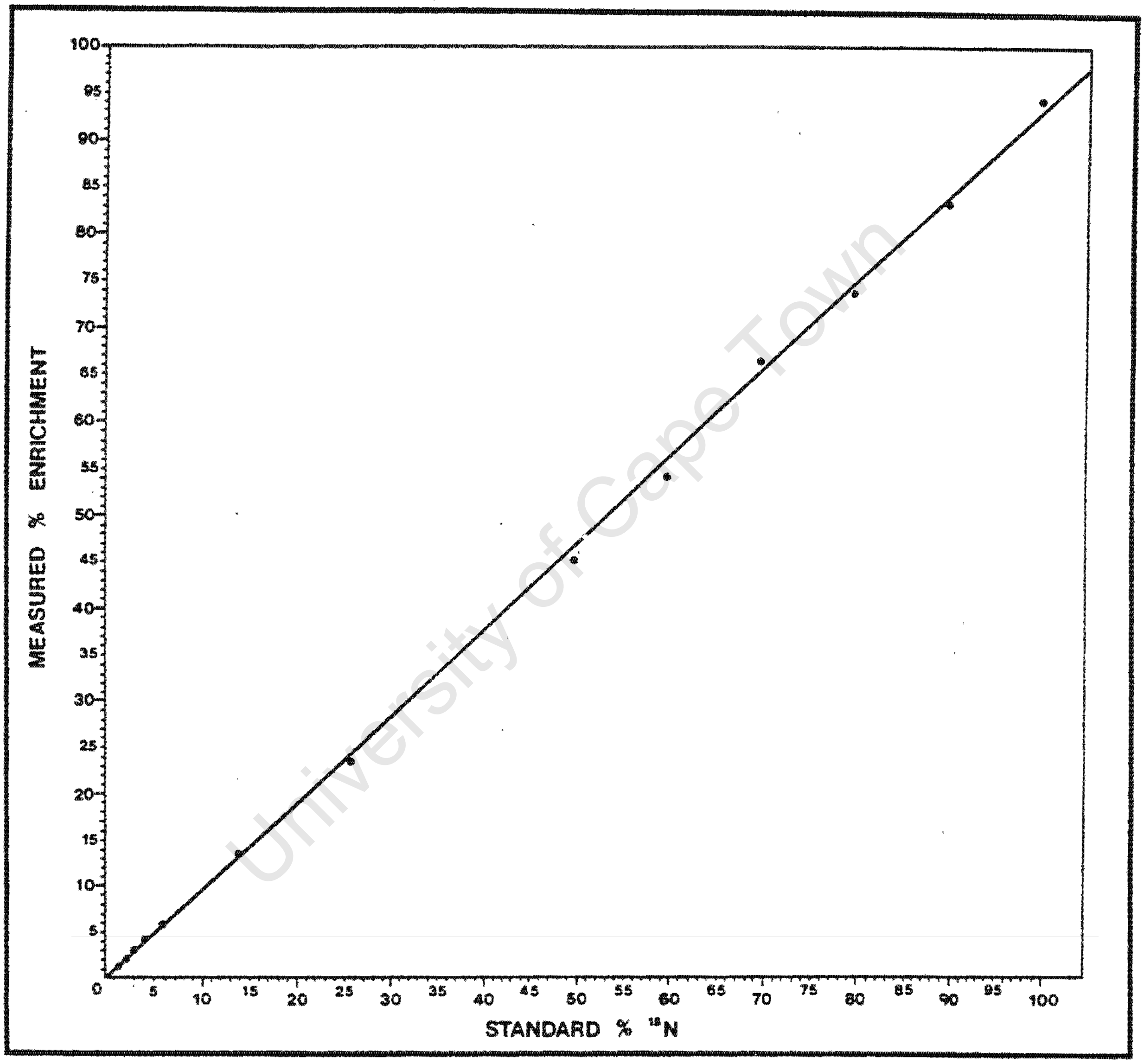


Figure 6. Standard curve for correction of ^{15}N enrichments determined with the Statron molecular emission spectrometer.

3.9. Xylem Sap Collection and Analysis.

Twenty-day-old plants were fed with the ^{15}N isotope as described in Section 3.4. After 3.5 and 7.5 hours of ^{15}N feeding the plants were de-topped below the insertion of the first leaf blade, the cut surface washed with distilled water, and blotted dry. On each occasion the sap exuded by 2 plants by root pressure was collected for a period of 1h, using a Pasteur pipette. The sap exuded originated in the xylem vessels (Pate, 1962), which are the main pathway for the transport of nitrogenous compounds from the roots (Pate, 1973). Collecting for no more than 1h prevents changes in sap composition caused by starvation effects (Koster, 1963; Pate & Wallace, 1964).

Samples were frozen until quantitative determinations of the nitrate, ammonium, and amino acid fractions were made (as described in Sections 3.7.1. - 3.7.3.) using diluted sap.

3.10. Enzyme Assays.

Assays for nitrate reductase (NR) and glutamine synthetase (GS) were performed on maize leaves and roots, using *in vitro* techniques.

3.10.1. Enzyme Extraction.

The extraction mixture described by Lewis *et al* (1982b), for the extraction of nitrate reductase, was used for the extraction of NR and GS. It contained 0.1M phosphate buffer (pH 7.5), 1mM disodium ethylenediaminetetraacetate (EDTA), 1mM dithiothtreitol, and 2.5% casein to stabilize the post-extraction decrease in NR activity reported by Wallace (1973 & 1975), Schrader _et al_ (1974), and Robin (1979) in maize and by other workers with differing species. Casein may stabilize the NR by protecting it from a proteolytic or inactivating enzyme (Schrader _et al_, 1974; Wallace, 1975; and Robin, 1979). The addition of casein to the extraction medium has also been reported to stabilize GS (Streit & Feller, 1983). In addition, 1% polyvinylpyrrolidone (Polyclar) was added to the extraction medium immediately prior to the enzyme extraction. This was found to enhance both NR and GS activity levels in preliminary experiments, and the results of work by Kaiser & Lewis (1984) reveal this yielded a 50% increase in the activities of both enzymes in _Helianthus_.

The enzymes were extracted from leaves and roots by grinding 1g of plant material and 8ml of extraction medium (and a pinch of acid-washed sand) with a chilled mortar and pestle (less than 5°C). The homogenate was filtered through a double layer of muslin and centrifuged at 2000g for 5 minutes in a refrigerated Beckman JU-21 centrifuge, the temperature being held at 2°C during this stage. The supernatent was decanted off and stored on ice, to be used as the crude enzyme source.

3.10.2. Incubation of Enzymes.

3.10.2.1. Nitrate Reductase.

The assay mixture was based on that of Bar Akiva & Sagiv (1967) :-

Ingredient	Volume (ml)
0.1M potassium nitrate	0.2
distilled water	1.4
1M phosphate buffer (pH 7.5)	0.1
NADH (1g ml^{-1})	0.1
Crude enzyme extract	0.2
Total Volume	2.0

The reaction was started by the addition of the crude extract to the incubation medium and allowed to proceed for 15min. in a 27°C water bath. The addition of 1.0ml of 1% (w/v) sulphanilimide in 1M HCl, followed by 1.0ml 0.01% (w/v) N-(1-napthyl)ethylenediamine hydrochloride (NED) solution, terminated the reaction and produced colour development for the colorimetric determination of the product nitrite. After standing for 15 minutes (to allow colour development) the assay samples were centrifuged at 2000g for 5 minutes, before reading the optical densities at 540nm on a Beckman Model 42 Spectrophotometer. In all experiments triplicate aliquots of each extract were assayed, and the resulting readings averaged. The actual amount of nitrite produced was calculated from the optical density readings by using the standard curve for nitrite shown in Figure 7.

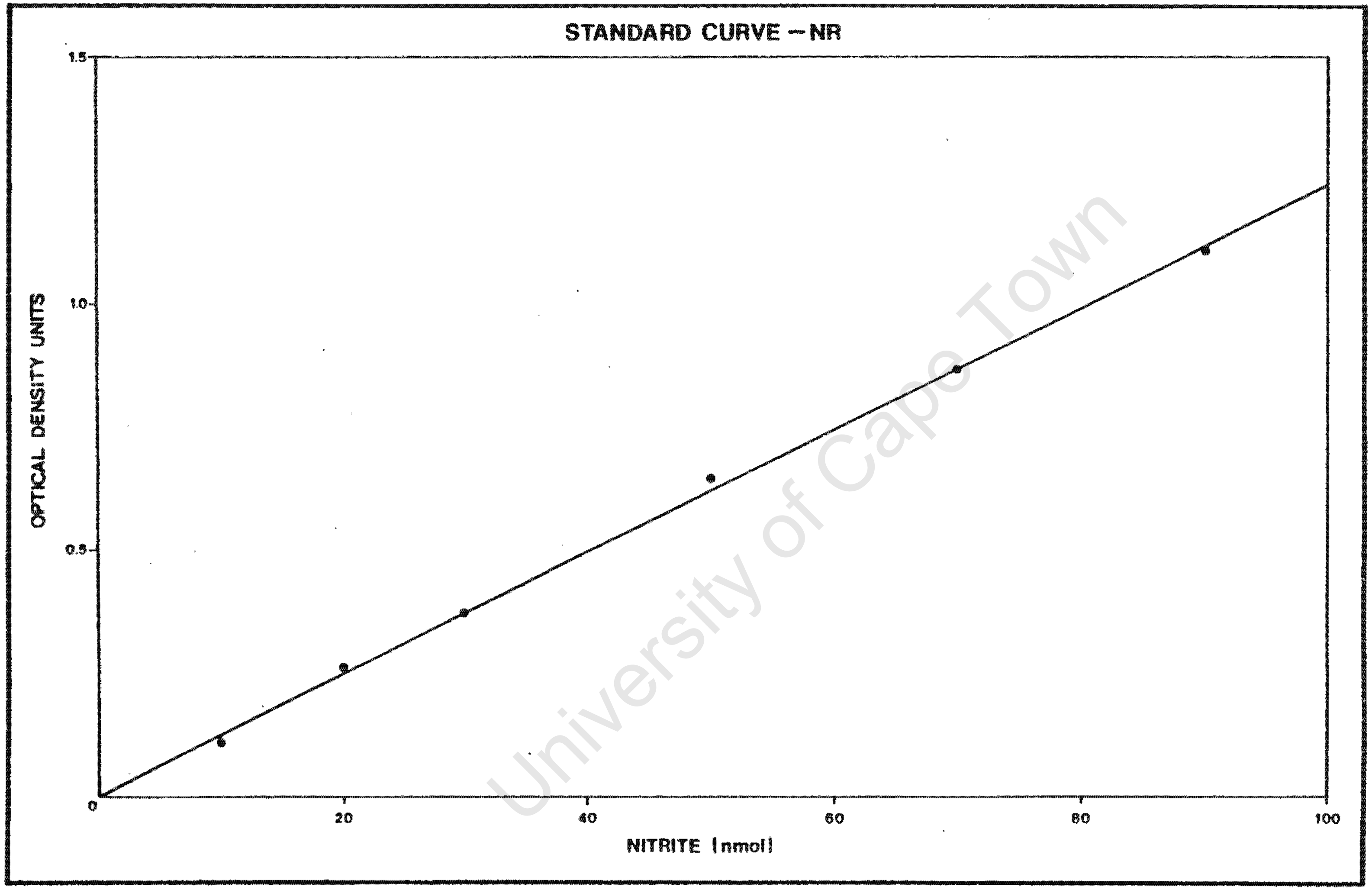
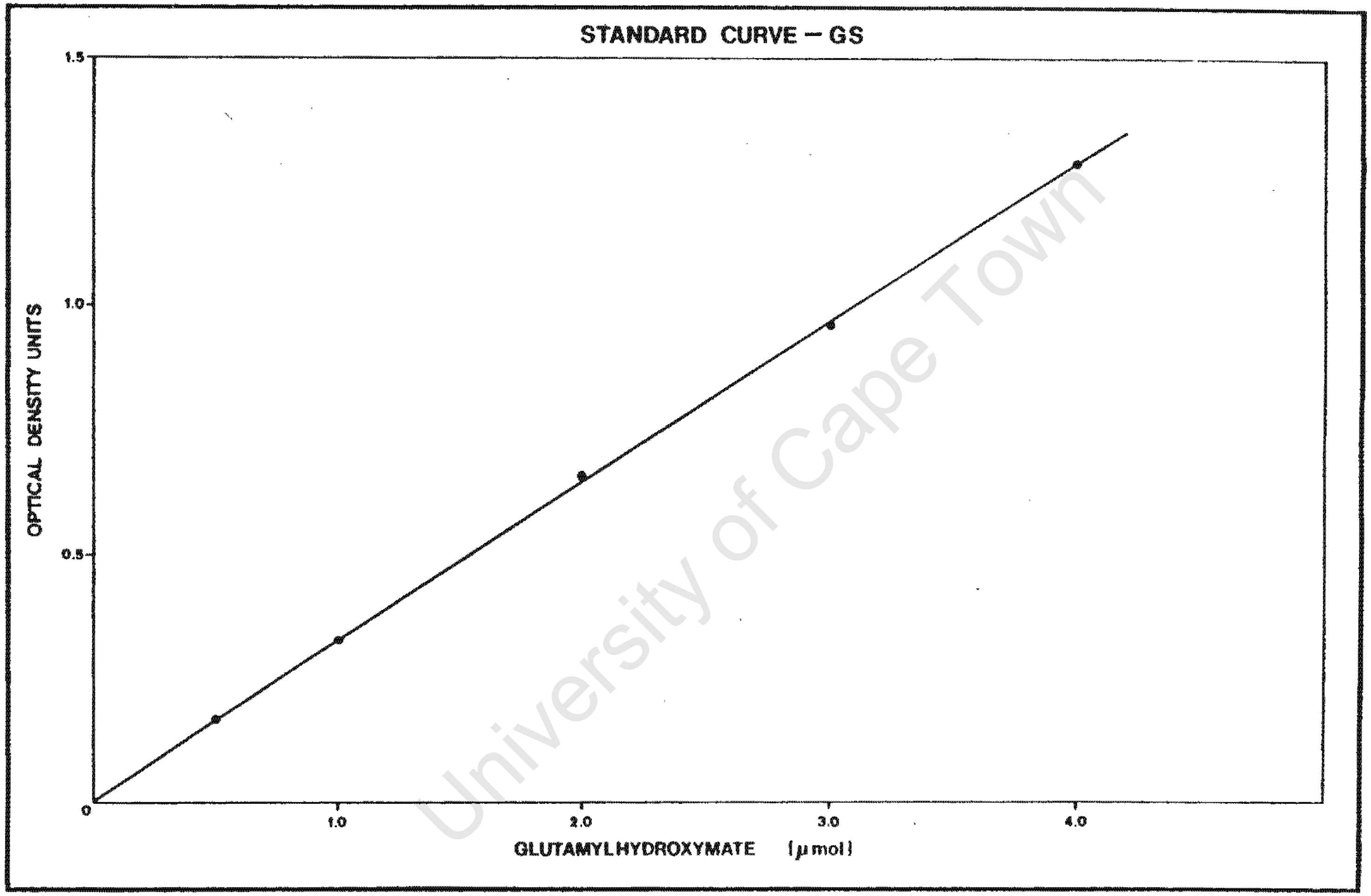


Figure 7. Standard curve for the determination of nitrite produced during the incubation period of the *in vitro* nitrate reductase assay.

3.10.2.2. Glutamine Synthetase.

The reaction mixture contained 36umol adenosine triphosphate, 90umol magnesium sulphate, 12umol hydroxylamine, 184umol L-glutamic acid, and 100umol imidazole hydrochloric acid. The addition of 0.2ml of crude extract brought the final reaction mixture to 2.0ml, at pH 7.2 (Rhodes *et al*, 1975). The reaction began with the addition of the enzyme extract to the incubation medium, and ran for 20 minutes at 27°C before termination by the addition of 1ml of a mixture of 10g trichloracetic acid and 8g ferric chloride (anhydrous) in 250ml 0.5M HCl (Ferguson & Sims, 1971). The reaction mixture was centrifuged at 2000g for 5 minutes before the optical density of each sample was read at 500nm with a Beckman Model 42 spectrophometer. Glutamine synthetase activity was determined from the amount of hydroximate formed in the reaction:-
L-glutamine + hydroxylamine + ATP ----> glutamylhydroxymate + ammonium (10)
(Hubbard & Stadtman, 1967). Standards containing the complete reaction mixture with known amounts of L-glutamic acid and monohydroximate were used to quantify the experimental absorbance readings in terms of product formation. The standard curve for this mixture is shown in Figure 8.

In each experiment triplicate aliquots of the crude extracts were assayed, and the results averaged.



<u>Figure 8.</u> Standard curve for the determination of glutamylhydroximate accumulated during the *in vitro* glutamine synthetase assay.

3.11. Growth Experiments and Monitoring of Nutrient Solutions.

Maize plants (var. R201) were grown under greenhouse conditions until the onset of flowering in nutrient solutions containing nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM N 1:1 mixture of both (Section 3.1). Five replicate plants were used for each nitrogen treatment. Calcium carbonate was added to the pots containing ammonium, and ammonium + nitrate, at 2g l^{-1}, after a preliminary experiment revealed that the nutrient solution pH could otherwise drop to as low as pH 3.7 before it was renewed (ie. after 2 days).

The nutrient solution pH was measured immediately before the solutions were renewed. The pH of the nitrate-containing solutions was in the range pH 6.4-7.0, that of ammonium-containing solutions was pH 6.0-6.6, whilst those nutrient solutions containing both nitrate and ammonium had a pH range of pH 6.3-6.9.

All pH measurements were made with an Orion Research Model 701 Digital pH meter using an Orion Research Ross combination electrode Model 81-02.

Testing the nutrient solutions with Szechrome NAS and Nessler's reagents (Sections 3.7.2. & 3.7.3.) showed that the nutrient solutions did not become devoid of nitrate or ammonium before they were renewed. No nitrate was detected in the ammonium feeding solutions.

When the plants were 48 days old (41 days after transfer to N-containing nutrient solutions) the first tassels appeared. The plants were divided into leaf blade, leaf base + stem, and root fractions. After washing the roots with distilled water and blotting dry with absorbent tissue, the fractions were weighed and oven-dried at 80°C for 48h. They were then weighed to obtain the dry masses, before milling in a Wiley Mill Standard Model No.3 (Arthur H. Thomas Co., Philadelphia, U.S.A.) with a 0.15mm mesh. A 0.2g sub-sample of each milled sample was taken for Kjeldahl digestion and steam distillation (as described in Section 3.6.) to determine the total organic nitrogen content (ie. amino acid + protein forms) of the various plant parts.

During the harvesting process two of the ammonium-fed plants were de-topped and xylem sap collected for 1h as described in Section 3.9. The exudate was tested for nitrate as described in Section 3.7.2. Only trace quantities of nitrate were detected.

CHAPTER 4

RESULTS

4.1. ANALYSIS OF XYLEM EXUDATE.

Xylem sap was collected from plants fed with a modified Long Ashton (Hewitt, 1966) nutrient solution containing nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM N 1:1 mixture of both. The sap was collected and analysed as described in Section 3.

The use of xylem sap studies in work on plant nutrition has certain inherent drawbacks. The flow rate is not the same as in whole plants, where the transpiration rate affects the upward movement of the xylem sap, but the relative concentrations of the nitrogen forms can give an accurate picture of the forms of nitrogen being exported by the roots. Shaner & Boyer's work (1976) suggests that the amount of N exported from the roots is unaffected by changes of water movement through the xylem, because their attempts to alter the nitrate flux by varying the transpiration rates were unsuccessful due to a relatively constant rate of nitrate delivery to the xylem as transpiration rates were decreased.

The possibility exists of contamination from surrounding tissues, eg phloem (Pate, 1976), or from stem tissue damaged by cutting. Ferguson (1980) found that cellular components from damaged cells did not contaminate sap obtained by vacuum extraction from Chinese goosberry, and it is likely that this is also the case with bleeding sap from maize. This probability is also supported by the close replication obtained by the analysis of exudate from duplicate plants during this project.

Xylem sap analysis may not show what is actually reaching the leaves. It has been found that some lateral movement of amino acids into stems and phloem can occur. Arginine may be absorbed by stems (70% of that exported by the root), asparagine, glutamine, serine, and valine may be absorbed effectively (40-60%), while aspartic and glutamic acids are ineffectively absorbed (McNeil et al, 1978; Pate, 1976). It has been suggested (Pate, 1980) that those amino acids absorbed efficiently by stems may be used in storage or in transfer from xylem to phloem, whereas those which are not absorbed so efficiently may be particularly important in leaf metabolism.

It is generally accepted, however, that xylem exudate can be used to gain an insight into the main nitrogen compounds exported by the root, provided that only the relative concentrations of nitrogenous compounds within the sap are considered.

4.1.1. Sap from ^{15}N Nitrate-fed Plants.

The composition of the sap from plants fed with nitrogen in the $^{15}NO_3^-$ form is given in Tables 3 & 4.

From Table 3 it can be seen that the nitrate concentration was between 14mM and 15mM, which is approximately 7-8 times the external nitrate concentration.

Bowling et al (1966) reported that some plants can accumulate nitrate from the external medium against a concentration gradient. Lewis et al (1982a) found that, at the 2mM feeding level, nitrate was concentrated to 27mM in barley xylem sap.

TABLE 3

Nitrogen content of xylem exudate of 20-day-old maize plants fed 2mM ^{15}N nitrate for 4h and 8h. Results from duplicate collections (performed simultaneously) were averaged, and the range is given.

Sap Fraction	Harvest	^{15}N content (μmoles ml^{-1})	Sap fraction ^{15}N as % of total ^{15}N	Sap fraction total N (μmoles ml^{-1})	Sap fraction N as % Total N
ammonium	4h	0.11 ± 0.01	3.5	1.68 ± 0.07	6.5
	8h	0.06 *	1.6	0.74 ± 0.06	3.0
nitrate	4h	2.81 ± 0.50	92.9	15.20 ± 1.90	58.6
	8h	3.34 ± 0.48	92.5	14.40 ± 1.75	59.5
amino nitrogen	4h	0.11 ± 0.03	3.5	9.07 ± 1.23	35.0
	8h	0.21 ± 0.02	5.9	9.06 ± 1.07	37.5

* Insufficient nitrogen for duplicate determinations.

Table 4.

Concentration (μmol ml^{-1}) of nitrogen in organic compounds in xylem sap from 20-day-old maize fed nitrogen as 2mM $^{15}NO_3^-$, or 2mM $^{15}NH_4^+$, or a 2mM N 1:1 mixture of both. Averaged from 2 plants, and collected at each of 4h and 8h ^{15}N feeding periods.

	Nitrogen Source and N Conc. (μmol. ml^{-1})					
	Nitrate		Nitrate + Ammonium		Ammonium	
Compound	4h	8h	4h	8h	4h	8h
Aspartic acid	0.069	0.140	0.156	0.203	0.158	0.051
Threonine	0.226	0.267	0.278	0.351	0.190	0.304
Serine	0.758	0.668	1.074	1.225	0.586	0.739
Asparagine	1.234	1.298	2.560	2.855	10.070	11.822
Glutamic acid	0.240	0.221	3.179	0.364	0.313	0.071
Glutamine	4.036	3.028	14.487	13.750	8.688	5.578
Glycine	0.213	0.269	0.252	0.257	0.338	0.182
Alanine	0.749	0.663	1.803	1.606	1.041	0.916
Valine	0.103	0.079	0.124	0.144	0.083	0.134
Methionine	0.010	Trace	0.023	0.041	Trace	Trace
Isoleucine	0.026	0.037	0.025	0.064	0.022	0.036
Leucine	0.031	0.037	0.051	0.056	0.033	0.038
Tyrosine	0.029	0.044	0.034	0.065	0.013	0.016
Phenylalanine	Trace	Trace	0.023	0.017	0.015	0.009
Lysine	0.435	0.542	0.326	0.760	0.606	0.682
Hystidine	0.164	0.369	0.318	0.476	0.434	0.383
Arginine	0.684	1.264	0.712	1.150	0.584	0.778
γ-Amino Butyric Acid	0.069	0.139	0.093	0.237	Trace	Trace
Total	9.076	9.065	25.519	23.621	23.174	21.739

Note: Small changes in the arginine concentration cause relatively large differences in the nitrogen levels of that amino acid as each arginine molecule has 4 nitrtogen atoms.

The total N content of each fraction of the exudate from maize fed 2mM ^{15}N-nitrate (Table 3) showed that ±59% of the nitrogen exported by the roots was in the nitrate form, 35-38% was as amino acids, and very little (3-7%) was ammonium. These figures do not accurately reflect the export of newly reduced nitrogen exported by the roots. The proportions of the ^{15}N forms in the sap (Table 3) indicate that 93% of the newly-absorbed nitrogen in the xylem stream, at both the 4h and 8h collections, was in the nitrate form, with only 4-6% in the organic form. Very little (2-4%) was in the ammonium form. Rufty _et al_ (1982) found that, in soybean, the relative proportions of the nitrogen in the nitrogen fractions overestimated the reduced nitrogen exported from the roots (38-59%) when compared with figures for reduced ^{15}N (5-17%). Under continued nitrate feeding, stored nitrate has been reported as being relatively unavailable for reduction in maize roots (MacKown _et al_, 1981; Shaner & Boyer, 1976), and in tobacco cells, barley aleurone layers, and _Zea mays_ leaf sections (Ferrari _et al_, 1973). The bulk of the reduced nitrogen in the xylem stream may be from endogenous sources such as protein turnover, discharge of root storage pools, or cycling through the root from the shoot as reported by Arima & Kumazawa, 1975; Muhammad & Kumuzawa, 1974; and Yoneyama, 1977 for rice, and Ivanko & Ingverson (1971) for maize.

The ^{15}N enrichment of the nitrate fraction of the xylem exudate at the 4h collection was 19.2% ± 3.7%, and that of the 8h collection was 23.2% ± 0.2% (not tabulated). Comparing these values with the 12% enrichment of the free nitrate fraction of the root of nitrate-fed maize (Table 12) suggests that newly-absorbed nitrate is loaded directly onto the xylem, with little input from the root nitrate storage pools.

Examining the levels of individual amino acids and amides (Table 4) shows that the composition of the organic fraction of the xylem sap was approximately the same at both the 4h and 8h collections. The amide glutamine carries the most nitrogen, 33-44% of the organic nitrogen being exported in this form. This is in keeping with the findings of other workers who have found glutamine to be the major nitrogen-carrying organic compound exported by the roots of many non-nodulated plants, eg. in sugar cane (Waldron, 1976), Datura (Probyn, 1978), sunflower (Kaiser & Lewis, 1979), and barley (Lewis et al, 1982a). Other important compounds are asparagine, arginine, alanine and serine. These organic compounds have a C:N ratio of 3:1 or lower and, with glutamine, account for 79% of the organic N in the xylem exudate. The roots rely heavily on the shoot for carbon skeletons for nitrogen assimilation (Dixon, 1976). The use of molecules with low C:N ratios for N transport reduces the loss of carbon by the roots, or alternately may function to allow leaves to acquire nitrogen with the minimal export of carbon.

The amino acid content of exudate from duplicate plants was almost identical, suggesting that contamination from damaged cells or phloem was low.

4.1.2. Sap From Ammonium-Fed Plants.

Tables 4 & 5 show the exudate composition of plants fed with a nutrient solution containing nitrogen in the $^{15}NH_4^+$ form.

From Table 5 it can be seen that 83-93% of the nitrogen in the sap was in the organic form, with very little ammonium being present. Lewis & Chadwick (1983) also observed low levels of ammonium in the xylem sap of barley. This restriction of the loading of ammonium onto the xylem may help to prevent the uncoupling of photosynthetic phosphorylation by ammonium, the work of Good (1960) having shown that this can occur at ammonium levels of 2mM.

The concentration of organic nitrogen in the sap was approximately 2.5x higher than in sap from nitrate-fed maize.

After 4h of ^{15}N feeding 66% of the newly-absorbed nitrogen in the xylem stream was present as amino acids, with the amount of translocated ^{15}N in organic forms increasing to 87% of the total after 8h of feeding with labelled ammonium (Table 5). It is therefore apparent that most of the absorbed ammonium is assimilated into nitrogenous organic compounds in the root.

From the values for the individual amino acids and amides in the xylem exudate it can be seen that the main organic nitrogen carriers were the amides asparagine and glutamine. Both have a C:N ratio lower than 3:1 which, as in the nitrate-fed plants, allows transfer of nitrogen from the roots to the shoot with a relatively low export of carbon.

It should be noted that in ammonium-fed plants asparagine was the largest carrier of organic N, whereas in nitrate-fed plants (and in plants grown on the mixed-nitrogen source) glutamine was the most important nitrogenous organic compound. The reason for this change-over is not apparent, but it was observed at the 4h and 8h harvests. This change-over is surprising when it is recalled that glutamine is normally considered to be an ammonium detoxifier, and a nitrogen storage compound (Haynes & Goh, 1978).

Table 5.

Nitrogen content of xylem exudate of 20-day-old maize plants fed 2mM ^{15}N ammonium for 4h and 8h. Results from duplicate samples were averaged for each time period and the range is given. Only trace quantities of nitrate were found.

Xylem Sap Fraction	Harvest	^{15}N Content (μmol ml^{-1})	Sap fraction ^{15}N as % of total ^{15}N	Sap fraction total N (μmol ml^{-1})	Sap Fraction N as % Total N
Ammonium	4h	1.46 ± 0.10	33.8	4.63 ± 1.08	16.6
	8h	0.48 ± 0.07	12.6	1.61 ± 0.42	7.3
Amino Acid	4h	2.86 ± 0.34	66.2	23.18 ± 3.54	83.4
	8h	3.31 ± 0.43	87.4	20.56 ± 1.73	92.7

4.1.3. Sap From Nitrate + Ammonium-Fed Plants.

Tables 4 & 6 show the composition of xylem sap collected from plants fed with a nutrient solution containing labelled nitrogen as 1mM nitrate + 1mM ammonium.

Examination of Table 6 reveals that 60-64% of the nitrogen in the exudate was in organic forms, 34-37% was detected as free nitrate, and only 2.5-3% in the ammonium form. The relative amounts of labelled nitrogen in each fraction fluctuated, with 35-55% of the ^{15}N being found as organic N, 43-60% being as nitrate, and 2.5-5% being in the free ammonium form.

The fraction of xylem-borne ^{15}N in the organic form was lower than the equivalent fraction in ammonium-fed maize due to the contribution of labelled nitrate exported by the root (35-55% compared with 66-87% respectively). The percentage of organic ^{15}N exported was much higher in plants fed nitrate + ammonium than that of nitrate-fed plants (35-55% as against 4-6%), indicating that most of the organic ^{15}N originated from the incorporation of absorbed $^{15}NH_4^+$ in those plants supplied with both nitrate and ammonium.

Glutamine was the most important nitrogen-containing organic compound in the xylem stream, 65% of the organic nitrogen exported by the roots being in that form. Asparagine, alanine, serine, and arginine were the other largest carriers of organic nitrogen. With glutamine, these molecules accounted for 94% of the total incorporated nitrogen in the xylem sap.

As with plants fed with nitrate or ammonium, most of the organic nitrogen was carried by molecules with a C:N ratio of 3:1 or lower.

The actual ^{15}N concentration of the amino and nitrate fractions in the exudate (Table 6) was much lower at the 8h collection than at the 4h collection. Since the total nitrogen concentrations were relatively unchanged, it is possible that the plants used for the 8h collection had, for some unknown reason, lower ^{15}N absorption rates than those used for the 4h collections.

Table 6

Nitrogen content of exudate of 20-day-old maize plants fed 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$ for 4h and 8h. Results from duplicate samples were averaged for each time period, and the range is given.

Xylem Sap Fraction	Harvest	^{15}N Content (μmol ml^{-1})	Sap fraction ^{15}N as % of total ^{15}N	Sap fraction total N (μmol ml^{-1})	Sap fraction N as % Total N
Ammonium	4h	0.29 ± 0.03	2.5	0.89 ± 0.07	2.5
	8h	0.15 ± 0.01	4.7	1.04 ± 0.20	2.7
Nitrate	4h	5.06 ± 0.58	42.6	12.00 ± 1.43	33.8
	8h	1.96 ± 0.22	60.4	14.50 ± 1.53	37.0
Amino Acid	4h	6.52 ± 0.40	54.9	22.64 ± 1.16	63.7
	8h	1.14 ± 0.11	34.9	23.64 ± 0.08	60.3

4.2. Growth Studies.

Maize plants were grown under greenhouse conditions in nutrient solutions containing nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM 1:1 mixture of both, as described in Section 3.10. The plants were harvested 48 days after the seeds were sown and processed as described in Section 3.10. The organic nitrogen contents were determined by the methods detailed in Section 3.5. and are shown in Tables 7 & 8. The fresh and dry weights of the plants are given in Table 9.

On examination of Tables 7-9 it can be seen that most organic nitrogen, per plant part, was found in the leaf blade (Table 7) and least in the roots. The ammonium-fed plants contained more organic nitrogen than the nitrate and mixed-feed plants. This applied to each of the plant parts, as well as to the whole plants. The mixed-feed plants had more organic nitrogen than those fed only nitrate-N. This was also found to be the case for all regions of the plants.

The application of a one-tailed paired-sample t-test, using Gosset's tables of t, showed that the mean total organic nitrogen contents of the plants grown on each of the 3 different nitrogen sources were significantly different (Table 8).

Table 9 shows that the ammonium-fed maize and plants grown on a mixed-nitrogen source had similar fresh weights, but nitrate-fed plants had lower fresh weights than plants supplied with ammonium or nitrate + ammonium. The shoot:root fresh weights were the same irrespective of the nitrogen source, the shoot:root ratio being almost 6:1 (Table 9).

Ammonium-fed plants had the most dry matter (Table 9), nitrate-fed plants had the least dry matter, and the mixed-feed plants were of

Table 7.

Organic nitrogen content (g) of 48-day-old maize grown in a nutrient solution containing nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM 1:1 mixture of both (± standard error).

	N content (g)		
	Nitrate-fed plants	Nitrate + ammonium-fed plants	Ammonium-fed plants
Leaf blade	0.617 ± 0.098	0.888 ± 0.032	1.000 ± 0.098
Leaf Blade + Stem	0.458 ± 0.055	0.642 ± 0.052	0.909 ± 0.094
Root	0.140 ± 0.012	0.174 ± 0.041	0.205 ± 0.032
Total	1.215 ± 0.151	1.704 ± 0.087	2.113 ± 0.165

Table 8.

Comparison of mean total organic nitrogen of maize grown in each of the treatments of Table 7. A one-tailed paired-sample t-test was applied.

Compared Treatments	t	Confidence Level of Significant Differences
Mixed N and Nitrate	7.45	0.001 P 0.0005
Ammonium and Nitrate	7.48	0.001 P 0.0005
Ammonium and Mixed N	3.78	0.01 P 0.005

Table 9.

Fresh and dry weights (g) of 48-day-old maize plants grown in a nutrient solution containing nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM N 1:1 mixture of both (average of 6 plants per treatment, ± standard error).

Feed	Fresh Wt (g)	Dry Wt (g)	FW/DW	Shoot/Root (FW)	Shoot/Root (DW)
Nitrate	541.4 ± 50.6	46.1 ± 4.2	11.7	5.9	7.5
Mixed N	636.4 ± 98.3	53.2 ± 7.9	12.0	5.8	6.5
Ammonium	652.7 ± 101.3	63.7 ± 5.4	10.2	5.9	8.7

intermediate dry matter content. The ammonium-fed plants had the largest shoot:root dry weight ratio, whilst the mixed-feed plants had the lowest.

The nitrate- and mixed-feed plants had similar fresh:dry weight ratios (11.7:1 and 12.0:1 respectively), whilst those plants fed with ammonium had a slightly lower fresh:dry weight ratio (10.2:1). This difference could indicate lower turgidity in the ammonium-fed plants than in those grown with nitrate and mixed nitrogen sources, and is one possible explanation for the relative softness of ammonium-fed maize in comparison to plants grown with the other nitrogen forms. Another possible explanation for the relative softness of ammonium-fed plants lies in the possible diversion of photosynthate from cell wall production to provide suitable carbon skeletons for the rapid assimilation of ammonium into organic compounds.

4.3. Enzyme Assays.

4.3.1. Diurnal Rhythm of Leaf blade in vitro Nitrate Reductase Activity.

The daily rhythm of *in vitro* leaf blade nitrate reductase activity (NRA) of 20- to 21-day-old maize plants was studied, using the techniques described in Section 3.9. Assays were performed on the 4th and 5th leaf blades, which were the two youngest fully formed and unrolled leaves. Extractions were made every 2 hours, except during the first 6 hours of the light period when determinations were made every hour. The sampling began 3h after the commencement of the light period on the 13th day after the seedlings had been transferred to the nitrate-containing nutrient

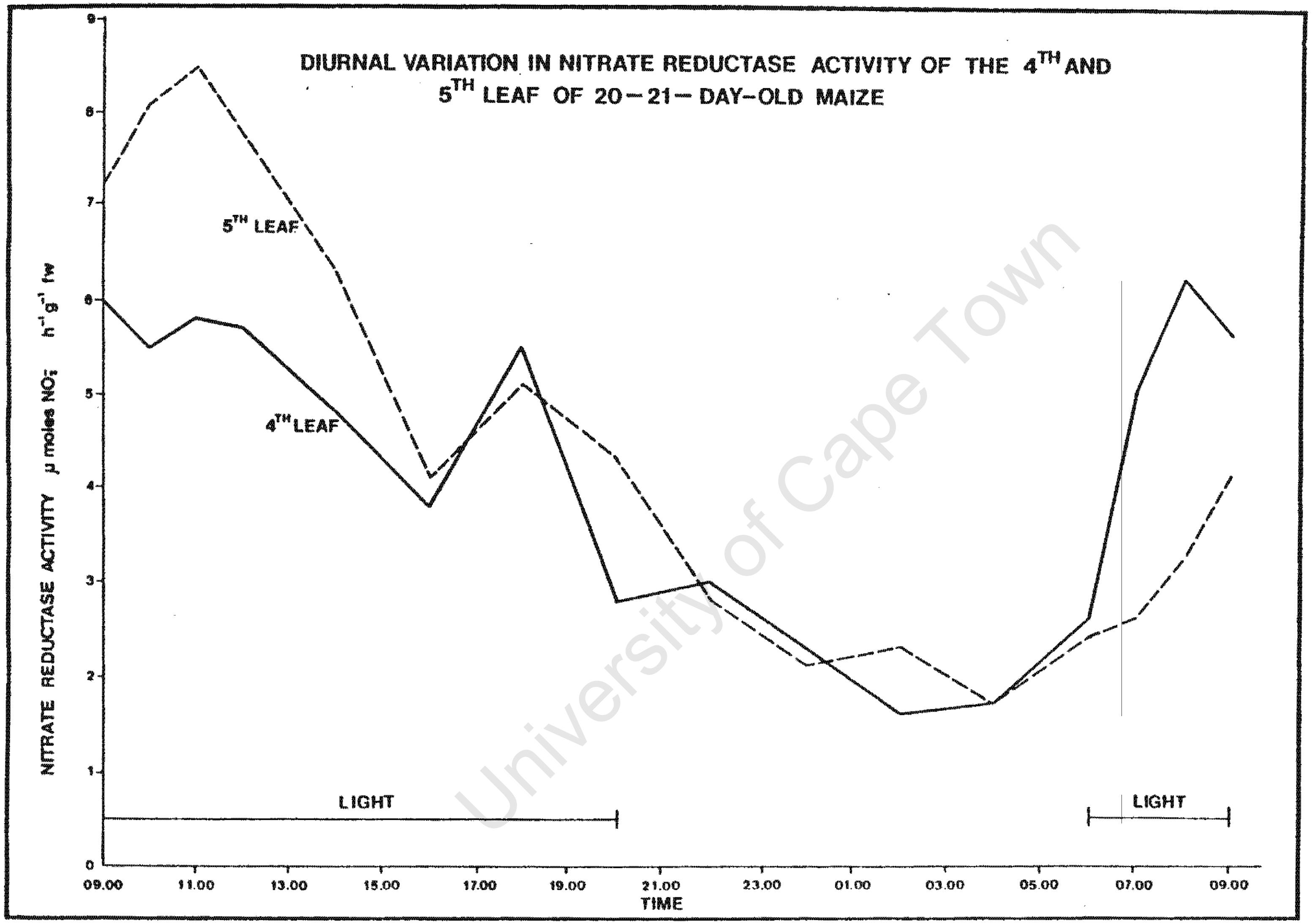


Figure 9. Diurnal rhythm of *in vitro* nitrate reductase activity in the 4th and 5th leaf blades of 20-21-day-old maize plants. The plants were supplied with a nutrient medium containing 2mM nitrate.

solutions, under the environmental conditions stipulated in Section 3.2. The results of the assays are presented in Figure 9.

On the first morning of the experiment, NRA in the 5th leaf blade rose to a peak of 8.5µmol NO_2^- h^{-1} g^{-1} fw, which occurred 5h after the beginning of the first light period. Activity thereafter decreased throughout the first light period, except for a smaller (and possibly not significant) peak of 5.1µmol NO_2^- h^{-1} g^{-1} fw which occurred 12h after the first light period began. The activity continued to decrease during the dark period until a minimum level of 1.7µmol NO_2^- h^{-1} g^{-1} fw was reached 20h after the commencement of the first light period. During the early hours of the second light phase NRA again rose rapidly.

The NRA of the 4th leaf blade appeared to have maximised at 6.0µmol NO_2^- h^{-1} g^{-1} fw 3h after the commencement of the first light period, and slowly fell throughout the light period except for a second, smaller peak (also possibly insignificant) of 5.5µmol NO_2^- h^{-1} g^{-1} fw which was observed 12h after the first light period began. The NRA decreased throughout the dark period to a minimum value of 1.6µmol NO_2^- h^{-1} g^{-1} fw, twenty hours after the start of the first light phase. Activity rose sharply during the early hours of the second light period.

Similar diurnal rhythms in NRA were found in maize and soybean leaves by Duke _et al_ (1978), and in barley leaves by Lewis _et al_ (1982b). Lillo (1983) found similar daily rhythms in barley grown under short days (8h light periods) with _in vivo_ and _in vitro_ assay techniques. Lillo (1983) also found that NRA decreased with leaf age. Chang _et al_ (1981) found diurnal rhythms of NRA in _Kalanchoë fedtschenkoi_ leaves, with higher activity in the light period than during the dark period. This rhythm continued for 6 days after the plants were transferred to continuous light or dark, suggesting that there is an endogenous rhythm of NRA. Although Kalanchoë is a Crassulacean acid metabolism (CAM) plant, the data supported the idea that NRA was independent of CAM.

NRA was higher in the 5th leaf blade than in the 4th during the first hours of the light periods, with the NRA of the 5th leaf blade increasing more rapidly after the light periods began than that of the 4th leaf blade. During the latter part of the first light period, and during the dark period, 4th and 5th leaf blade NRA's were similar. Reports have been made of NRA varying with leaf age in soybean (Harper & Hageman, 1972; Carelli & Magalhães, 1981; Santoro & Magalhães, 1983).

Because of differences in NRA between leaves at different stages of development, and at various times throughout the day, it is essential when making comparative studies to keep these factors constant. This may also apply to other enzymes of nitrogen assimilation (Duke et al, 1978). Consequently, in all further studies on the enzymes of N assimilation, extractions were performed 6h after the light period commenced. These were performed on the 5th leaf blade of 20-day-old maize plants, or with a 1g random sample of 21-day-old roots.

4.3.2. Effect of Nitrogen Source on Leaf Blade and Root NRA and GSA.

Comparative studies were made of leaf blade and root NRA and GSA, using the methodology described in Section 3.9. The results of these experiments are presented in Tables 10 & 11.

Table 10.

Nitrate reductase activity of the 5th leaf blade of 20-day-old maize, and of the roots of 21- day-old maize (µmol NO_2^- h^{-1} g^{-1} fw), fed 2mM nitrate, or 2mM ammonium, or a 2mM 1:1 mixture of both. Extractions were performed 6h after the light period commenced. Each figure is the average of triplicate determinations of NRA in each extract.

	NRA (µmol NO_2^- h^{-1} g^{-1} fw)		
	Nitrate-fed plants	Mixed N-fed plants	Ammonium-fed plants
Leaf Blade	14.8	15.6	2.4
Root	2.2	2.8	0.9

Table 11.

Glutamine synthetase activity of the 5th leaf blade of 20-day-old maize, and of the roots of 21-day -old maize (µmol glutamylhydroxymate h^{-1} g^{-1} fw), fed a nutrient solution containing 2mM nitrate, or 2mM ammonium, or a 2mM 1:1 mixture of both. Extractions were performed 6h after the commencement of the light period. Each figure is the average of triplicate determinations of GSA in each extract.

	GSA (µmol glutamylhydroxymate h^{-1} g^{-1} fw)		
	Nitrate-fed plants	Mixed N-fed plants	Ammonium-fed plants
Leaf Blade	49.5	48.9	44.1
Root	45.1	44.1	44.4

From Table 10 it can be seen that leaf blade NRA levels for nitrate and nitrate + ammonium-fed plants were similar (14.8 and 15.6µmol NO_2^- h^{-1} g^{-1} fw respectively), but NRA in leaf blades of ammonium-fed plants was much lower (2.4µmol NO_2^- h^{-1} g^{-1} fw). These results indicate that NRA is enhanced by nitrate feeding [a generally accepted view, eg. Sahulka (1977); Buczek (1979)], but that NRA in leaves is not reduced by the presence of ammonium in the nutrient medium [see second column (nitrate + ammonium feed) of Table 10]. The latter point is in agreement with the findings of Afridi & Hewitt (1964), Beevers et al (1965), Schrader et al (1972), and Stewart et al (1974). Mehta and Srivastava (1982) established that ammonium enhanced the NRA of maize leaves when nitrate was supplied, and suggested that this could be a result of any of 3 factors: (1) Ammonium could increase the synthesis of inactive NR, which is then activated by endogenous nitrate. (2) It could increase the synthesis of the reductant NADH. (3) It may mobilize nitrate from endogenous storage pools and consequently cause activation of the inactive enzyme.

In roots, NRAs in plants fed 2mM nitrate and 2mM 1:1 nitrate + ammonium were similar (2.2 and 2.8µmol NO_2^- h^{-1} g^{-1} fw respectively), but NRA was lower in plants fed 2mM ammonium nitrogen only (0.9µmol NO_2^- h^{-1} g^{-1} fw). This indicates that NRA in roots was induced by nitrate feeding. The results also indicate that the addition of ammonium to a nitrate-containing nutrient solution does not result in decreased nitrate reductase activity. The results differ from the findings of Smith & Thompson (1971) and Frith (1972) who reported that ammonium caused depression of root NRA in barley and in apple seedlings when supplied together with nitrate.

Reports of the effects of ammonium on nitrate-induced NRA are inconclusive. Radin (1975) found that ammonium inhibited NRA in cotton roots and shoots more at pH7 than at pH5. Oaks _et al_ (1977) reported that maize root NRA was depressed by ammonium at pH7.5 but was stimulated by ammonium at pH5.8. Mengel _et al_ (1983), working with maize, observed that ammonium reduced NRA in shoots and roots. In the experiments of Mengel and his co-workers the nutrient solutions containing both nitrate and ammonium were in the range pH4.6 - 5.5, whilst those containing only nitrate-N ranged from pH6 to pH7. Mengel _et al_ (1983) suggested that the nutrient pH may have a more important influence on NRA than the mere presence of ammonium, based on the observation that the addition of bicarbonate to the nutrient solution, and the presence of excess hydroxyl ions, also resulted in increased root NRA.

In maize fed nitrate-N, leaf blade NRA was almost 7 times that of the roots, whilst in plants fed with nitrate + ammonium, leaf blade NRA was almost 6 times root NRA. This suggests that the leaf blade is likely to be the larger centre of nitrate reduction when nitrate is supplied.

From Table 12, the ^{15}N absorption rate of nitrate-fed plants was 98 µmol ^{15}N h^{-1} $plant^{-1}$ at the 4h harvest. If Tables 10 and 12 are used to calculate the total leaf blade NRA of the nitrate-fed plants used for ^{15}N feeding, a value of 186 µmol NO_2^- h^{-1} $plant^{-1}$ is obtained, which is double the rate of nitrate absorption. The _in vitro_ nitrate reductase activities indicate that the leaf blade NRA is sufficiently high to cope with the reduction of all the absorbed nitrate, although it should be noted from Table 15 that nitrate storage pools do exist in all plant regions. The actual concentration of free nitrate within the plants fell during the period between the 4h and 8h harvests (Table 15). It may be that nitrate accumulation in the tissues takes place during the hours of darkness, when NRA activities are lower than during the light period (Figure 9).

By consulting Table 11 it can be seen that little variation in glutamine synthetase activity occurred between leaf blades and roots of plants grown with all 3 nitrogen sources, and that leaf and root GSA levels were unaffected by the nitrogen source. This is similar to the findings of Lewis et al (1982a) for barley. The activities of GS are higher than those of NR, also in agreement with the findings of Lewis et al (1982a).

The relatively high GS activities, in comparison to NR activities, may contribute to the control of free ammonium levels within the plant tissues, although GS also has to incorporate ammonium from photorespiration and amino acid turnover into glutamine.

4.4. Extracts of Whole Plants Fed With ^{15}N-Nitrogen.

Six ^{15}N-feeding experiments were performed, and extractions and analyses of the whole plants carried out, as described in Sections 3.4. to 3.8.

In two cases the plants were fed 2mM nitrate; in one of these the maize plants were grown hydroponically, whilst in the second the plants were grown rooted in builder's stone, as described in Section 3.2. This was to verify that the improved growth obtained by the use of the gravel culture method could be shown in terms of the nitrogen nutrition of the plant material.

Two experiments were performed on maize grown in each of 2mM ammonium, and 1mM nitrate + 1mM ammonium feeding solutions. For each of these two nitrogen sources, one experiment was performed with $CaCO_3$ added to the nutrient solution, and one was carried out without calcium carbonate being present (see Section 3.11). The difference between plants grown with calcium carbonate and those grown without indicated whether pH control is advantageous when plants undergo ammonium nutrition.

During the period of growth, the maximum changes in nutrient pH from the pre-adjusted pH 5 were as follows: in solutions containing ammonium nitrogen only, the pH dropped to ±pH 4 between changes of nutrients (every 2 days) when calcium carbonate was absent. In those solutions with nitrate + ammonium nitrogen, the pH decreased to as low as pH 4.5 when $CaCO_3$ was omitted from the nutrient medium. In all other solutions the pH, immediately prior to replenishment, was in the range pH 6.0-6.5.

The plants grown on different nitrogen sources had different appearances, these being detailed in Section 4.1.

The seedlings were observed to be capable of survival for 12-14 days on seed reserves if only water was supplied, so the period of reliance on nutrient nitrogen is too small to allow meaningful comparisons between the masses of 20-day-old maize plants grown on the various nitrogen sources. Differences in stored seed reserves could contribute significantly to any observed differences in plant size at 20 days. Consequently, although fresh weights were recorded at the time of harvesting the ^{15}N-fed plants, the effect of nitrogen source on plant size was compared using material grown for 48 days (Section 4.1.).

For the same reasons figures for total plant nitrogen in the 20-day-old maize plants fed with the 3 different nitrogen sources are not used as an indicator of the preference of the plants for any of the nitrogen sources. Total plant N contents are compared on 48-day-old plants in Section 4.1.

4.4.1. Effect of Culture Method on ^{15}N Assimilation Rates.

The effects of culture method on ^{15}N assimilation rates were studied to verify that the changes in the culture techniques described in Section 3.2. did, in fact, result in improvements in the nitrogen nutrition of maize grown in nutrient culture. Assimilation rates of ^{15}N in plants grown hydroponically, and in gravel culture, were compared by supplying labelled nitrate to maize plants grown using both feeding techniques as described in Section 3.4. The effects of calcium carbonate in ammonium-containing nutrient solutions were investigated by performing ^{15}N feeding experiments (as detailed in Section 3.4.) on maize supplied with ammonium, or ammonium + nitrate, both in the presence and in the absence of calcium carbonate.

4.4.1.1. Effects of Gravel Culture and Hydroponic Cultivation Methods on The Production of Organic N from ^{15}N-Nitrate.

The assimilation rates of ^{15}N from labelled nitrate into organic nitrogen (free + bound) are shown in Table 12.

At the 4h and 8h harvests the assimilation rate in maize grown in gravel culture greatly surpassed that of maize grown hydroponically. The incorporation of ^{15}N into organic N in the leaf was approximately 3x faster, and in the root 2x faster, in the gravel culture plants. The overall incorporation of ^{15}N into organic forms was roughly 2.5x more rapid with the gravel culture plants. These figures clearly demonstrate that nitrogen uptake in hydroponically grown plants is limited in comparison to that observed in plants grown in gravel culture. This may be related to the availability of oxygen in the root region (nitrate absorption being an active process, as discussed in Section 2.2.) even though the hydroponic nutrient solutions were vigorously aerated. Hewitt & Smith (1975) stated that the level of aeration of the roots of plants grown in water-culture greatly affects the absorption of nutrients and water.

Table 12.

Assimilation rate of ^{15}N into organic (free + bound) forms (μg ^{15}N h^{-1} g^{-1} fw) in 20-day-old maize plants fed with a nutrient solution containing 2mM $^{15}NO_3^-$ for 4h and 8h. Plants were grown hydroponically, or in gravel culture. Each value is the mean of duplicated determinations performed on each of 2 plants, ± range.

	4h Harvest			8h Harvest		
Culture Technique	Leaf Blade + Leaf Base	Root	Plant Total	Leaf Blade + Leaf Base	Root	Plant Total
Hydroponic	5.6 ± 0.2	7.2 ± 0.9	6.1 ± 0.5	6.4 ± 0.2	6.4 ± 0.4	6.4 ± 0.1
Gravel Culture	14.5 ± 0.7	14.1 ± 1.0	14.3 ± 0.2	19.6 ± 3.4	10.2 ± 1.3	15.7 ± 2.7

4.4.1.2. Effects of Calcium Carbonate on the Incorporation of Labelled N in Maize Fed with Labelled Ammonium.

The rates of incorporation of ^{15}N into organic forms, after feeding maize with ammonium-containing nutrient solutions both in the presence and in the absence of calcium carbonate for 4h and 8h, are presented in Table 13.

At the 4h harvest the labelled organic compound production rate was higher in the plants which were grown on nutrient solutions which contained calcium carbonate. This was unsurprising: the absorption of ammonium in the absence of pH control leads to a nutrient pH decrease, and consequent reduction in the absorption rate, as discussed in Chapter 2. At the 8h harvest the incorporation rates were similar, this being the result of a decrease in the ^{15}N assimilation rate of the plants which were supplied with calcium carbonate (32.7 and 20.2µg $^{15}N\ h^{-1}g^{-1}$ fw respectively at the 4h and 8h harvests). This diminished ^{15}N assimilation rate may have been due to a decrease in the $^{15}NH_4^+$ concentration as the ^{15}N feeding experiment progressed.

Table 13.

Assimilation rate (µg ^{15}N h^{-1} g^{-1} fw) of ^{15}N into organic (free + bound) forms in 20-day-old maize plants fed with a nutrient solution containing 2mM ^{15}N ammonium fro 4h and 8h. Plants were grown in gravel culture with, or without, calcium carbonate being present. Each value is the mean of duplicate determinations on each of 2 plants, ± range.

	4h Harvest			8h Harvest		
Calcium Carbonate	Leaf Blade + Leaf Base	Root	Plant Total	Leaf Blade + Leaf Base	Root	Plant Total
+	32.9 ± 3.3	33.6 ± 5.1	32.7 ± 0.7	22.9 ± 3.0	20.6 ± 2.0	20.2 ± 1.1
-	16.0 ± 0.4	36.3 ± 1.3	22.5 ± 1.1	19.8 ± 3.2	24.3 ± 3.2	20.8 ± 3.3

4.4.1.3. Effect of Calcium Carbonate on the Incorporation of Labelled Nitrogen in Maize Fed ^{15}N Nitrate + ^{15}N Ammonium.

Table 14 shows the rates of incorporation of ^{15}N into organic forms in maize fed with 2mM 1:1 $^{15}NO_3^-$ + $^{15}NH_4^+$ for 4h and 8h. In one experiment the nutrient solution pH was stabilized with calcium carbonate, whilst in the other it was not.

The incorporation rates in plants grown in pH-stabilized nutrient solutions were similar, at both the 4h and 8h harvests, to those of plants grown without $CaCO_3$ in the nutrient medium. This is predictable, as the absorption of nitrate and ammonium ions have antagonistic effects on the nutrient pH, as discussed in Chapter 2. Even in the absence of calcium carbonate the nutrient solutions are, to some extent, buffered, so the effects of pH changes on organic ion absorption are relatively small.

Table 14.

Assimilation rate (µg $^{15}N\ h^{-1}\ g^{-1}$ fw) of ^{15}N into organic (free + bound) N in 20-day-old maize plants fed with a nutrient solution containing 2mM 1:1 $^{15}NO_3^-$ + $^{15}NH_4^+$ for 4h and 8h. Plants were grown in gravel culture with, or without, calcium carbonate in the nutrient medium. Each value is the mean of duplicate determinations on each of 2 plants, ± range.

	4h Harvest			8h Harvest		
Calcium Carbonate	Leaf Blade + Leaf Base	Root	Plant Total	Leaf Blade + Leaf Base	Root	Plant Total
+	22.9 ± 0.6	21.2 ± 0.9	22.4 ± 0.9	25.0 ± 1.8	19.1 ± 0.5	23.2 ± 1.1
–	26.7 ± 2.7	19.7 ± 2.7	24.8 ± 1.3	20.2 ± 0.2	13.3 ± 0.0	18.2 ± 0.2

4.5. Effect of Nitrogen Source on the Incorporation of Labelled Nitrogen into Organic Forms.

By comparing the results shown in Tables 12-14 it is possible to determine the effects of nitrogen source on the rate of incorporation of ^{15}N into nitrogenous organic compounds.

The $^{15}NO_3^-$-fed plants (gravel culture) have lower incorporation rates than those plants which were grown in mixed nitrogen source solutions (ave. 15.0µg ^{15}N h^{-1} g^{-1} fw in the nitrate-fed plants, and 22.8µg ^{15}N h^{-1} g^{-1} fw in the mixed-feed plants with calcium carbonate. The ammonium-fed maize, with calcium carbonate present, displayed the highest incorporation rate (32.7µg ^{15}N h^{-1} g^{-1} fw). These figures indicate that nutrient ammonium is assimilated more rapidly into organic N than absorbed nitrate, which is in agreement with the findings of the 48-day growth experiments (Section 4.2).

4.6. Distribution of N and ^{15}N Within Plants.

In view of the results of sections 4.4.1.1.-4.4.1.3. the experiments which were used to compare the distribution of ^{15}N when feeding 2mM nitrate, or 2mM ammonium, or a 2mM N 1:1 mixture of both, were those where the maize was grown rooted in builder's stone (see Section 3.1.), and with calcium carbonate added to the nutrient solutions to prevent the pH from becoming too low (see pH change report in Section 4.4.). This allowed comparison under the growth conditions which proved to result in the best plant growth, and also permitted a study of plants which were grown at similar nutrient pH.

The nitrogen concentrations and ^{15}N enrichments of maize plants fed with the three different nitrogen sources for the 4h and 8h feeding periods are shown in Table 15 (nitrate feed), Table 16 (ammonium feed), and Table 17 (1:1 mixed nitrate + ammonium). All feeding solutions containing ammonium or ammonium + nitrate contained calcium carbonate (see Section 3.10). The masses of the plants used to collect the data presented in Tables 15-17 are given in Table 18. Figure 10 has been constructed from Tables 15-17, and illustrates the distribution of ^{15}N in organic and inorganic forms in the different compartments of the maize plants. The free nitrate and ammonium concentrations (μmol g^{-1} fw) for each of the plant regions are shown in Table 19.

Table 15.

Concentrations and ^{15}N enrichments (A%E) of the nitrate, ammonium, soluble organic, and bound organic nitrogen fractions of individual leaf blade, leaf base + stem, and root regions of 20-day-old maize plants fed with a nutrient solution containing 2mM $^{15}NO_3^-$ for 4h and 8h. Figures have been obtained by averaging duplicated determinations on duplicate plants, and are expressed as mean ± range.

		Leaf Blade			Leaf Base + Stem			Root		
N Fraction	Harvest	N Conc. (µgN/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)
Ammonium	4h	98 ± 11	10.3 ± 0.1	10 ± 1	179 ± 26	13.5 ± 0.9	24 ± 2	452 ± 15	13.3 ± 1.0	61 ± 7
	8h	106 ± 4	13.1 ± 1.1	14 ± 1	452 ± 15	24.5 ± 0.8	112 ± 8	397 ± 120	16.2 ± 0.3	65 ± 19
Nitrate	4h	2 388 ± 92	12.1 ± 1.5	289 ± 25	5 341 ± 565	14.9 ± 1.2	788 ± 22	6 927 ± 365	29.0 ± 1.4	2 003 ± 11
	8h	1 212 ± 173	12.0 ± 0.5	144 ± 15	5 408 ± 428	18.4 ± 1.6	986 ± 6	6 226 ± 720	33.0 ± 1.2	2 045 ± 161
Soluble Organic Nitrogen	4h	1 668 ± 355	13.7 ± 0.6	231 ± 58	3 716 ± 294	11.2 ± 0.9	415 ± 2	2 707 ± 119	16.5 ± 0.1	447 ± 16
	8h	1 750 ± 474	26.2 ± 0.6	464 ± 137	3 236 ± 118	24.9 ± 0.9	804 3	2 251 ± 647	19.3 ± 1.2	428 ± 98
Bound Nitrogen	4h	40 196 ± 2 832	1.3 ± 0.1	501 ± 58	16 058 ± 211	1.9 ± 0.2	300 ± 32	18 417 ± 1 071	2.4 ± 0.1	443 ± 5
	8h	54 180 ± 3 937	3.3 ± 0.0	1 809 ± 135	19 616 ± 773	5.2 ± 0.2	1 013 ± 1	20 841 ± 1 324	5.2 ± 0.3	1 071 ± 6

* Plant masses are tabulated in Table 18.

Table 16.

Concentrations and ^{15}N enrichments (A%E) of the nitrate, ammonium, soluble organic, and bound organic nitrogen fractions of individual leaf blade, leaf base + stem, and root regions of 20-day-old maize plants fed with a nutrient solution containing 2mM $^{15}NH_4^+$ for 4h and 8h. Figures have been obtained by averaging duplicated determinations of duplicate plants, and are expressed as mean ± range.

		Leaf Blade			Leaf Base + Stem			Root		
N Fraction	Harvest	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)	N Conc. (µgN/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)
Ammonium	4h	263 ± 22	14.0 ± 0.1	37 ± 3	368 ± 40	20.8 ± 1.4	76 ± 3	849 ± 91	62.5 ± 1.1	530 ± 48
	8h	77 ± 19	13.8 ± 2.0	10 ± 1	387 ± 73	22.2 ± 1.9	84 ± 9	153 ± 7	28.8 ± 0.3	44 ± 2
Soluble Organic Nitrogen	4h	7 386 ± 29	15.5 ± 1.6	1 144 ± 110	8 168 ± 975	14.1 ± 0.1	1 154 ± 149	5 385 ± 304	21.2 ± 1.9	1 136 ± 39
	8h	7 755 ± 511	15.4 ± 1.2	1 201 ± 173	8 335 ± 861	17.4 ± 1.9	1 434 ± 11	6 055 ± 435	20.7 ± 1.1	1 245 ± 23
Bound Nitrogen	4h	52 808 ± 2 015	2.2 ± 0.1	1 173 ± 91	16 270 ± 1 119	3.5 ± 0.0	566 ± 46	18 616 ± 4 099	4.2 ± 0.1	792 ± 186
	8h	53 693 ± 8 725	3.7 ± 0.0	2 011 ± 347	14 714 ± 19	7.7 ± 0.1	1 137 ± 22	16 101 ± 1 895	8.2 ± 0.9	1 299 ± 8

* The masses of plants used in this experiment are given in Table 18.

Table 17.

Concentrations and ^{15}N enrichments (A%E) of the nitrate, ammonium, soluble organic, and bound organic nitrogen fractions of individual leaf blade, leaf base + stem, and root regions of 20-day-old maize plants fed with a nutrient solution containing 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$ for 4h and 8h. Figures have been averaged from duplicated determinations of duplicate plants, and are expressed as mean ± range.

		Leaf Blade			Leaf Base + Stem			Root		
N Fraction	Harvest	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)	N Conc. (µg N/ Plant)	^{15}N (A%E)	^{15}N Conc. (µg/ Plant)
Ammonium	4h	204 ± 29	16.5 ± 1.7	34 ± 2	292 ± 14	22.0 ± 0.6	65 ± 5	332 ± 11	49.8 ± 4.5	166 ± 21
	8h	171 ± 11	18.1 ± 0.3	31 ± 2	244 ± 62	27.8 ± 1.5	69 ± 21	321 ± 29	45.6 ± 0.9	146 ± 10
Nitrate	4h	2 899 ± 884	5.0 ± 0.0	144 ± 45	5 072 ± 670	8.4 ± 0.3	425 ± 43	2 999 ± 264	14.6 ± 0.7	439 ± 59
	8h	2 780 ± 501	7.8 ± 1.8	227 ± 88	5 651 ± 1 276	13.8 ± 0.8	769 ± 132	3 791 ± 219	23.4 ± 1.2	889 ± 97
Soluble Organic Nitrogen	4h	2 534 ± 310	15.2 ± 0.1	384 ± 45	2 324 ± 647	14.4 ± 1.1	328 ± 68	884 ± 119	16.7 ± 0.1	148 ± 21
	8h	2 588 ± 802	21.1 ± 2.5	567 ± 134	2 990 ± 167	22.4 ± 0.5	665 ± 146	1 677 ± 401	22.6 ± 0.3	380 ± 96
Bound Nitrogen	4h	44 869 ± 7 893	1.6 ± 0.2	707 ± 24	19 861 ± 1 514	3.2 ± 0.0	629 ± 51	13 844 ± 2 237	3.6 ± 0.1	504 ± 96
	8h	46 409 ± 9 782	2.9 ± 0.3	1 365 ± 439	20 682 ± 4 872	7.3 ± 0.3	1 514 ± 407	15 565 ± 2 263	6.1 ± 0.1	943 ± 129

* Plant masses are given in Table 18.

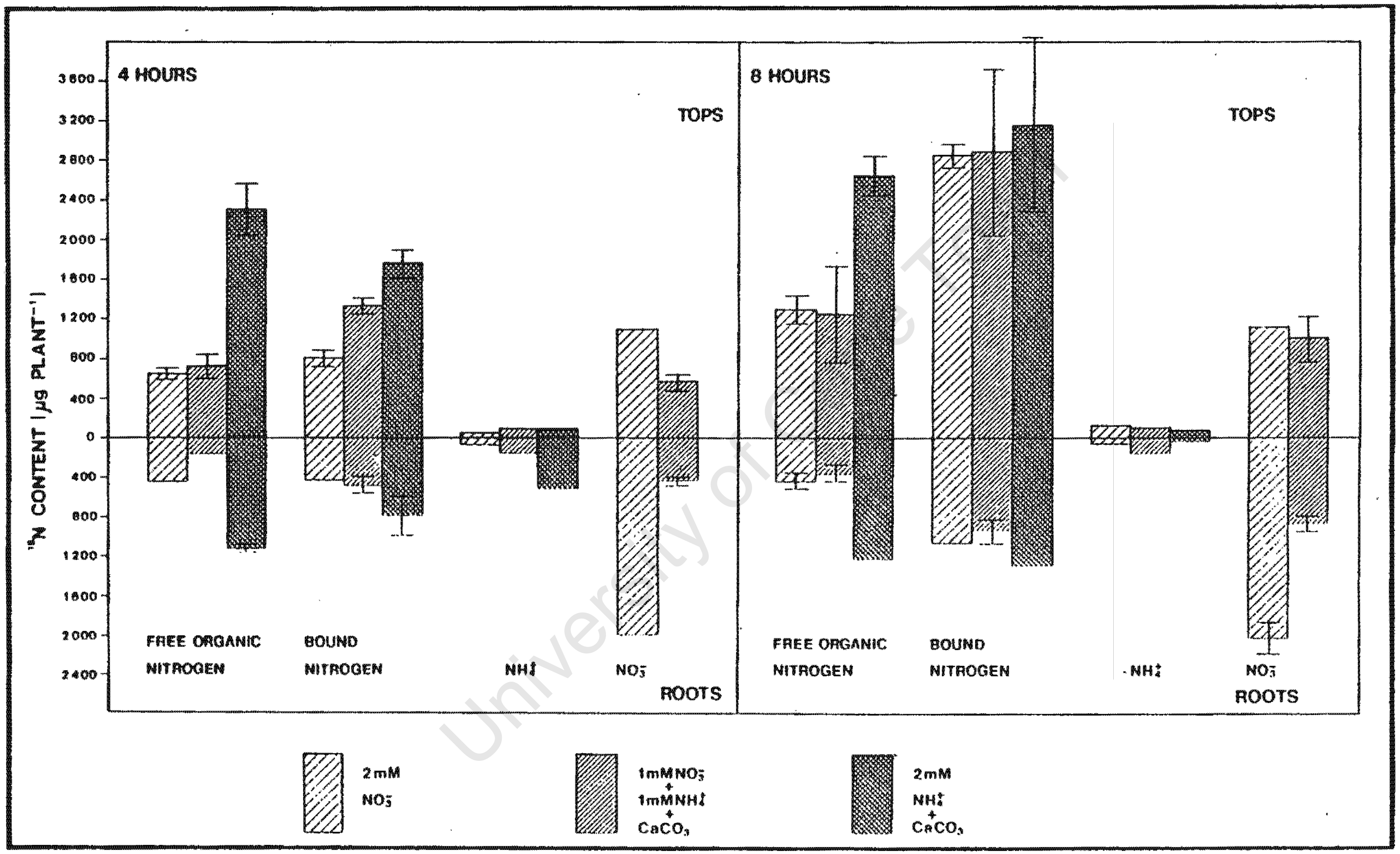


Figure 10. Distribution of ^{15}N in nitrate, ammonium, soluble organic and bound organic fractions of the shoot and the root of individual 20-day-old maize plants fed 2mM nitrate, or 2mM ammonium, or a 2mM 1:1 mixture of both. Plants were harvested 4h and 8h after the commencement of ^{15}N feeding. Each bar represents the mean of duplicate determinations performed on duplicate individuals, and the error bars indicate the upper and lower labelled N levels. Where no errors are indicated, the difference between replicates was too small to be shown.

Table 18.

Masses (g) of 20-day-old maize fed ^{15}N-nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM N 1:1 mixture of both. Figures are the mean of duplicate plants, ± range.

		Nitrogen Form		
Plant Part	Harvest	Nitrate	Mixed N	Ammonium
Leaf Blade	4h	12.6 ± 1.6	11.6 ± 1.4	16.6 ± 0.9
	8h	13.0 ± 0.9	9.9 ± 2.5	14.9 ± 1.5
Leaf Base + Stem	4h	12.3 ± 0.3	10.9 ± 1.2	14.1 ± 0.8
	8h	13.6 ± 1.8	10.3 ± 2.7	15.4 ± 1.9
Root	4h	15.9 ± 0.7	7.7 ± 1.0	14.8 ± 3.3
	8h	19.0 ± 3.6	8.7 ± 1.7	15.6 ± 1.7
Total	4h	41.2 ± 2.6	30.2 ± 3.6	45.6 ± 3.2
	8h	45.6 ± 6.4	29.0 ± 7.0	45.9 ± 5.1

Table 19.

Nitrate and ammonium concentrations (μmol g^{-1} fw) in leaf blade, leaf base + stem, and root regions of 20-day-old maize plants fed with a nutrient solution containing labelled nitrogen as 2mM nitrate, or 2mM ammonium, or a 2mM N 1:1 mixture of both. Results were averaged from 4 plants (2 x 4h and 2 x 8h harvests), ± Standard Error.

Plant Part	Nitrate Feed		Mixed N Feed		Ammonium Feed	
	Nitrate	Ammonium	Nitrate	Ammonium	Nitrate	Ammonium
Leaf Blade	143.0 ± 64.2	8.0 ± 0.8	264.6 ± 48.7	17.9 ± 3.1	0.0 ± ---	10.5 ± 6.5
Leaf Base + Stem	417.4 ± 39.7	24.3 ± 12.3	508.6 ± 54.9	25.1 ± 2.4	0.0 ± ---	25.4 ± 1.8
Root	386.1 ± 73.2	24.5 ± 5.1	421.0 ± 61.9	41.1 ± 7.7	0.0 ± ---	34.9 ± 29.5

4.6.1. ^{15}N Nitrate-fed plants.

In the nitrate-fed plants the major portion of the organic ^{15}N at the 4h and 8h harvests was found in the shoot, this being the case for both the free and bound organic N forms (Figure 10 & Table 15).

From Figure 10 and Table 15, it appears that shoot assimilatory nitrate pools were saturated after 4h of ^{15}N feeding, as the ^{15}N enrichments at the 4h and 8h harvests were approximately the same (12A%E ^{15}N in the leaf blade and 15A%E ^{15}N in the leaf base + stem) but the amount of soluble organic ^{15}N in the shoot increased between the 4h and 8h harvests, with leaf blade and leaf base enrichments doubling (approximately 14A%E ^{15}N to 26A%E ^{15}N, and 11A%E ^{15}N to 25A%E ^{15}N respectively). This indicates that the shoot assimilates nitrate into organic N very rapidly, bearing in mind that the xylem sap analysis showed that 93% of the ^{15}N delivered to the shoot was in the nitrate form.

Root assimilatory nitrate pools and free organic N assimilatory pools were both saturated, or almost saturated, after 4h of ^{15}N feeding (29.0-33.0A%E and 16.5-19.3A%E respectively) indicating that separate assimilatory and storage pools exist for both nitrate and amino acids.

Leaf blade nitrate storage pools were smaller than those of the leaf base and root, as can be seen from Tables 12 and 16. Free ammonium levels were lower in leaf blades than in leaf bases and roots, 8.0 μmol g^{-1} fw compared with 24.3 and 24.5 μmol g^{-1} fw respectively, this observation being in harmony with the known ability of ammonium to disrupt photosynthesis at comparatively low internal concentrations.

At the 4h harvest ^{15}N in the bound fraction of the shoot was approximately double that of the bound fraction of the root (801 and 443µg ^{15}N plant^{-1} respectively). At the 8h harvest, bound ^{15}N in the shoot had increased 3.5 fold to 2822µg ^{15}N plant^{-1} , whilst that of the root had risen 2.4 fold to 1071µg ^{15}N plant^{-1}. The 3.5 fold increase in bound ^{15}N in the shoot at the 8h harvest, over that of the 4h harvest, is indicative of a considerable delay in the incorporation of newly absorbed ^{15}N into the bound forms in the shoot. This lag period appears to be related to a delay in absorbed nitrate being reduced, as no such delay occurred in maize fed with labelled ammonium.

Leaf blade and leaf base nitrate assimilatory pools are relatively small in comparison to the storage pools, this being deduced from their saturation after 4h of ^{15}N feeding at 12 and 15-18A%E ^{15}N respectively. Leaf blade and leaf base soluble organic N assimilatory pools were not saturated at the 4h harvest, their ^{15}N enrichments doubling at the 8h harvest from 13.7 to 26.2A%E ^{15}N and from 11.2 to 24.9A%E ^{15}N respectively. It is not possible to determine whether the leaf blade and leaf base soluble organic pools had saturated at the 8h harvest, nor is it possible to estimate the size of the assimilatory pools because of the possibility of a turnover of bound organic nitrogen.

At the 4h harvest 64% of the bound ^{15}N was in the shoot, and at the 8h harvest 72% of the bound ^{15}N was in the shoot, clearly showing the shoot to be the final destination of most of the newly-absorbed nitrogen. The total ^{15}N in organic forms in the shoot was greater than that of the root, and since 93% of the newly-absorbed nitrogen in the xylem stream was in the nitrate form, the bulk of the newly-absorbed nitrate must be assimilated into nitrogenous organic compounds in the shoot.

4.6.2. ^{15}N Ammonium-fed Plants.

At the 4h harvest the assimilated ^{15}N in the shoots was already greater than in the roots, in both the soluble and insoluble organic nitrogen fractions, showing the shoot to be the final destination of most of the newly-absorbed nitrogen (Figure 10 & Table 16).

The free organic N assimilatory pools of the root were already saturated at the 4h harvest (±21A%E ^{15}N), indicating that newly absorbed ^{15}N was rapidly incorporated into soluble organic compounds in the root. Most of these are immediately exported to the shoot, though some are used in the synthesis of protein required by the roots. Lewis & Chadwick (1983) found the root to be the principal region of N assimilation in ammonium-fed barley plants, and also observed that the main destination of the organic ^{15}N was the shoot.

In both root and shoot the amount of ^{15}N in the bound form was predictably greater at 8h than at 4h, with the amount in the shoot exceeding that in the root at both harvests. In both shoot and root, ^{15}N in the soluble organic fraction exceeded that of the bound fraction after 4h of ^{15}N feeding, whereas the reverse situation was observed at the 8h harvest.

Bound ^{15}N increased by a factor of 1.6 in the root and 1.8 in the shoot between the 4h and 8h harvests. As the plant masses (Table 18) and free organic N pool sizes (Table 16) were almost the same, this may be indicative of a reduced ammonium absorption rate between the 4h and 8h harvests.

Soluble organic N pools in the leaf blade, leaf base, and root were larger than those of the nitrate-fed plants (average approximately 7.6, 8.3, and 5.7mg N plant^{-1} respectively for the ammonium-fed plants, compared with 1.7, 3.5, and 2.5 mg N plant^{-1} for the nitrate-fed maize. This phenomenon has been frequently reported (Haynes & Goh, 1978), and it is believed to be the result of the detoxification of absorbed ammonium by rapid incorporation into amino acids. Unlike the free organic assimilatory pools of the nitrate-fed maize plants, the assimilatory pools were saturated at the 4h harvest at 15.5, 14-17 and 21A%E ^{15}N for the leaf blade, leaf base, and root regions respectively. No positive conclusions can be made regarding the assimilatory pool size because of the possibility of the turnover of bound organic nitrogen or cycling of amino acids from the shoot via the root. It is likely, however, that the latter organic nitrogen sources do not contribute large quantities of nitrogen to the soluble organic nitrogen pools, and consequently soluble organic N storage pools are probably considerably larger than the assimilatory pools.

Free ammonium levels (Table 19) were higher in the roots than in the leaf bases, which in turn had higher levels than the leaf blades. This was similar to the situation in nitrate-fed plants, the leaf blade and leaf base levels being comparable. As discussed in the Literature Review, the accumulation of free ammonium in the tissues (and particularly the leaf tissues) is detrimental to the plant, and it was therefore anticipated that levels of uncombined ammonium would be low. Root ammonium pools were larger than in nitrate-fed plants, though it should be noted that in ammonium fed plants the levels fluctuated greatly, with the levels at 4h exceeding those recorded at the 8h harvest.

4.6.3. Mixed Feed - 1:1 ^{15}N Ammonium + ^{15}N Nitrate.

The bound ^{15}N in the tops exceeded that in the roots at both the 4h and the 8h harvests. Between the harvests, bound ^{15}N in the shoot increased by a factor of 2.2 and that in the shoot by a factor of 1.9. The relative increase in the bound ^{15}N in the shoot after 8h of ^{15}N feeding was less than that of the ^{15}N nitrate-fed plants (where a 3.5 fold increase was observed) and more than that observed in the ammonium-fed plants (1.8 fold increase). There is clearly a delay in the incorporation of newly-absorbed nitrogen into bound organic forms with nitrate feeding, but very little or no delay with ammonium or nitrate + ammonium feeding. This delay is presumably associated with nitrate reduction, and as almost no delay was observed when nitrate + ammonium nitrogen were supplied, it appears that root-assimilated $^{15}NH_4^+$ plays a significant, and perhaps major, role in the nitrogen nutrition of the plants reared on the mixed N source.

At both the 4h and the 8h harvests the quantity of ^{15}N in the free organic fraction of the shoots was greater than the equivalent root fraction. The ^{15}N enrichments of the free organic pools increased at the 8h harvest, when compared with those of the 4h harvest, in all plant regions. Soluble organic N pools in the roots and leaf base were smaller than in nitrate-fed material, but those of the leaf blade were greater. Soluble organic N pools in all 3 plant regions were much smaller than in ammonium-fed plants. However, the leaf base, leaf blade, and root soluble organic nitrogen pools were not saturated at the 4h harvest, whereas those of the ammonium-fed plants were, suggesting that uptake of ammonium from the mixed-source nutrient solution was slower than the uptake from those nutrient media which contained only ammoniacal nitrogen. This may be the result of antagonism between nitrate and ammonium absorption, as deduced by Lewis et al (1982a) from their results with barley, or could simply be a concentration effect (when ammonium was the sole nitrogen source, it was supplied at 2mM, whereas it was only present in nitrate + ammonium nutrient solutions at a concentration of 1mM).

The free $^{15}NO_3^-$ in both tops and roots was higher at the 8h harvest than at the 4h harvest, that of the shoot exceeding that of the root in both cases. Leaf base nitrate pools were larger than those of the root, which in turn were larger than those of the leaf blade. This differed from the nitrate-fed plants, where the root contained the biggest free nitrate pools. The leaf blade nitrate pools were bigger than in nitrate-fed plants (ave. 2.8 and 1.8mg N plant^{-1} in the mixed-feed and nitrate-fed plants respectively), which is curious considering the similarity in NR activities found between maize plants grown on the two nitrogen sources.

The free nitrate pools were not saturated at the 4h harvest, the ^{15}N enrichments increasing from 5.0 to 7.8, 8.4 to 13.8, and 14.6 to 23.7A%E ^{15}N in the leaf blade, leaf base, and root regions respectively. These figures are lower than those of the nitrate-fed maize, which were saturated, or almost so, at the 4h harvest at enrichment values of approx. 12, 15-18, and 29-33A%E ^{15}N for the leaf blade, leaf base, and roots respectively. It appears that the absorption of nitrate in the mixed-feed plants was lower than in the nitrate-fed plants, bearing in mind that NR activities were similar in the two situations. This may result from repression of nitrate absorption, as suggested by Lewis et al (1982a), or could be a concentration effect resulting from the reduced nitrate concentration of the mixed-nitrogen nutrient solutions.

Free ammonium concentrations (Table 19) in the leaf blade were lower than in the leaf base, with the root having the highest free ammonium levels of all the regions. This is similar to the findings in the ammonium-fed plants, though the levels were slightly higher in the leaf blade and leaf base than those of the ammonium-fed plants. Shoot and root ammonium assimilatory pools were saturated at the 4h harvest (Figure 10 and Table 17). The ^{15}N enrichment of the free ammonium pools was higher in the roots than in the leaf base and leaf blade (46-50A%E ^{15}N in roots, 22-28A%E ^{15}N in leaf base, and 17-18A%E ^{15}N in leaf blade).

CHAPTER 5

CONCLUSIONS.

1. The growth of nitrate-fed Zea mays L. var. R201, from the seedling through to the fruiting stage, was improved by the addition of a ferric citrate supplement to the Long Ashton nutrient solution, together with a lowering of the pH of the supplied nutrient solution to pH 5.

2. A further improvement in the growth of nitrate-fed plants was obtained by rooting the maize in 9mm diameter builder's stone, instead of using a hydroponic culture system, and cycling the nutrient solution through the rooting medium from a reservoir below.

3. In vitro nitrate reductase activity of the leaf blade of 20- to 21-day-old maize was found to exhibit a diurnal rhythm, with the activity rising during the early hours of the light period to a maximum level which occurred several hours after the commencement of the light period. The NRA fell throughout the rest of the light period and during most of the dark period.

4. In 20- to 21-day-old plants in vitro nitrate reductase activity of the 5th leaf was higher than that of the 4th leaf during most of the light period, although towards the end of the light period and during the hours of darkness the activity levels of the 4th and 5th leaf blades were similar.

5. On a unit fresh weight basis, the nitrate reductase activity of the leaf blade was 7 times that of the root in nitrate-fed plants, whilst leaf blade NRA exceeded root NRA by a factor of 6 in maize plants fed nitrate + ammonium (ie plants fed with nitrate or nitrate + ammonium nitrogen had similar ratios of leaf blade:root NRA). This implies that the leaf is more likely to be the major centre of nitrate reduction than is the root when nitrate is supplied to maize.

6. Analysis of xylem exudate collected from plants fed with ^{15}N-nitrate for 4h and 8h revealed that 93% of the newly absorbed nitrate in the xylem stream was in the nitrate form, indicating that the shoot is the major region of nitrogen assimilation for nitrate-fed maize.

7. When maize was fed with ^{15}N-labelled ammonium, 67-87% of the ^{15}N in the xylem exudate was in organic forms, the remainder being detected in the ammonium form, indicating that the root is the major region of assimilation of absorbed ammonium (only trace quantities of nitrate were detected).

8. Xylem exudate collected from plants fed with labelled nitrate + labelled ammonium contained 35-55% of the newly-absorbed ^{15}N in the form of amino acids. This is considerably less than the equivalent fraction from ammonium-fed maize. The xylem exudate from the mixed-feed plants contained 43-60% of the labelled nitrogen as nitrate, this being much smaller than the equivalent fraction of sap from nitrate-fed maize. These figures indicate that both root-assimilated ammonium and translocated nitrate contribute to the newly-absorbed nitrogen exported by the roots in

plants fed with a nitrate + ammonium N source.

9. The main amino acids found in xylem sap were glutamine and asparagine, with arginine, alanine and serine also carrying a significant fraction of the organic nitrogen. These compounds have a low C:N ratio, and the translocation of nitrogen in these forms allows the movement of nitrogen from the roots to the shoots with a relatively small carbon requirement in comparison to that which would be needed were other amino acids used to carry translocated organic nitrogen.

10. A study of the effects of nitrate, ammonium, and nitrate + ammonium nitrogen sources on the growth of maize plants revealed that 48-day-old plants fed with ammonium or nitrate + ammonium had higher masses than those fed with nitrate-N as the sole nitrogen source. When ammonium was the sole nitrogen source the organic nitrogen content was larger than when nitrate or nitrate + ammonium was supplied. Nitrate nutrition produced maize with lower organic N content than feeding nitrate + ammonium.

Ammonium-fed maize was softer than maize grown with nitrate or nitrate + ammonium. This may be due to the diversion of photosynthate from cell wall production to the provision of carbon skeletons for the rapid incorporation of absorbed ammonium. This hypothesis is supported by the presence of larger free soluble organic nitrogen pools in ammonium-fed plants than were found in nitrate- and nitrate + ammonium-fed plants.

11. Comparisons of the rate of assimilation of absorbed ^{15}N showed that ammonium-fed maize had higher ^{15}N assimilation rates than plants fed with a 1:1 mixed nitrogen source, while plants fed with labelled

nitrate exhibited the lowest rate of incorporation of newly-absorbed ^{15}N.

12. Analysis of the various N fractions of plants fed with ^{15}N-nitrate revealed the existence of separate assimilatory and storage pools for nitrate and soluble nitrogenous organic compounds, and it is likely that the storage pools are considerably larger than the assimilatory pools.

The shoot was seen to be the final destination of the newly-absorbed nitrate-nitrogen, and most of the reduction and assimilation of nitrate appears to take place in the shoot.

There is an apparent lag in the appearance of newly-absorbed nitrogen in the bound fraction of the shoot of nitrate-fed maize, this possibly being due to a delay in the reduction of recently-absorbed nitrogen.

13. Extraction and analysis of the nitrogenous compounds of maize supplied with labelled ammonium revealed that most of the newly-absorbed nitrogen was destined for the shoot, but the bulk of the ^{15}N was assimilated into organic compounds in the root.

Soluble organic nitrogen pools were larger than those of nitrate-fed plants, this probably serving as a mechanism for the detoxification of the rapidly-absorbed nutrient ammonium. This hypothesis is further supported by the finding that ammonium did not accumulate in the tissues to any great extent.

14. The results obtained when ^{15}N nitrate and ammonium were supplied in the nutrient solution simultaneously were indicative of both nitrate and ammonium being absorbed and assimilated into organic compounds. It appears that the absorption of both nitrate and ammonium was reduced when compared to the rate of absorption when either one was supplied alone; this could be the result of suppression by the other inorganic nitrogen form, or simply a result of a lower rate of supply of nitrate and of ammonium than in the single-source situations (when nitrate or ammonium was supplied as the sole nitrogen source, each was present in the nutrient solution at a 2mM concentration, whereas each inorganic nitrogen form was only present at a 1mM concentration when nitrate + ammonium was supplied to the plant material.

Nutrient ammonium seemed to provide the greater portion of the newly-assimilated nitrogen in plants fed with nitrate + ammonium. This was deduced from the observation that no delay was found in the appearance of labelled nitrogen in the bound fraction, as was noted with ammonium as the sole N source; with nitrate nutrition there was a delay in the incorporation of recently-absorbed nitrogen into bound organic compounds.

15. *Zea mays* L. var. R201, grown with a water culture technique, displayed superior growth with ammonium nutrition to that obtained with nitrate nutrition. Ammonium nutrition resulted in larger plants, higher total organic nitrogen concentrations, and more rapid assimilation rates than nitrate feeding. Plants grown on a mixed-nitrogen source exhibited intermediate growth characteristics.

In field-grown plants, however, different responses to nitrate, ammonium, and nitrate + ammonium sources may be observed because of the ammonium-retentive capacities of soils, particularly those with high ion-exchange capacities where ammonium absorption could be severely retarded in comparison to that of nitrate.

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