

**A ^{14}C and ^{15}N Study of the Effects of
Ammonium or Nitrate Nutrition on Carbon Allocation
in Triticum aestivum L. and Zea mays L.**

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ABSTRACT

The poor response of some plant species, e.g. wheat, to ammonium nutrition has been attributed to a diversion of carbon allocation from structural material for root extension to functions associated with the assimilation and translocation of ammonium in the root. The aim of this research was to investigate carbon allocation in response to ammonium or nitrate nutrition in wheat, an ammonium intolerant species, and maize, which exhibits ammonium tolerance. Experiments were carried out at 4mM and 12mM nitrogen feeding levels in sand and hydroponic culture respectively. pH of growth media was maintained at 6.0 to 6.5. Measurements made included shoot : root ratios, photosynthetic and root respiratory rates, plant water content, xylem sap analysis, and ^{14}C and ^{15}N allocation to soluble and bound nitrogen compounds, and soluble, storage and structural carbohydrates.

Stunted root growth occurred in ammonium-fed wheat, which was exacerbated by increasing the NH_4 concentration. No difference in growth response was evident between ammonium- and nitrate-fed maize. Photosynthetic rates of ammonium- and nitrate-fed plants within both species were similar but maize showed a 3-fold higher photosynthetic rate than wheat. Root respiration of ammonium- and nitrate-fed wheat was similar, while nitrate-fed maize appeared to have a higher root respiratory rate than ammonium-fed maize. Xylem sap analysis showed that for both species, ammonium-fed plants translocated more amino compounds and more carbon to the shoots than nitrate-fed plants, although maize appeared to have a more rapid translocation rate than wheat.

^{14}C allocation to nitrogenous compounds in roots of ammonium-fed plants was greater than that in nitrate-fed counterparts for both species. In wheat this increase appeared to be accommodated by a larger initial diversion of ^{14}C to the root. In maize, reserve carbon in the root appeared to accommodate this increase. A reduction in ^{14}C allocation to structural material in ammonium-fed plants compared to nitrate-fed counterparts was not evident in either species.

^{15}N tracing in maize showed that significantly more nitrogen was taken up by ammonium-fed plants in comparison to nitrate-fed plants. The difference in total N between plants fed ammonium or nitrate was, however, not nearly as pronounced, suggesting that ammonium may be cycled out of the plant again.

The response of wheat and maize to ammonium or nitrate nutrition is discussed independently, and suggestions for further research are made.

PREFACE

The work reported in this thesis was carried out as part of the research directive of the Plant Nitrogen Physiology Research Grant at the University of Cape Town Botany Department, headed by Professor O.A.M. Lewis. The broad framework of this directive is outlined in chapter 1. Chapter 2 provides a literature survey of relevant research, including work carried out by the Research Grant, to put the research reported here in context. Aims and scope of the research are outlined at the end of this chapter. Chapters 3 and 4 deal respectively with materials and methods used, and results obtained. These two chapters have been structured to follow the same format, so that the methods used and results obtained from them are easily cross referenced. To maintain clarity, pilot studies carried out for the selection of suitable methods have been placed in appendices. In chapter 5, the results obtained for the two plant species under study are discussed independently, in an effort to draw together the overall strategy employed by each in response to the nitrogen feeding regimes imposed. This chapter concludes with suggestions for future research. Brief concluding remarks are made.

This research was presented by the author at the 11th International Nitrogen Physiology Congress, held in Naples in 1989. This was made possible by generous funding from the F.R.D. Aspects of this research are published in the Conference Proceedings (Lewis *et al.*, 1990).

DECLARATION

I declare that this is my own, unaided work.

It has not been submitted before for any degree or examination in any other university.

Signed by candidate

Martina van der Leij

May, 1991

University of Cape Town

TABLE OF CONTENTS

	Page
Abstract	ii
Preface	iii
Declaration	iv
Acknowledgements	x
Abbreviations and Symbols	xi
List of Figures	xii
List of Tables	xiv
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: BACKGROUND	4
2.1. Nitrogen in the Soil.....	4
2.2. Ammonium vs. Nitrate Nutrition.....	5
2.2.1. Energy Considerations	5
2.2.2. Potential Advantages of Ammonium over Nitrate as a Nitrogen Source.....	8
2.2.3. Plant Response to Ammonium Nutrition	9
2.3. Acquisition of Nitrogen.....	9
2.3.1. Nitrate Nutrition and Assimilation	10
2.3.2. Ammonium Nutrition and Assimilation.....	12
2.4. Ammonium Toxicity and Proposed Explanations for Poor Response to Ammonium Nutrition	14
2.5. Scope, Aims and Structure of this Research.....	18

CHAPTER 3: MATERIALS AND METHODS	21
3.1. Plant Cultivation.....	21
3.1.1. Sand Culture	22
3.1.2. Hydroponic Culture.....	23
3.1.3. Growth Conditions.....	25
3.1.3.1. Maize	26
3.1.3.2. Wheat.....	26
3.2. Experimental Measurements and Procedures.....	29
3.2.1. Photosynthesis.....	29
3.2.2. Root Respiration.....	29
3.2.3. Xylem Sap	30
3.2.4. Plant Water Content.....	30
3.2.5. Labelling with ^{14}C	31
3.2.5.1. ^{14}C Feeding Apparatus	31
3.2.5.2. ^{14}C Feeding Procedure	32
3.2.6. Dual Labelling with ^{15}N and ^{14}C	33
3.2.7. Harvesting and Ethanol Extraction.....	35
3.2.8. Separation and Quantification of Carbon Fractions.....	35
3.2.8.1. The Ethanol-Soluble Fraction	36
3.2.8.2. The Ethanol-Insoluble Fraction	36
3.2.8.3. ^{14}C Sample Analysis	37
3.2.9. Separation and Quantification of Nitrogen Fractions.....	40
3.2.9.1. The Ethanol-Soluble Fraction	40
3.2.9.2. The Ethanol-Insoluble Fraction	41
3.2.9.3. ^{15}N Sample Analysis	41
3.3. Statistical Analysis.....	43

CHAPTER 4: RESULTS	44
4.1. Plant Growth Responses.....	44
4.2. Photosynthesis.....	45
4.3. Root Respiration.....	45
4.4. Xylem Sap.....	45
4.5. Plant Water Content.....	46
4.6. ^{14}C Carbon Distribution.....	52
4.6.1. Ratio of Total ^{14}C Distribution between Root and Shoot	52
4.6.2. ^{14}C Allocation to Carbohydrate and Nitrogen Compounds within the Root and Shoot.....	53
4.6.2.1. Wheat.....	53
4.6.2.1.1. ^{14}C Distribution within the Wheat Root.....	53
4.6.2.1.2. ^{14}C Distribution within the Wheat Shoot	55
4.6.2.2. Maize	56
4.6.2.2.1. ^{14}C Distribution within the Maize Root	56
4.6.2.2.2. ^{14}C Distribution within the Maize Shoot.....	58
4.6.3. ^{14}C Allocation to Carbohydrate and Nitrogenous Compounds on a Whole Plant Basis.....	65
4.6.3.1. ^{14}C Allocation in Wheat on a Whole Plant Basis.....	65
4.6.3.2. ^{14}C Allocation in Maize on a Whole Plant Basis.....	67
4.7. Total N and ^{15}N Distribution in Maize.....	71

CHAPTER 5 : DISCUSSION	73
5.1. Wheat	73
5.1.1. Plant Growth Responses.....	73
5.1.2. Photosynthesis.....	73
5.1.3. Root Respiration.....	74
5.1.4. Xylem Sap	75
5.1.5. Plant Water Content.....	76
5.1.6. ^{14}C Allocation to Nitrogenous Compounds.....	77
5.1.7. ^{14}C Allocation to Structural Material.....	77
5.1.8. Summary of Wheat Response to Nitrogen Form.....	78
5.2. Maize	80
5.2.1. Plant Growth Responses.....	80
5.2.2. Photosynthesis.....	80
5.2.3. Root Respiration.....	80
5.2.4. Xylem Sap	81
5.2.5. Plant Water Content.....	82
5.2.6. ^{14}C Allocation to Nitrogenous Compounds.....	82
5.2.7. ^{14}C Allocation to Structural Compounds.....	83
5.2.8. ^{15}N and Total Nitrogen Content of Maize.....	83
5.2.9. Summary of Maize Response to Nitrogen Form	84
5.3. Comparison of Wheat and Maize Response to Nitrogen Form.....	85
5.4. Suggestions for Further Research.....	86

CONCLUSIONS	88
APPENDICES	90
Appendix 1: Pilot Study of the Maintenance of pH in Sand Culture Experiments..	90
Appendix 2: Pilot Studies optimizing Hydroponic Cultivation of Maize	91
REFERENCES	93

University of Cape Town

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ABBREVIATIONS AND SYMBOLS

A%E	Atom percent excess
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
GOGAT	Glutamate synthase
GS	Glutamine synthetase
IRGA	Infra red gas analyser
NADH	Nicotinamide adenine dinucleotide (reduced)
NADPH	Nicotinamide adenine dinucleotide phosphate
NiR	Nitrite reductase
Nitrapyrin	2-chloro-6(trichloromethyl) pyridine
NR	Nitrate Reductase
p.p.m.	Parts per million
S.E.	Standard error

LIST OF FIGURES

	Page
Figure 1: ^{14}C Feeding apparatus	34
Figure 2: Flowchart showing the process of fractionation carried out for quantification of ^{14}C allocation ratios to ethanol-soluble and -insoluble nitrogenous and carbohydrate fractions.	39
Figure 3: Shoot:root ratio of plant fresh mass for wheat and maize fed ammonium or nitrate at 4mM and 12mM concentrations.	47
Figure 4: Photosynthetic rate of wheat and maize supplied either ammonium or nitrate.	48
Figure 5: Root respiration of wheat and maize supplied either ammonium or nitrate.	49
Figure 6: Ratio of shoot:root ^{14}C allocation in wheat and maize fed ammonium or nitrate at 4mM or 12mM concentrations.	60

Figure 7: Ratio of ^{14}C allocation to carbohydrate : nitrogenous compounds in the roots and shoots of wheat fed ammonium or nitrate at 4mM or 12mM concentrations.

61

Figure 8: Ratio of ^{14}C allocation to soluble and insoluble nitrogenous- and carbohydrate fractions within the root and shoot of wheat supplied ammonium or nitrate at 4mM or 12mM concentrations.

62

Figure 9: Ratio of ^{14}C allocation to carbohydrate : nitrogenous compounds in the roots and shoots of maize fed ammonium or nitrate at 4mM or 12mM concentrations.

63

Figure 10: Ratio of ^{14}C allocation to soluble and insoluble nitrogenous- and carbohydrate fractions within the root and shoot of maize supplied ammonium or nitrate at 4mM or 12mM concentrations.

64

LIST OF TABLES

	Page
Table 1: Total nitrogen and ^{15}N concentration in the roots and shoots of wheat supplied either ammonium as 12mM 99A% ^{15}N NH_4Cl or nitrate as 12mM 99A% ^{15}N KNO_3 (data from Cramer, M. unpublished).	20
Table 2: Macro- and micro-nutrients in 10dm ³ of final feeding solution supplied to wheat (Hewitt, 1966).	27
Table 3: Macro- and micro-nutrients in 10dm ³ of final feeding solution supplied to maize (modified from Hewitt, 1966).	28
Table 4: C : N ratio, total amino acid and ammonium content of xylem sap from wheat or maize fed ammonium or nitrate.	50
Table 5: Dry mass as a percentage of fresh mass of wheat and maize fed 4mM ammonium or nitrate.	51
Table 6: Allocation of ^{14}C on a whole-plant percentage basis, to the carbon fractions of wheat fed either ammonium or nitrate at 4mM and 12mM concentrations.	69

CHAPTER 1: INTRODUCTION

With human population numbers constantly increasing and malnutrition a serious problem in third world countries, the provision of cost-effective, high quality, protein-rich crops is at a premium in these areas. This is particularly relevant in the South African context, where diseases stemming from protein deficiency, such as kwashiorkor and dysentery, are a common problem.

Nitrogen is the principal constituent of plants and accounts for at least half of the total ions absorbed. It is a structural component of protein, as well as vital molecules such as nucleic acids and co-enzymes. Available nitrogen is often the most limiting factor for plant growth, severely restricting the yield of crop plants that could supply dietary protein for humans.

Nitrogen fertilizer efficiency is low (less than 50%) because of nitrate leaching and volatilization. With increasing fertilizer costs, methods of improving fertilizer efficiency, and thus crop yield, would be advantageous.

Since ammonium is not readily lost from the soil due to its positive charge, ammonium fertilizers used in conjunction with nitrification inhibitors have the potential to increase fertilizer efficiency, as well as reducing pollution of waterways.

Nitrogen use efficiency of plants may be increased by using ammonium, since this nitrogen form, unlike nitrate, does not require energetically costly reduction before incorporation into nitrogen compounds. Protein and mineral composition of cereal crops may also be enhanced by the supply of ammonium fertilizers (De Kock, 1970; Cox and Reisenauer, 1973; Ikeda *et al.*, 1974).

Despite these apparent advantages of ammonium nutrition, plant response is not always favourable. Some species (wheat, barley) exhibit poor response to this nitrogen form and yield is severely reduced (Lewis and Chadwick, 1983; Lewis *et al.*, 1987), while others (including maize) respond favourably with enhanced productivity (Blair *et al.*, 1970; Ikeda and Osawa, 1979, 1980, 1981; Murphy and Lewis , 1987).

The understanding of the mechanisms governing these responses would be invaluable for the prescription of fertilizer regimes and for the possible genetic manipulation of crop species to increase nitrogen use efficiency, yield and cost effectiveness of fertilization.

The poor response of some species to ammonium nutrition has been ascribed to an alteration of carbon allocation, where carbon is diverted from structural incorporation (growth) to compulsory ammonium assimilation to prevent ammonium toxicity in the root (Lewis and Chadwick, 1983; Lewis *et al.*, 1987).

It was the purpose of this research to test this explanation of the poor response of some plants to ammonium nutrition. With this aim, the carbon allocation of an ammonium tolerant (maize) and an ammonium intolerant (wheat) species supplied either ammonium or nitrate as a nitrogen source was investigated. Maize was selected since it is also an agronomically important cereal crop and exhibits ammonium tolerance without being pre-adapted to acidic- or waterlogged soils, where ammonium may be the predominant form of nitrogen.

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CHAPTER 2: BACKGROUND

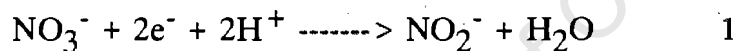
2.1. Nitrogen in the Soil

The decomposition of plant material in the soil releases nitrogen in the form of ammonium. However, the most predominant form of nitrogen in the soil is nitrate because of the presence of nitrifying bacteria such as *Nitrosomonas*, which oxidise ammonium to nitrite and *Nitrobacter*, which oxidise nitrite to nitrate. Both bacteria are chemosynthetic and oxidise ammonium to nitrate for the production of ATP. The activity of these bacteria is dependent on good aeration, since molecular oxygen is the terminal electron acceptor from the first oxidation (ammonium to nitrite), and good water availability, since the oxygen atom which facilitates the formation of nitrate from nitrite is derived from water (Aleem, 1970). In addition to good aeration and good water availability, bacterial activity is also favoured by pH's close to neutral, and by fairly high temperatures. These are the characteristics of fertile soils. Working with intact bacteria, Aleem (1970) showed that both of these nitrifying organisms recover energy from the oxidation of ammonium to nitrate inefficiently. This suggests that vigorous oxidation is necessary to support metabolism in these organisms and explains the rapid conversion of ammonium to nitrate under favourable conditions.

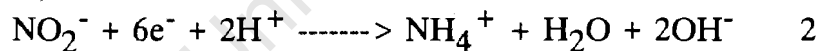
2.2. Ammonium versus Nitrate Nutrition

2.2.1. Energy Considerations

Assimilatory nitrate reduction in higher plants involves the reduction of nitrate via nitrite to ammonium and its subsequent incorporation into organic nitrogenous compounds (reviewed in Milfin, 1980). This is in essence a reversal of the processes occurring in the soil. This reduction of nitrate from its state of maximum oxidation to its state of maximum reduction requires eight electrons and is catalysed by the action of two enzymes, nitrate reductase (NR : E.C. 1.6.6.1.) and nitrite reductase (NiR : E.C. 1.7.7.1.). NR catalyses the following reaction:

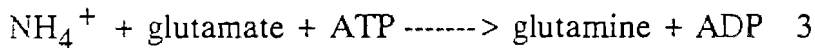


with NADH acting as the electron donor (Beever and Hageman, 1969). Nitrite reductase catalyses the further reduction of the nitrite produced as follows:

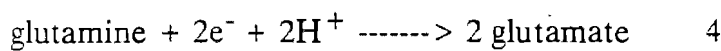


These two reactions require a total of $350 \text{ kJmol}^{-1} \text{NO}_3^-$, the energy equivalent of 15-16 ATP molecules per NH_4^+ molecule.

The incorporation of ammonium into nitrogenous compounds occurs serially via two enzymes; glutamine synthetase (GS : E.C. 6.3.1.2.; L-Glutamate:ammonia ligase), which catalyses the reaction:



and glutamate synthase (GOGAT : E.C. 2.6.1.53.; L-glutamine 2-oxoglutarate aminotransferase), which catalyses the subsequent reaction:



where the electron donor in leaves is reduced ferredoxin, and in roots NADH. This pathway is known as the GS/GOGAT pathway and consumes only 5 ATP molecules per ammonia molecule.

From these equations, it appears that in terms of energy considerations, it would be more beneficial to plants to utilize ammonium as a nitrogen source. However, for most agricultural plants, nitrate is the principal source of nitrogen. This appears to be a direct consequence of nitrate being the predominant form of nitrogen available in fertile soils (i.e. non-acidic soils with good aeration and available water : Section 2.1.).

In addition to the energetic costs of reducing nitrate to ammonium, the active uptake of nitrate also requires energy, unlike ammonium, which enters the plant by passive diffusion. The uptake of the nitrate anion is opposed by the difference in transmembrane electrical potential, and energy is required to overcome this

repulsion. Also, given that plants are able to store nitrate, there exists a higher concentration within the plant cells than in the external medium, which necessitates nitrate transport against a concentration gradient. Although a membrane carrier for nitrate has not been identified, the existence of nitrate permeases has been postulated (Jackson, 1978). The active, energy-requiring nature of nitrate uptake is evidenced by the sensitivity of the process to anaerobic conditions (Trought and Drew, 1981) and to uncoupling of respiration (Dean-Drummond and Glass, 1983).

It therefore appears that reduction and utilization of nitrate is energetically more expensive than that of ammonium. This metabolic energy is produced photosynthetically : the photosynthetic light reaction provides reductants directly in the form of reduced ferredoxin, and indirectly as NADPH and NADH for the conversion of nitrate to ammonium (Foyer, 1984). As a consequence of this, it may be inferred that if it is possible to ensure a supply of ammonium to plants in the field, then these plants will have a greater source of energy and carbon available for other functions. These may include the biosynthesis of macromolecules which would be reflected in a greater production of dry matter in plants fed ammonium.

2.2.2. Potential Advantages of Ammonium over Nitrate as a Nitrogen Source

The development of nitrification inhibitors, for example Nitrapyrin (trade name N-Serve, Dow Chemical Company) facilitates the persistence of ammonium in the soil by slowing bacterial nitrification (section 2.1.). An advantage is that ammonium is less easily leached from the soil than nitrate, due to its positive charge (Reisenauer, 1978) and therefore remains available to plants for longer (Chancy and Kamprath, 1982). Eutrophication of rivers, a pollution problem caused by leaching of nitrate fertilizers (van Dienst, 1986), will also be reduced if ammonium is used.

In addition to the proposed advantages of ammonium over nitrate in relation to fertilizer application, this nitrogen form may enhance nitrogen availability at crucial developmental stages, for example at grain filling, when nitrate reducing potential is low (Soares and Lewis, 1986). Ammonium may also enhance cold tolerance in areas prone to frost, since ammonium absorption is not affected by low temperatures while nitrate absorption is (MacDuff *et al.*, 1987). Photosynthetic rate has been reported to be higher in ammonium- than in nitrate-fed plants (Field and Mooney, 1986, and references therein). This is postulated to be due to the higher nitrogen content of ammonium-fed plants (due to free diffusion of this ion into the plant) since photosynthetic rate is linearly related to nitrogen content of the shoot (Field and Mooney, 1986).

2.2.3. Plant Response to Ammonium Nutrition

Superficially, the utilization of ammonium fertilizers in tandem with nitrification inhibitors appears to be an excellent approach to nitrogen fertilization. In reality however, numerous species (for example bean, cucumber, pea, radish, barley, wheat) appear to be adversely affected by ammonium nutrition, and often exhibit a large decrease in yield (Maynard and Barker, 1969; Haynes and Goh 1978; McElhannon and Mills, 1978). This is very prominently manifested in retarded root growth (Bennett, *et al.*, 1964; Haynes and Goh, 1977, 1978; Lewis and Chadwick, 1983; Lewis *et al.*, 1987). Other species such as maize (Morris and Giddens, 1963; Blair *et al.*, 1970; Murphy and Lewis 1987), rice (Wahhab and Bhatti, 1957), and members of the Ericaceae (Colgrove and Roberts, 1956) are ammonium tolerant and may even show a preference for ammonium nutrition.

It seems that the apparent advantages afforded by ammonium nutrition are a simplification and that a more detailed account of the mechanisms of nitrate and ammonium nutrition is required.

2.3. Acquisition of Nitrogen

Plant acquisition of nitrogen in either ionic form, requires three basic steps - absorption of the ion from the soil, translocation of the ion within the plant, and its assimilation into an organic form such as amino acids. These three processes differ greatly, depending on whether the ion is nitrate or ammonium.

2.3.1. Nitrate Nutrition and Assimilation

Uptake of nitrate is an active, energy-requiring process (Huffaker and Rains, 1978) associated with alkalization of the external medium. This alkalization was explained by Lips *et al.* (1970) in a model for nitrate uptake where malate is transported in conjunction with potassium ions from the shoot to the root. In the root, malate is oxidized and decarboxylated to form bicarbonate. This bicarbonate ion is exchanged for nitrate from the external medium resulting in a pH increase of the rhizosphere. Although supporting evidence has been published (Kirkby and Armstrong, 1980), no direct evidence confirms this mechanism (Schrader and Thomas, 1981). With the large amounts of nitrate absorbed by the plant, the scheme proposed by Lips *et al.* (1970) suggests that large amounts of carbon will be lost from the plant during nitrate uptake. Various other models have been proposed more recently. For example, Ulrich and Novacky (1981) put forward a $2\text{H}^+ : 1\text{NO}_3^-$ symport model; Thibaud and Grignon (1981) proposed a $2\text{NO}_3^- : 1\text{OH}^-$ antiport model and Dean-Drummond (1984) a $\text{NO}_3^- : \text{NO}_3^-$ exchange system. There is however, no consensus to date on the most representative model of nitrate uptake.

Nitrate nutrition in plants is also associated with an increase in malate production (Ben Zioni *et al.*, 1971). Comparison of malate production in nitrate-fed maize with or without tungstate application to inhibit nitrate reductase, has shown that the increase in malate is due to the presence of the nitrate ion, rather than its assimilation (Blackwood and Mifflin, 1976). The precise reason for this increase in malate is not known, although it has been viewed as a necessary disadvantage of nitrate nutrition to compensate for the development of a cation/anion imbalance

when the nitrate ion is absorbed. This malate may be stored in vacuoles or catabolised in the root to maintain ionic balance. The production and fate of malate may use about 2% of the available energy (Raven and Smith, 1976) but is considered by some workers to be necessary for osmoregulation (Smirnov and Stewart, 1985; Chaillou *et al.*, 1986; Stienstra, 1986).

Nitrate ions are taken up in large amounts and may accumulate in the vacuole of cells of both roots and shoots (Oaks, 1986). This accumulation may serve as a reservoir for a continued supply of nitrogen if conditions become unfavourable for nitrogen uptake.

The first step of nitrate reduction, from nitrate to nitrite, is catalysed by the inducible enzyme NR using NADH as a reductant (Equation 1). NR is a water soluble enzyme located in the cytoplasm of both leaf and root tissue (Lee, 1980). The reduction and assimilation of nitrate may take place in the root and/or shoot, depending on the species (Pate, 1980). Wheat appears to assimilate nitrate mainly in the shoot (Lewis *et al.*, 1987), although Champigny and Talouizte (1986) found that both uptake and reduction of nitrate was correlated with root carbohydrate status in this species. Maize given nitrate was shown to assimilate 93% of newly absorbed nitrogen in the shoots (Murphy and Lewis, 1987).

Schrader and Thomas (1981) noted that if excess reductant is present in the chloroplasts, then nitrate reduction in green leaves in the light may be essentially 'free' to plants. Reduction in the absence of photosynthetic reductants (in the dark, in light that limits photosynthesis, or in non-chlorophyllous tissue) requires the oxidation of carbohydrates or organic acids. Under the latter conditions, nitrate

assimilation consumes up to 15% of the total energy production of the plant (Penning de Fries *et al.*, 1974). Although the latter process may be necessary to provide nitrogen during the night, it may be energetically more economical to reduce the bulk of nitrogen required during the day in the shoot, which would require its transport from the root. The energy cost of transport and accumulation of nitrate in the plant is relatively low ($10\text{-}30 \text{ kJmol}^{-1} \text{ NO}_3^-$; Touraine and Grignon, 1982). However, even if this low-cost nitrate reduction does occur, the reduction of nitrate to ammonium is still dependent on photosynthetically-generated carbon for the provision of carbon skeletons and energy for the production of the enzymes nitrate- and nitrite reductase (Stulen, 1986).

NiR, which converts nitrite to ammonium (Equation 2), is found within the chloroplasts of leaf tissue (Wallsgrave *et al.* 1979), and associated with proplastids in non-green tissue (Emes and Fowler, 1979). This ensures that whether nitrate is reduced in the root or in the shoot, NiR is in close proximity to continue this reduction, thus preventing the accumulation of nitrite. Ammonium formed via the action of this enzyme is then immediately incorporated into nitrogen compounds as described below (Section 2.3.2.).

2.3.2. Ammonium Nutrition and Assimilation

Although its subsequent assimilation is essentially the same regardless of source, ammonium generated within the plant via nitrate assimilation presents a different situation from ammonium which has an exogenous source. When generated via nitrate assimilation, the steady-state concentrations of ammonium are low due to its

enzymatically controlled production both spatially and quantitatively (Neyra and Hageman, 1978; Goyal and Huffaker, 1981). Exogenous ammonium may be absorbed in large amounts compared to nitrate ions (Lewis and Chadwick, 1983) and may not immediately be restricted to the sites of ammonium assimilation in the cell (Goyal and Huffaker, 1984). The following discussion pertains mainly to the fate and effects of exogenous ammonium, although the mechanism of amino acid production is applicable to ammonium regardless of origin.

Ammonium transport through the plasmalemma is a passive process, although it is affected by the availability of counter-ions (Ruffy, *et al.*, 1982) and their membrane permeability (Lycklama, 1963). Since the accumulation of ammonium is toxic (see section 2.4.1.), translocation or storage of the ion in the vacuole is not possible. Very little or no free ammonium is transported in the xylem (Ivanko and Ingversen, 1971). Ammonium is rapidly assimilated in the roots (Pate, 1980; Lewis and Chadwick, 1983) by conversion to organic compounds, thus ensuring a constantly high diffusion gradient (Mifflin and Lea, 1976; Givan, 1979). The formation of these compounds liberates protons that must be eliminated from the cytoplasm to prevent acidification. This H^+ ion is liberated into the rhizosphere causing an increase in external medium pH (Raven and Smith, 1976).

The assimilation of ammonium into organic compounds occurs via a cycle that requires the catalysis of one ATP molecule and two electrons per ammonium molecule (Oaks and Hirel, 1985). GS is localized in the plastids and cytosol of photosynthetic and non-photosynthetic tissue (Lee, 1980), which makes it available for the immediate detoxification of ammonium formed by NiR activity (Equation 3), as well as that entering the roots by diffusion.

Glutamine formed by GS is converted to two glutamate molecules via GOGAT, an enzyme localized in the chloroplasts of leaf tissue (Wallsgrave *et al.*, 1979) and associated with proplastids in non-green tissue (Emes and Fowler, 1979). GOGAT utilizes reduced ferredoxin from the photosynthetic light reaction to produce glutamate in the leaves, while in the roots, NADH is the electron donor (Equation 4). The energy required for the assimilation of ammonium in the roots is supplied by respiration, and may consume 2-5% of the total energy production of the plant (Oaks and Hirel, 1985).

2.4. Ammonium Toxicity and Proposed Explanations for the poor Response to Ammonium Nutrition

The toxic effects of ammonium ions were originally thought to be caused by the increased acidity of the growth medium due to the expulsion of hydrogen ions during ammonium assimilation (Hewitt, 1966; Maynard and Barker, 1969). Some workers found that the use of CaCO_3 in the feeding solution prevented ammonium inhibition of plant growth (Barker *et al.*, 1966a, b; 1967; Maynard and Barker, 1969). As an explanation for the restoration of growth rate when pH was regulated, it was suggested that lowered pH affected growth indirectly, by increasing the concentration of aqueous ammonia in solution (Bennett and Adams, 1970). Since ammonium nutrition is known to lower medium pH, however, and since a low pH causes the ratio of $\text{NH}_4^+:\text{NH}_3(\text{aq})$ to favour NH_4^+ (Weir *et al.*, 1972), it is not clear how buffering the solution improved growth if the toxic effect was due to higher levels of exogenous ammonia. However, other workers found that buffering of nutrient solutions did not ameliorate the effects of ammonium, and root growth

was still stunted (Lewis and Chadwick, 1983; Lewis *et al.*, 1987). Ammonium is also considered to have a toxic effect on cells by creating high free ammonium concentrations within cells, rather than by lowering external pH. However, high levels of internal ammonium are very rarely found, since plants have efficient mechanisms that ensure the rapid conversion of ammonium to innocuous compounds. These processes have been identified by Givan (1979) as the glutamine synthetase/glutamate synthase cycle (operating at normal ammonium levels) and the glutamate dehydrogenase and asparagine synthetase reactions (operating at high ammonium levels).

The toxic effects of ammonium have been ascribed to its uncoupling of cyclic phosphorylation (Mills and Jones, 1979 and references therein). However, most of the experiments exhibiting this, have been carried out on isolated leaf discs or chloroplasts (Gibbs and Calo, 1959; Krogmann *et al.*, 1959; Avron, 1960). Analyses of xylem and phloem fluids from whole plants fed ammonium imply that very little, if any, free ammonium is transported to the shoots (Pate, 1980) due to efficient assimilatory cycles. Uncoupling of cyclic phosphorylation, although possible, would therefore very rarely occur in intact plants. Free ammonium does, however, appear to cause collapse of the transmembrane proton gradients required by many metabolic processes (Kleiner, 1981). This may occur in the root or shoot, but is more likely to disrupt root functioning, due to the unlikelihood of ammonium being transported to the shoot.

The priority avoidance of ammonium toxicity by the plant is widely believed to be the cause for the poor response of some species to ammonium nutrition. The reduced amount of carbon available for growth, especially in the root, due to assimilation of ammonium taken up from the growing medium, leads to stunting (Kirkby and Hughes, 1970; Micheal *et al.*, 1970; Lewis and Chadwick, 1983; Lewis *et al.*, 1987). Processes that might cause a reduction in carbon allocation to root growth are the following:

- 1) The carbon supply to the root of ammonium-fed plants has to be diverted from structural material (for root extension) to the provision of carbon skeletons for the products of nitrogen assimilation (Lewis *et al.*, 1987).
- 2) Nitrogen is exported to the shoot accompanied by carbon. This could cause a further decrease in carbon available for root extension (Lewis *et al.*, 1987).
- 3) Ammonium-fed plants require immediate energy for large-scale ammonium assimilation and detoxification in their roots, causing a greater loss of carbon via increased root respiration in comparison to nitrate-fed plants (Willis and Yemm, 1955; Givan, 1979 and references therein). In contrast, when species are supplied nitrate, they are able to store and assimilate it as they require. In those species where assimilation occurs predominantly in the leaf, this occurs in close proximity to the photosynthetic source of energy and carbon skeletons.

Recent ^{15}N -tracing experiments carried out in our laboratory (Cramer, M. unpublished), have shown that ammonium-fed plants have far higher total nitrogen and newly-assimilated nitrogen contents in both root and shoot, compared to nitrate-fed plants. These data, kindly provided by M. Cramer, are shown in Table 1. The total bound nitrogen in the root is 4.4mg/g for ammonium-fed, and 1.8 for nitrate-fed plants. In the shoot, these high levels of bound nitrogen are not evident in ammonium- as against nitrate-fed plants (4.6 as against 4.9mg/g respectively). Newly-assimilated nitrogen is present in far higher levels in ammonium- than in nitrate-fed roots (Table 1). This higher uptake of newly assimilated nitrogen in ammonium-fed plants, compared with nitrate-fed plants, is reflected in the total nitrogen content of the root. The ratio of newly-assimilated nitrogen of ammonium- : nitrate-fed plants is 3.0 : 1 for roots and 0.96 : 1 for shoots. The ratio of total nitrogen of ammonium- : nitrate-fed plants, is somewhat lower in the roots than the ratio for newly assimilated nitrogen above, at 2.55 : 1, and slightly higher in the shoots at 1.2 : 1. This implies that not all the newly assimilated nitrogen is accounted for in the total nitrogen content for ammonium-fed plants, and that translocation to the shoot occurs fairly slowly.

From these data, it can be seen that ammonium-fed wheat appear to take up more nitrogen than wheat supplied nitrate, and that this is reflected in the overall nitrogen status of these plants, especially the roots. Since this ammonium requires detoxification in the root before transport to the shoot, it is suggested that the combined effects of root respiratory energy loss, loss of carbon skeletons to the immediate synthesis of nitrogen compounds, and their subsequent translocation to the shoot in this form, retard root growth severely, thus causing the poor response to ammonium nutrition frequently observed.

Ammonium-tolerant plants such as maize may be able to cope with this increased demand for carbon through their greater carbon-gaining capacity afforded by the C-4 photosynthetic pathway.

2.5. Scope, Aims and Structure of this Research

This work investigates aspects of carbon allocation in response to ammonium and nitrate nutrition in maize and wheat, in an effort to explain observed differences in growth response to these nitrogen regimes. More specifically, the questions addressed are:

- 1) Is carbon diverted from structural material to nitrogen compounds in the roots of ammonium-fed plants, thus causing a retardation of root growth?
- 2) Compared to nitrate-fed plants, does the necessary assimilation of ammonium by the roots of ammonium-fed plants cause an increase in carbon lost to root respiration?
- 3) Does the necessary transportation of ammonium-derived nitrogen to the shoot in conjunction with carbon deplete available carbon supplies in the root?
- 4) How does ammonium-intolerant wheat response differ from that of ammonium-tolerant maize?

Firstly, the carbon gain (by photosynthesis) and carbon losses (via root respiration) were measured for ammonium- and nitrate-fed plants, and the nitrogen content of the xylem sap determined. ^{14}C -tracing experiments were then used to see if there were any differences in carbon allocation to root and shoot between the two treatments, and to detect differences in carbon allocation to nitrogen and carbohydrate compounds within the root and the shoot. The carbon fractions isolated were the soluble nitrogen fraction (mainly amino compounds but including the enzyme Rubisco); the insoluble nitrogen fraction (mainly protein); the soluble carbohydrate fraction (sugars); the storage carbohydrate fraction (starch) and the structural carbohydrate (cellulose). Since all but the structural carbohydrate fraction may be translocated from the root to the shoot and *vice versa*, the carbon allocation to these pools on a whole-plant basis was also calculated. The aim was to use these measurements to clarify the proposed strategies of carbon allocation in wheat fed either ammonium or nitrate.

Direct statistical comparison of wheat and maize (an ammonium tolerant species) was not possible, due to the higher carbon-gaining capacity of maize afforded by the C-4 photosynthetic pathway, and because of the different growth requirements of the two species. Carbon allocation in maize was therefore analysed independently, using the same methods as for wheat. The total nitrogen and ^{15}N (newly-assimilated) allocation to soluble and bound nitrogen fractions was also determined. These data provided information on carbon allocation in response to nitrate or ammonium nutrition in an ammonium-tolerant species.

Table 1: Total nitrogen (mg N g^{-1} fw) and ^{15}N concentration ($\mu\text{g N g}^{-1}$ fw) in roots and shoots of wheat supplied either ammonium as $12\text{mM } 99\text{A}\% \text{E } ^{15}\text{N NH}_4\text{Cl}$, or nitrate as $12\text{mM } 99\text{A}\% \text{E } ^{15}\text{N KNO}_3$ (data from Cramer, M. unpublished, means of 3 replicates).

N-Fraction	Nitrogen Treatment	ROOT		SHOOT	
		N conc. mgN/g fw	^{15}N conc. $\mu\text{gN/g fw}$	N conc. mgN/g fw	^{15}N conc $\mu\text{gN/g fw}$
Soluble	12mM ammonium	2.68	391.68	2.60	147.74
	12mM nitrate	0.97	126.61	1.48	142.04
Bound	12mM ammonium	4.35	170.06	4.59	66.18
	12mM nitrate	1.79	61.91	4.78	80.27

CHAPTER 3: MATERIALS AND METHODS

3.1. Plant Cultivation

^{14}C Feeding experiments were carried out on both wheat and maize using the following two plant culture regimes:

a) A soil-grown plant culture fed either 4mM ammonium (as NH_4Cl) or 4mM nitrate (as KNO_3).

b) A hydroponic plant culture fed either 12mM ammonium (as NH_4Cl) or 12mM nitrate (as KNO_3).

Regime b) provided a high nitrogen supply and a fast nitrogen diffusion rate relative to regime a). Any differences in carbon allocation patterns observed between nitrate- and ammonium-fed plants in the first experiment should be emphasized in the second experiment, if they were attributable to an increased stress imposed by ammonium nutrition. The latter experiments also allowed for the simultaneous feeding and tracing of ^{15}N .

3.1.1. Sand Culture

Plastic pots of 1.7dm³ capacity were acid-washed with a solution of 5% HCl and rinsed four times with de-ionized water. The bottom of the pots were lined with net (to retain the sand), covered with a layer of coarse builders' stone (for good drainage), and filled with sand.

The sand used for the soil culture experiments was highly leached, fine river sand (Consol Glass, Cape Town). This was first tested for ammonium (indolphenol blue method), nitrate (copper cadmium reduction followed by Greiss Ilosvay determination) and nitrite content (Greiss Ilosvay determination) using the methods described by Stock (1983). No nitrite or ammonium was found; negligible amounts ($<0.04\mu\text{g g}^{-1}$) of nitrate were found in some solutions only. It was decided that these amounts were sufficiently small not to interfere with experimentation.

To buffer fluctuations in pH, about 7g of calcium carbonate was added to the sand for each pot and mixed well. The results of a pilot study (Appendix 1) showed that this maintained pH at a level of ± 6.5 throughout the growing period.

To prevent nitrification of ammonium, 1mg l⁻¹ N-Serve (trade name for nitrapyrin; Dow Chemical Company) was added to the feeding solution every day. After feeding, 0.5ml of the eluate from the bottom of pots containing ammonium-fed plants was collected and added to 5ml Szechrome NAS reagent (Stock, 1983). The development of a mauve-purple colour was a qualitative indication of nitrate presence. This N-Serve treatment prevented the formation of any nitrate during the course of the pilot experiments and was therefore used as a method to prevent

nitrification in pots containing ammonium-fed plants. Addition of N-Serve has been reported to have an adverse effect on plant growth (Murphy, 1984). Although the plants did not appear to be adversely affected in this study, N-Serve was given to the nitrate-fed plants as well, to avoid introducing another possible source of variation between treatments.

Maize (*Zea mays* L. var PNR 394) and wheat (*Triticum aestivum* L. var Gamtoos) seeds were germinated by leaving them to imbibe aerated, distilled water overnight. Viable seeds showing emergence of the radical after this treatment were planted out in seed trays containing moist vermiculite. Week-old seedlings were planted out in 1.7dm³ pots (two per pot for maize and 10 to 14 per pot for wheat). The two treatments administered were 4mM nitrate as KNO₃ or 4mM ammonium as NH₄Cl in standard Long Ashton solution (Hewitt, 1966 : Table 2). Three pots of each species were planted per treatment. The plants were watered daily with 350ml of solution, which was sufficient volume to saturate the sand and cause a flushing out of liquid from the bottom of the pot.

3.1.2. Hydroponic Culture

Wheat cultivation in a hydroponic medium has been carried out in the past with relative ease (Lewis *et al.*, 1987). However, the poor response of maize to hydroponic culture required extensive experimentation to obtain a suitable growing method. The main problems encountered were chlorosis and striation of the leaves after 3-5 days in hydroponic solution, especially in plants fed nitrate. A brief report of the procedures carried out to remedy this problem is given in Appendix 2.

Plastic troughs of 20dm³ capacity were provided with hard, white plastic lids. The colour of the lids helped to reflect the high light intensities and prevent excessive heating of the nutrient solution. The lids had 8, 3cm-diameter holes drilled into them, situated well away from a hole for the air inlet. The troughs had a stoppered hole introduced into a bottom corner to facilitate solution changing. Both troughs and lids were acid washed with 5% HCl solution and scrubbed with a 5% sodium hypochlorite solution. They were then rinsed four times with de-ionized water before use.

The final growth medium used for maize cultivation was modified Long Ashton solution (Table 3) with 0.7mM ferric citrate replacing the usual iron source. For wheat cultivation, the standard Long Ashton solution (Table 2) was used.

Calcium carbonate was found to be unsuitable as a pH regulator in hydroponic culture, since it tended to clog the air regulation apparatus. pH was therefore monitored once every two days and regulated by the addition of either 1M HCl or 1M CaCO₃ to pH 6.0.

To detect any nitrification occurring in the ammonium-containing troughs, samples of the solution were removed on a regular basis and tested qualitatively for the presence of nitrate using Szechrome NAS reagent according to the method of Stock (1983). No nitrification occurred during the course of the experiments.

Week-old maize and wheat seedlings, germinated as for the soil culture experiments, were inserted into the lids of the troughs and held in place with strips of polythene foam, which allowed for expansion of the shoots as they grew. The lids were then suspended over the troughs containing the relevant Long Ashton solution for the species and either 12mM KNO_3 or 12mM NH_4Cl . Three troughs were prepared for each nitrogen treatment for each species. During the experiments, levels of nitrate and ammonium were checked qualitatively using Szechrome NAS (Stock, 1983) and Nessler's (A.O.A.C., 1965) reagents respectively. 5ml of reagent was added to 0.5ml of solution and the development of colour (purple in the case of Szechrome and yellow in the case of Nessler's) was taken as an indication of the amount of nitrogen source remaining. It was found to be sufficient to change the solutions once a week to avoid nitrogen depletion occurring between feed changes.

3.1.3. Growth Conditions

All experiments were carried out in constant environment chambers (Furcold (Pty) Ltd) fitted with a REX-P100 RKC programming unit and a seconic hybrid SD-50H temperature and humidity recorder. Lighting was provided by high power sodium, high power metal halide and incandescent lamps.

During the course of the experiments, each species was grown under its respective optimum growth conditions, taking into consideration their differing photosynthetic pathways (wheat being a C-3 plant and maize a C-4 plant).

3.1.3.1. Maize

All maize experiments were carried out at an irradiance of $1400\mu\text{mol m}^{-2}\text{s}^{-1}$ with a day length of 14 hours at 35°C and a night temperature of 20°C . The relative humidity was kept constant at 60%. Plants were grown for two weeks under these conditions, making them 21 days old (including germination time) at harvest.

3.1.3.2. Wheat

All wheat experiments were carried out at an irradiance of $500\mu\text{mol m}^{-2}\text{s}^{-1}$ with a day length of 14 hours at 25°C and a night temperature of 15°C . The relative humidity was kept constant at 60%. Plants were grown for three weeks under these conditions to allow for the development of sufficient experimental material. This made the plants 28 days old (including germination time) at harvest.

Table 2: Macro- and micro-nutrients in 10dm³ of final feeding solution supplied to wheat (Hewitt, 1966).

Macronutrient	Mass (g)
Na ₂ HPO ₄ · 12H ₂ O	1.42
MgSO ₄ · 7H ₂ O	2.48
CaCl ₂ · 2H ₂ O	3.36
K ₂ SO ₄	1.58
Micronutrient	Mass (mg)
HBO ₃	85.80
MnSO ₄ · 4H ₂ O	46.40
ZnSO ₄ · 7H ₂ O	6.60
CuSO ₄ · 5H ₂ O	2.40
Na ₂ MoO ₄ · 2H ₂ O	0.60
[CH ₂ N(CH ₂ COO) ₂] ₂ FeNa ₂ (FeEDTA)	330.00

Table 3: Macro- and micro-nutrients in 10dm³ of final feeding solution supplied to maize (modified from Hewitt, 1966).

Macronutrient	Mass (g)
Na ₂ HPO ₄ · 12H ₂ O	1.24
MgSO ₄ · 7H ₂ O	2.48
CaCl ₂ · 2H ₂ O	5.92
K ₂ SO ₄	1.58
Micronutrient	Mass (mg)
HBO ₃	83.00
MnSO ₄ · 4H ₂ O	35.20
ZnSO ₄ · 7H ₂ O	6.60
CuSO ₄ · 5H ₂ O	2.40
Na ₂ MoO ₄ · 2H ₂ O	6.00
C ₆ H ₅ O ₇ Fe · 5H ₂ O (Ferric citrate)	2336.00

3.2. Experimental Measurements and Procedures

3.2.1. Photosynthesis

Photosynthetic measurements were carried out on two- and three-week old soil-grown maize and wheat respectively. A portable ADC infra red gas analyzer (IRGA) Model LCA 2 (Analytical Development Co., Hoddeston, England) connected to a Parkinson's leaf chamber was used to record 12 replicate measurements (four per plant) for each treatment within each species. Results were expressed in $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$.

3.2.2. Root Respiration

The roots of hydroponically grown plants were enclosed in 1dm^3 airtight containers for the determination of root respiration rate. The container had an air inlet fitted with a flow regulator which bubbled air through the feeding solution and an air outlet at the top of the container. The lid was sealed with agar around the plant and the inlets. A constant airstream (450ml min^{-1}) was drawn through the container and the CO_2 content of the emerging airstream was monitored using an IRGA (ADC 225 MK 3, Analytical Development Co.). Once set up, the system required approximately an hour to reach equilibrium (when the exchange of CO_2 with the nutrient solution was constant). To determine when this point was reached, an Omniscribe data recorder (Houston Instruments, Austen, Texas, U.S.A.) was connected to the IRGA. From these readings, root respiration was calculated as nmols CO_2 per gram fresh mass per second. Correction for temperature was made using Boyle's Gas Law.

3.2.3. Xylem Sap

Xylem sap was collected from soil-grown plants of each species. Shoots were severed at a point about 4cm above soil level. The stumps were washed with de-ionized water and blotted dry with filter paper. Xylem sap was collected using a Pasteur pipette during the following hour.

The amino acid composition of the xylem sap was determined using a Beckman 120C Amino Acid Analyser (Beckman Instruments Inc., Fullerton, California, U.S.A.) employing the lithium buffer method described by Kedenburg (1971) to separate glutamate and the amides.

3.2.4. Plant Water Content

Sand-grown maize and wheat plants supplied with 4mM ammonium or nitrate were harvested for the determination of water content. After the growth period (21 days for maize and 28 days for wheat), the plants were divided into root and shoot subsamples. The roots were washed thoroughly to remove all sand and blotted dry. Fresh mass of roots and shoots was recorded, after which plant material was oven-dried for 24 hours at 60°C. Four replicates for each nitrogen treatment within each species were carried out. Expression of water content was dry mass as a percentage of fresh mass.

3.2.5. Labelling with ^{14}C

3.2.5.1. ^{14}C Feeding Apparatus

The ^{14}C feeding apparatus consisted of a clear perspex feeding chamber measuring 650x240x600mm, the open end of which rested in a trough in a thick perspex plate (Figure 1). The trough was filled with water once the plants were enclosed, ensuring an airtight seal. The chamber was fitted with three air inlets (towards the top of the side wall) and three air outlets (towards the bottom of the opposite wall), corresponding to the positions of the three pots. This aided air circulation around the plants. Air was drawn into the chamber using an IRGA (ADC 225 MK 3 : Analytical Development Co.), which allowed the CO_2 levels inside the chamber to be monitored. The outlet line, from the chamber to the IRGA, contained a water vapour trap of magnesium perchloride and a bypassable serial connection to the ^{14}C source consisting of two ^{14}C generating towers. The vapour trap dried the air before it entered the IRGA, while the bypassable connection to the ^{14}C source allowed regulation of the introduction of ^{14}C into the airstream. The inlet line, from the IRGA to the chamber, contained another bypassable serial connection to a CO_2 scrub of soda lime, which was used at the end of feeding time to remove any remaining $^{14}\text{CO}_2$.

3.2.5.2. ^{14}C Feeding Procedure

On the morning of the ^{14}C feed, sand-grown plants were fed with nutrient solution. In the case of the hydroponically-grown plants, three from each treatment were removed from the troughs and the roots carefully fed into 1dm^3 jars, allowing the lids through which they were grown to be screwed onto the jars containing ^{15}N nutrient solution (section 3.2.6.). Containers of hydroponic- or sand-grown plants were placed in batches of three into the feeding chamber. A batch always consisted of a combination of nitrogen treatments to prevent any differences in batch feeding being interpreted as real differences between N feeding regimes.

Initially, the serial connections to the ^{14}C source and the CO_2 scrub were closed. Once the chamber was sealed, it was normal to find CO_2 levels of up to 450ppm in the system, due to human exhalation of CO_2 . The plants were left to photosynthesize until the CO_2 concentration was down to $\pm 300\text{ppm}$. The feeding chamber was then covered with black plastic to halt photosynthesis, and the taps to the circuit containing the ^{14}C generating towers were opened. This caused 50ml of 10% lactic acid from the first tower to be drawn over into the second tower containing 50uCi of $\text{Na}^{14}\text{CO}_3$ (Amersham International, Buckinghamshire, U.K.). The $^{14}\text{CO}_2$ generated was allowed to diffuse evenly through the system for about three minutes before the feeding chamber was uncovered. Plants were left to photosynthesize until the CO_2 level neared the CO_2 compensation point which was $\pm 20\text{p.p.m.}$ for maize and 80p.p.m. for wheat. This usually occurred after 15- and 30 minutes respectively. At this point, the taps to the CO_2 scrub were opened to remove any residual $^{14}\text{CO}_2$ before the chamber was opened. The plants were then left to grow for a further 24 hours before harvest.

3.2.6. Dual Labelling with ^{15}N and ^{14}C

Before the commencement of the $^{15}\text{N}/^{14}\text{C}$ feeding of maize, fresh nutrient solution (Table 2) was prepared. The nitrogen source was provided as 12mM 99A%E ^{15}N KNO_3 or 12mM 99A%E ^{15}N NH_4Cl (BOC-Prochem, London U.K.). These solutions were placed in the growth cabinets overnight to equilibrate with the ambient temperature. The jars to which plants were transplanted for the ^{14}C feed were then filled with these nutrient solutions. Thus plants were supplied with ^{15}N during the ^{14}C feed. After ^{14}C feeding, the plants were left to grow in the solutions for a further 24 hours before harvesting.

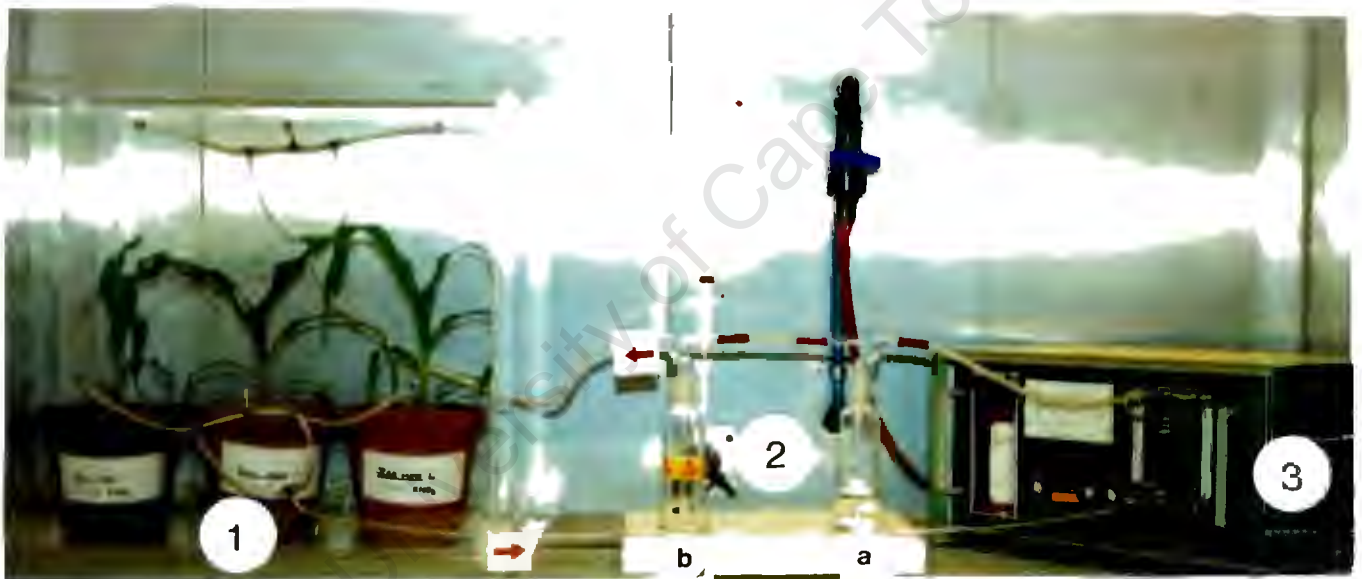


Figure 1: ^{14}C Feeding apparatus

1) perspex chamber, 2) ^{14}C generating towers : a) containing 10% lactic acid, b) containing $50\mu\text{Ci Na}^{14}\text{CO}_3$, 3) IRGA. H_2O and CO_2 scrubs not shown. Arrows indicate direction of flow.

3.2.7. Harvesting and Ethanol Extraction

Root systems of sand-grown plants were washed very thoroughly in de-ionized water to ensure removal of all sand particles. The root systems of hydroponically-grown plants were rinsed in de-ionized water and blotted dry. Plants were then divided into root and shoot subsamples. The fresh mass of each subsample and, in the case of the sand-grown wheat plants, the number of plants per pot, was recorded. From these data, shoot:root fresh mass ratios were calculated.

The tissue from each subsample was then coarsely chopped before being homogenized in 100ml of 80% ethanol-water. Alcohol extraction was carried out for 24h at 0°C, during which the homogenate was shaken occasionally to resuspend the plant tissue and aid the extraction process. After 24h, the homogenate was filtered through tared, oven dried Whatman no.1 filter paper. The residue was dried at 80°C for 24h and reweighed. The filtrate was blown down to 20ml under an airstream and then shaken with 20ml petroleum ether to remove chlorophyll and lipid material. This suspension was left to stand for 24h at 0°C to ensure good separation of the petroleum ether from the ethanol extract. Samples were then frozen and the petroleum ether decanted.

3.2.8. Separation and Quantification of Carbon Fractions

Figure 2 is a flow chart depicting the procedure followed during the separation of the plant extracts into carbon fractions.

3.2.8.1. The Ethanol-Soluble Fraction

To separate nitrogen and carbohydrate components of the ethanol-extracted fraction, 4ml of filtrate was passed through a 6x1 cm Dowex 50W-X8 standard H⁺ (100-200 mesh partical size) ion exchange column (Atkins and Canvin, 1971). Soluble carbohydrates were eluted from the column using 50ml distilled water, while the soluble nitrogen fraction was eluted using 100ml 2M HCl. Duplicate samples for each plant extract were run through the columns. The resulting fractions (termed the soluble nitrogen- and soluble carbohydrate fractions) were stored at 0°C for later scintillation counting.

3.2.8.2 The Ethanol-Insoluble Fraction

After drying and reweighing, the residue from the ethanol extraction was milled to 20 mesh using a Wiley tissue mill. 0.5g of milled sample and 10ml of 6M HCl were added to tared, oven-dried McCartney bottles. The samples were sealed and hydrolysed at 103°C for 24h and then filtered through tared, oven dried Whatman no. 1 filter paper. The filtrate was dried under vacuum twice using an Evapomix (Buchler Instruments, Port Lee, New York, U.S.A.) connected to a high vacuum pump and fitted with a liquid nitrogen cold trap. The samples were then made up to 10ml using distilled water and neutralized by addition of 1M NaOH. The final volumes of the samples were then measured and recorded. The nitrogen and carbohydrate fractions were then separated on an ion exchange column following the procedure used for the ethanol-extracted fraction (3.2.1.). The two fractions obtained were termed the insoluble nitrogen- and the storage carbohydrate compounds.

Residue from the hydrolysis was dried for 24h at 80°C and weighed. Samples of 0.05g were weighed out onto Whatman no.1 filter paper and oxidized using a Packard Tricarb Oxidizer Model 306 (Packard International, Zurich, Switzerland) as described in section 3.2.8.3. This fraction was termed structural carbohydrate.

3.2.8.3. ^{14}C Sample Analysis

Using the methods described above, five carbon fractions were isolated for analysis of ^{14}C content. These were the soluble nitrogen- (amino compounds and soluble proteins) and soluble non-nitrogen or carbohydrate carbon (sugars) from the ethanol-water soluble (labile) extraction; the insoluble nitrogen (proteins) and insoluble non nitrogen or storage carbohydrate carbon (starch) from the acid digested extraction, and the structural carbohydrate (cellulose).

The radioactivity of duplicate samples from all of these fractions for both species and both growing media was counted using a Beckman LS 5000 liquid scintillation counter (Beckman Instruments) with an automatic quench correction. For all fractions except the structural carbohydrates, 0.5ml of sample was placed in a plastic mini "Econovial" (Packard International) with 5ml "Instagel" (Packard International), a universal liquid scintillation cocktail for aqueous and non-aqueous samples which has a 50% water holding capacity. The samples were counted immediately after mixing, to prevent any reaction with the wall of the plastic vial. The radioactivity of each sample was counted for 20 minutes. Blank samples, consisting of scintillation fluid mixed with the appropriate volume of elution fluid (either distilled water or 2M HCl), were run concurrently. The mean of counts

obtained for duplicate samples (in DPM) was calculated (after correction with counts obtained for blank samples) if duplicates did not differ by more than 5%. If larger differences were obtained between duplicates, the sample was recounted.

$^{14}\text{CO}_2$ from the structural material was trapped in "Carbo-Sorb" (Packard International) upon oxidation, and counted in glass vials with 12ml "Permafluor" scintillation cocktail (Packard International). Duplicate oxidations were carried out for each sample and the means calculated after correction for blank readings obtained by oxidizing oven-dried filter paper.

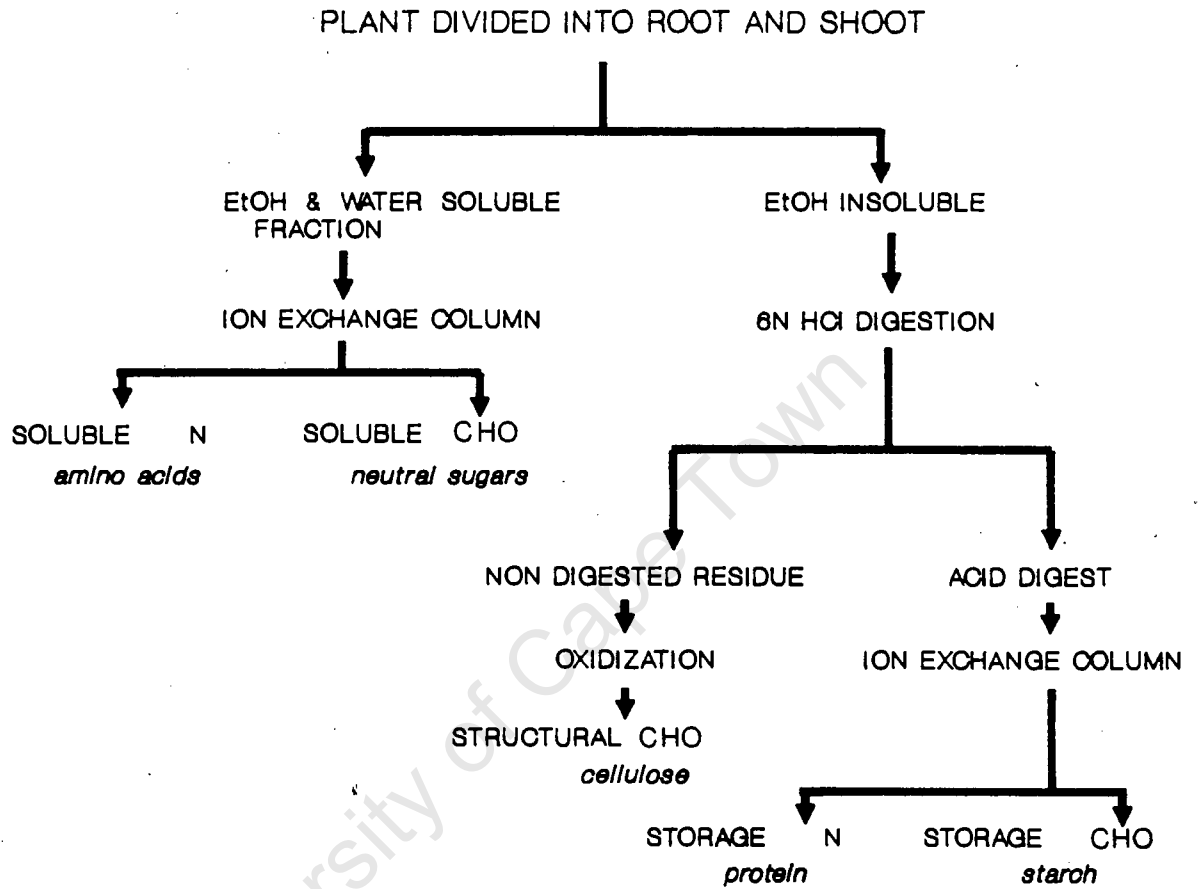


Figure 2: Flowchart showing the process of fractionation carried out for quantification of ^{14}C allocation ratios to ethanol-soluble and -insoluble nitrogenous (N) and carbohydrate (CHO) fractions.

3.2.9. Separation and Quantification of Nitrogen Fractions

3.2.9.1. The Ethanol-Soluble Fraction

After passing the ethanol-soluble filtrate through the ion exchange column (section 3.2.7.1.), nitrogen compounds contained in the eluate were converted to ammonium sulphate by Kjeldahl digestion. 3ml of the eluate, a mercury catalyst tablet containing the equivalent of 0.1g of mercury (BDH, Poole, Dorset, U.K.) and 3ml of concentrated, nitrogen-free sulphuric acid containing 34g of salicylic acid per liter were added to each Kjeldahl flask. The salicylic acid converts nitrate to ammonium and so facilitates the formation of ammonium sulphate (Fiedler and Proksch, 1975).

Duplicate digestions for each sample were carried out for 2h at 200°C. The temperature was then increased to 375°C and digestion was continued for 2h after the samples had become clear. After cooling, the samples were made up to 25ml using distilled water and stored at 0°C.

After addition of an excess (15ml) of alkaline solution (50% sodium hydroxide with 2.5% sodium thiosulphate (w/v)), ammonia was recovered by steam distillation of the acid mixture using a Markham's still. Sodium thiosulphate was added to precipitate the mercury contained in the Kjeldahl catalyst tablet.

During steam distillation, the first 30ml of ammonia distillate was collected in 2ml of 0.05M HCl. The excess acid was back-titrated to pH 5.2 with a solution of sodium hydroxide and a Schott T80/20 Auto Titrator. This solution was then re-acidified with about 2ml of 0.5M hydrochloric acid to prevent loss of ammonia,

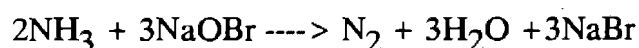
which is insoluble in alkaline solution. Duplicate distillations of all samples were carried out and duplicates repeated if back-titration values differed by more than 0.1ml. These values were then used to calculate total nitrogen content of samples. The distillate was then blown down under an air stream to a volume suitable for ^{15}N determination, the optimal total nitrogen content for this being $25\mu\text{g}$ per 0.2ml of sample.

3.2.9.2. The Ethanol-Insoluble Fraction

The nitrogen compounds contained in the milled, ethanol-insoluble plant material (section 3.2.7.2.) were converted to ammonium sulphate by Kjeldahl digestion as described above (section 3.2.9.1.). Samples of 0.1g were used per digestion and two replicates were digested for each sample. Distillates were collected, titrated and prepared for ^{15}N analysis as described above.

3.2.9.3. ^{15}N Sample Analysis

^{15}N analysis was carried out using a Jasco Atomic Emission Spectrophotometer (Japan Spectroscopic Company Ltd, Hachioji City, Tokyo), following the methods described by Faust (1967). The oxidant used was an alkaline hypobromite solution which reacted with the sample under vacuum to release nitrogen gas according to the following reaction:



The vacuum system used comprised an Edwards high-vacuum, single-stage pump to establish a good pre-vacuum, connected in series to an oil immersion pump which brought the pressure down to a final value of 1×10^{-3} mbar. The vapour pressure of the system was reduced by three liquid nitrogen cold traps.

For each sample, 0.2ml of hypobromite and 0.2ml sample were pipetted into the separate lobes of a small Rittenburg vessel. This was frozen and de-gased three times to ensure removal of all water vapour and air. The vessel was then rotated to react the solutions. Nitrogen gas liberated was collected in a glass discharge tube. The tube was sealed over an oxygen flame and the sample ionized using a Tesla coil. A frequency oscillator (Jasco ^{15}N Atomic Emission Spectrophotometer) was then used to excite the gas molecules trapped in the discharge tube, emitting a typical violet colour. A blue-white colour was obtained if there was a presence of water vapour (due to inefficient de-gassing) or bromine (produced during the final oxidation step of the reaction of the sample with the hypobromite, if the sample is too acid). This method is based on the photoelectric recording of bandheads emitted by the three isotopic molecules $^{15}\text{N}^{15}\text{N}$, $^{14}\text{N}^{15}\text{N}$ and $^{14}\text{N}^{14}\text{N}$ on excitation. Percentage enrichment of the sample was calculated from the following formula:

$$\text{En}\% = \frac{100}{2(A/B+C)+1}$$

Where A and B are the bandheads of the $^{14}\text{N}^{14}\text{N}$ and $^{14}\text{N}^{15}\text{N}$ molecules respectively, and C is the attenuation or gain setting on the spectrophotometer at

which bandheads A and B were recorded. All ^{15}N enrichment figures obtained were averages of three complete traces and were corrected using a calibration curve constructed for the spectrophotometer. Percentage enrichment in excess of natural abundance (A%E) was obtained by subtracting the natural abundance (0.37%) from the corrected percentage enrichment (Yoneyama *et al*, 1975). The ^{15}N content of the sample ($\mu\text{g } ^{15}\text{N g}^{-1} \text{ fw}$) was calculated as the product of the total nitrogen content (obtained from the distillation) and the A%E value.

3.3. Statistical Analysis

^{14}C allocation to the various carbon pools (figure 2), was calculated as a percentage of total radioactivity contained in the respective carbon fractions. Since percentage expression is a ratio, it has a bimodal, rather than a normal distribution (Zar, 1984). The data were therefore arcsin transformed to obtain a normal distribution (Zar, 1984) before means, standard errors (S.E.) and statistical tests were computed. Statistically significant differences between treatments for each species were determined using Student's t-test.

CHAPTER 4 : RESULTS

4.1. Plant Growth Responses

In 4mM nitrogen-fed wheat, a significantly greater ($p < 0.05$) shoot : root fresh mass ratio was found in ammonium-fed plants (2.2 : 1) in comparison to nitrate-fed plants (1.7 : 1). This was accentuated in the 12mM nitrogen-fed plants (with ratios of 3.5 : 1 for ammonium- compared to 1.1 : 1 for nitrate-fed plants : Figure 3). This increase in shoot : root ratio was attributable to a large decrease in root mass, as the shoots of ammonium-fed plants were also smaller in comparison to nitrate-fed plants. In addition to the difference in size, ammonium-fed wheat differed in appearance from nitrate-fed wheat in that the shoots appeared darker green and had a tendency to lodge. The root system was small, brownish in colour and fibrous. Nitrate-fed plants were a lighter green and more erect. The root system was extensive, white in colour and very dense.

Maize showed no significant difference in shoot : root ratio of fresh mass between nitrate and ammonium treatments at either 4mM or 12mM nitrogen levels, although the roots of ammonium-fed plants tended to be slightly larger than that of the nitrate-fed plants at the 4mM level (Figure 3). The shoots were large and erect. An increase in nitrogen concentration had no adverse effect on the plants subjected to either nitrate or ammonium nutrition.

4.2. Photosynthesis

In both wheat and maize, no significant differences were found between the carbon assimilatory rates of ammonium- and nitrate-fed plants as measured in $\mu\text{mol CO}_2$ fixed $\text{m}^{-2}\text{s}^{-1}$ (Figure 4). This similarity within species in carbon gaining capacity when supplied one or other nitrogen form allowed for comparison of quantitative carbon allocation patterns between ammonium- and nitrate-fed plants within each species. As expected, due to the C-4 photosynthetic pathway of maize, there was a large difference in carbon assimilatory rate between wheat and maize, with maize having close to a three-fold higher rate per unit area (Figure 4).

4.3 Root Respiration

There was no significant difference evident in root respiration between nitrate- and ammonium-fed wheat plants (Figure 5) as measured by nmol CO_2 respired $\text{g}^{-1}\text{s}^{-1}$. In maize plants, however, the nitrate- and ammonium-fed treatments exhibited a significantly different ($p < 0.05$) respiration rate (Figure 5), the roots of nitrate-fed plants showing a greater respiratory carbon loss.

4.4. Xylem Sap

Xylem sap analysis from wheat supplied ammonium or nitrate (Table 4) showed that ammonium-fed wheat transported significantly more ($p < 0.05$) amino compounds to the shoot than nitrate-fed wheat ($3.97 \mu\text{mol N/ml}$ as opposed to $3.11 \mu\text{mol N/ml}$), and that the C:N ratio of xylem sap was lower in ammonium-fed plants (2.4) than in nitrate-fed plants (2.75). More carbon was translocated to the shoot in

ammonium-fed wheat ($9.53\mu\text{mol C/ml}$) than in nitrate-fed wheat ($8.55\mu\text{mol C/ml}$) although this was not a significant difference.

Analysis of maize xylem sap showed similar trends. Ammonium-fed maize transported significantly more ($p < 0.05$) amino compounds ($20.09\mu\text{mol N/ml}$) than those fed nitrate ($13.61\mu\text{mol N/ml}$). The C:N ratios were 2.33 and 2.42 for ammonium- and nitrate-fed plants respectively (Table 4). Significantly more carbon ($p < 0.05$) was translocated to the shoots of ammonium-fed maize ($46.8\mu\text{mol C/ml}$) in comparison to nitrate-fed maize ($31.9\mu\text{mol C/ml}$).

For both wheat and maize, the amount of free ammonium in the xylem sap was low ($\pm 0.46\mu\text{mol/ml}$) and not significantly different for ammonium- and nitrate-fed plants (Table 4).

4.5. Plant Water Content

The water content of maize and wheat plants, as determined by the difference in fresh mass and dry mass, is shown in Table 5. A significant difference ($p < 0.05$) in water content was found in ammonium-fed wheat in comparison to nitrate-fed wheat, with that of ammonium-fed plants being lower. No significant difference was found between the water content of maize fed ammonium or nitrate.

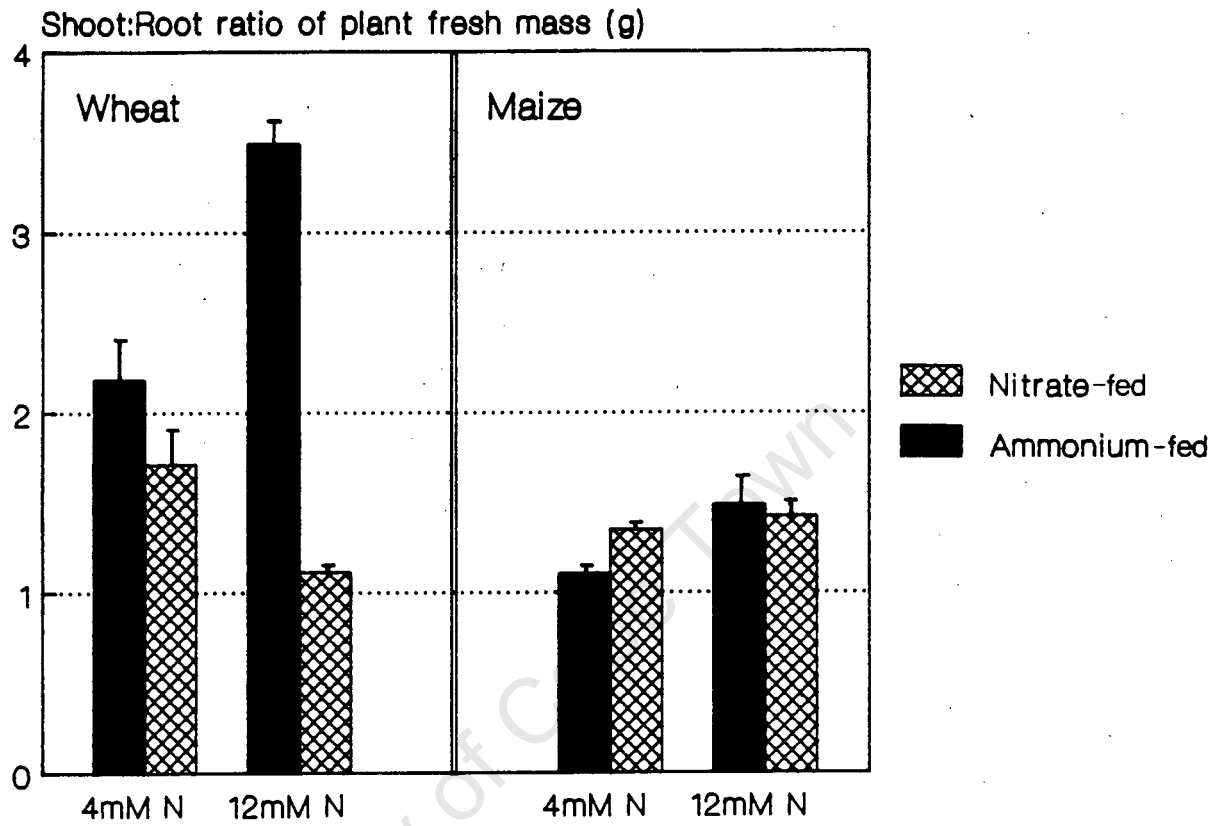


Figure 3: Shoot:root ratio of plant fresh mass for wheat and maize fed ammonium or nitrate at 4mM and 12mM concentrations. (Means of 3 replicates of 10 plants each for wheat and 2 plants each for maize. Bar heights represent S.E. Means and S.E. calculated after arcsin square root transformation).

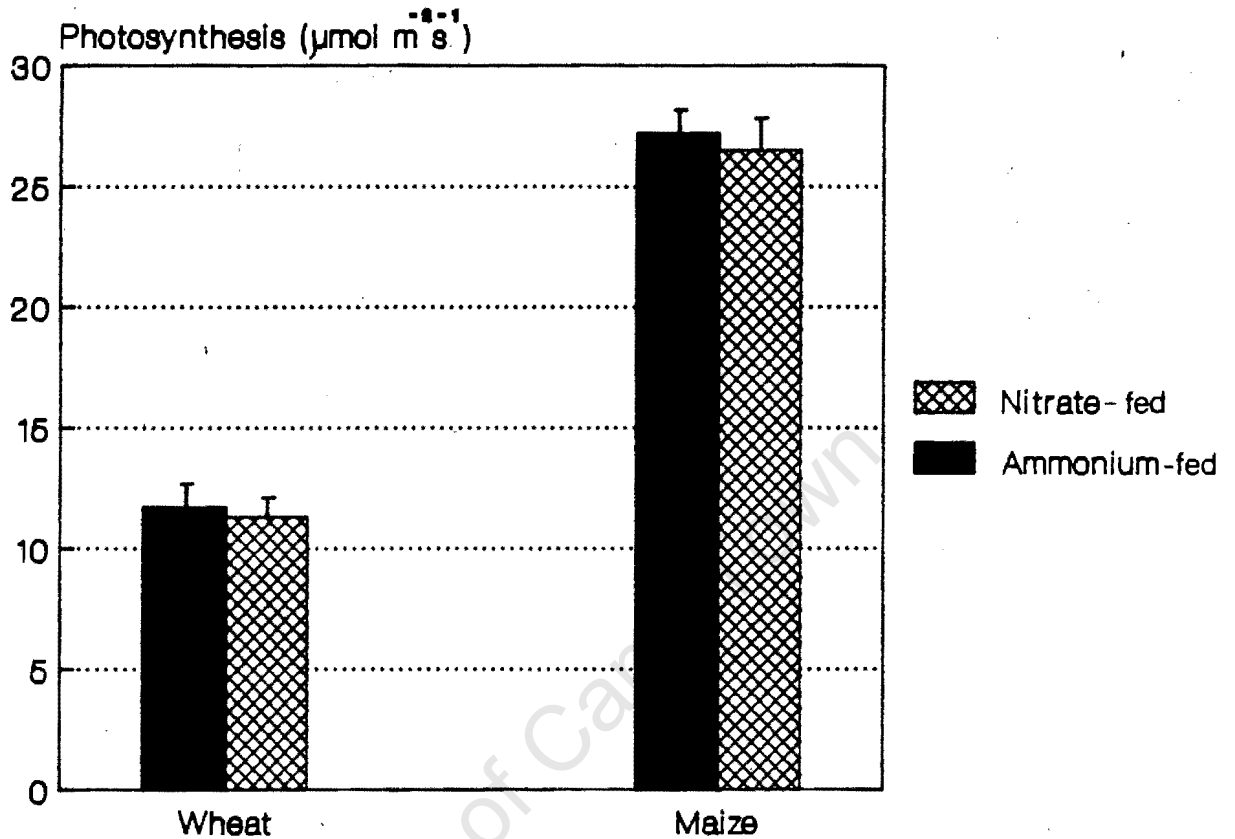


Figure 4: Photosynthetic rate ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) of wheat and maize supplied either ammonium or nitrate. Measurements for wheat were recorded at an irradiance of $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 25°C , while those for maize were recorded at an irradiance of $1400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 35°C . (Means of readings from 3 plants, 4 readings per plant. Bar heights represent S.E.).

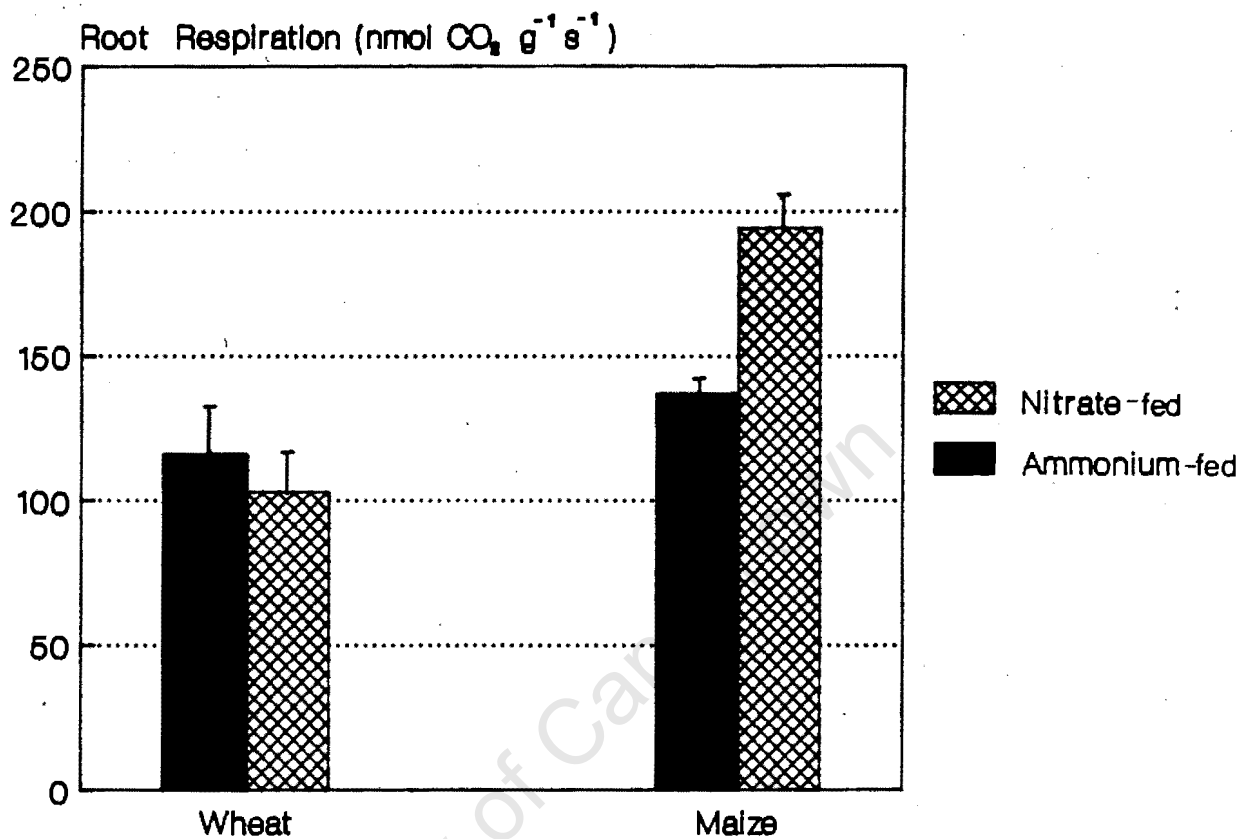


Figure 5: Root respiration ($\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) of wheat and maize supplied either ammonium or nitrate. Measurements recorded as described in section 3.2.2. (Means of four replicates, bar heights represent S.E.).

Table 4: Total amino compounds ($\mu\text{mol N/ml}$), C:N ratio, total carbon ($\mu\text{mol C/ml}$) and ammonium content ($\mu\text{mol N/ml}$) of xylem sap from wheat or maize fed ammonium or nitrate. (Means of 3 replicates \pm S.E.).

	Wheat		Maize	
	Ammonium-fed	Nitrate-fed	Ammonium-fed	Nitrate-fed
Total Amino Compounds $\mu\text{mol N/ml}$	3.97 ± 0.17	3.11 ± 0.12	20.09 ± 1.21	13.16 ± 1.35
C/N Ratio	2.40 ± 0.02	2.75 ± 0.09	2.33 ± 0.03	2.42 ± 0.04
NH_3 $\mu\text{mol N/ml}$	0.40 ± 0.04	0.32 ± 0.01	0.66 ± 0.01	0.45 ± 0.04
Total C $\mu\text{mol C/ml}$	9.53 ± 0.46	8.55 ± 0.39	46.81 ± 2.28	31.85 ± 1.52

Table 5: Dry mass as a percentage fresh mass of wheat and maize fed 4mM ammonium or nitrate.
 (Means of 4 replicates of 10 plants each for wheat and 2 plants each for maize \pm S.E.).

Nitrogen Treatment	Wheat		Maize	
	Root	Shoot	Root	Shoot
Ammonium	10.95 ± 1.77	18.93 ± 0.61	7.63 ± 0.66	10.43 ± 0.61
Nitrate	6.82 ± 0.98	16.07 ± 1.68	6.88 ± 0.51	12.76 ± 0.64

4.6. ^{14}C Carbon Distribution

4.6.1. Ratio of Total ^{14}C Distribution between Root and Shoot

In wheat, plants fed 4mM ammonium showed a statistically significant difference ($p < 0.05$) in shoot : root ratio of ^{14}C allocation (2.6 : 1) compared to 4mM nitrate-fed plants (3.2 : 1) 24h after ^{14}C feeding (Figure 6). This was accentuated in the 12mM feed, with ratios of 2.3 : 1 for ammonium- and 4.6 : 1 for nitrate-fed plants (significant difference, $p < 0.05$). This indicates a greater diversion of newly assimilated carbon to the root of ammonium-fed plants compared to nitrate-fed plants and suggests that the roots of ammonium-fed plants act as a greater sink for this carbon. The nitrate-fed plants retained a greater proportion of newly assimilated carbon in the shoots (relative to the roots) in the 12mM nitrogen treatment when compared to the 4mM treatment, suggesting that more carbon is required for the assimilation of greater amounts of nitrate nitrogen, which occurs predominantly in the shoot.

In maize, plants supplied 4mM nitrogen showed no significant difference in the shoot : root ^{14}C allocation ratios between ammonium- and nitrate-fed plants (Figure 6). The ^{14}C allocation to the root relative to the shoot for this nitrogen concentration was high, reflected in the relatively low shoot : root ratio of ^{14}C allocation (below 2 : 1) for maize fed 4mM N. This indicates rapid transport of ^{14}C to the root.

When maize was supplied with 12mM nitrogen, however, a marked increase in the shoot : root ratio of ^{14}C allocation occurred (from below 2:1 in plants supplied 4mM N to over 3:1 in plants supplied 12mM N : Figure 6). In plants supplied the higher nitrogen concentration, this ratio was significantly different ($p < 0.05$) between nitrate- and ammonium-fed plants. This is possibly a result of an increased requirement for carbon skeletons in shoots of nitrate-fed plants for nitrate assimilation, which occurs predominantly in this organ.

4.6.2. ^{14}C Allocation to Carbohydrate and Nitrogen Compounds within the Root and Shoot

4.6.2.1. Wheat

4.6.2.1.1. ^{14}C Distribution within the Wheat Root

At the 4mM nitrogen feeding level, the roots of ammonium-fed wheat apparently allocated a larger (although not significantly greater) proportion of newly assimilated carbon in the root to nitrogen compounds relative to carbohydrate compounds than did nitrate-fed plants (^{14}C allocation ratio for carbohydrate : nitrogen compounds being 2.9 : 1 in ammonium-fed wheat and 3.4 : 1 in nitrate-fed wheat) (Figure 7). This allocation trend was accentuated when the nitrogen concentration was increased to 12mM, with relative ^{14}C allocation ratios of 2.4 : 1 in ammonium- and 3.6 : 1 in nitrate-fed wheat roots being significantly different ($p < 0.05$) (Figure 7).

This ^{14}C allocation ratio is evident in the percentage ^{14}C allocation to total nitrogen compounds (soluble and insoluble), which was slightly higher in ammonium-fed roots (25.6%) than in the nitrate-fed roots (23.2%) but not significantly so (Figure 8). This difference was due to an increase in ^{14}C allocation to soluble nitrogen compounds. This difference may confirm the additional need for carbon in roots of ammonium-fed wheat for immediate assimilation of this N form. ^{14}C appears to have been diverted from the carbohydrate pool (both soluble and storage) which drew 34.5% ^{14}C in ammonium- and 37.5% in nitrate-fed plants. Proportional allocation of ^{14}C to structural carbohydrate was the same for the two N forms (40%) (Figure 8).

At the 12mM N feeding level, these trends were accentuated, with a significantly greater proportional ^{14}C allocation ($p < 0.05$) to the soluble nitrogen pool (16.2%), and a total ^{14}C allocation of 29.9% to nitrogen compounds in the case of ammonium-fed plants when compared to nitrate-fed plants. Nitrate-fed plants showed a ^{14}C allocation of 7.5% to the soluble pool, and 21.5% to soluble plus insoluble nitrogen compounds (Figure 8). Proportional ^{14}C allocation to the soluble plus storage carbohydrate pools was correspondingly reduced (35.6% in ammonium- and 42.1% in nitrate-fed plants significantly different : $p < 0.05$). Allocation of ^{14}C to structural material was also slightly lower in ammonium-fed plants (34.1%) when compared to the nitrate-fed plants (36.0%) but not significantly so. ^{14}C allocation to structural carbohydrate was lower for both treatments at the 12mM N feeding level when compared to the 4mM level (Figure 8).

4.6.2.1.2. ^{14}C Distribution within the Wheat Shoot

In the shoots of wheat fed 4mM nitrogen, there was no difference in the proportion of ^{14}C allocated to nitrogen- and carbohydrate compounds between nitrate- and ammonium-fed plants (Figure 7). Both treatments showed a ^{14}C allocation ratio of $\pm 3 : 1$ for carbohydrate- : nitrogen compounds. At the 12mM N level, the shoots appeared to allocate a slightly higher proportion of ^{14}C to nitrogen compounds, when compared to 4mM N-fed wheat shoots, and 12mM nitrate-fed wheat appeared to have a higher proportion of ^{14}C allocated to nitrogen compounds than their 12mM ammonium-fed counterparts (^{14}C allocation ratio to carbohydrate- : nitrogen compounds being 2.4 : 1 for ammonium-fed wheat shoots and 1.7 : 1 for nitrate-fed wheat shoots) but these differences were not significantly different.

This ratio was reflected in the ^{14}C allocation on a percentage basis (Figure 8). In the shoots of 4mM N-fed wheat, there was very little difference between ammonium- and nitrate-fed plants in the relative proportions of ^{14}C allocated to the various fractions. Approximately 25% was allocated to nitrogen compounds, 31% to non-structural carbohydrate compounds and 43% to structural carbohydrate (Figure 8).

When the N concentration was increased to 12mM, there was a significant increase ($p < 0.05$) in ^{14}C allocation to soluble nitrogen compounds in the shoots of ammonium-fed wheat (from 4% in the 4mM ammonium treatment to 10% in the 12mM ammonium treatment). Since the increase in ^{14}C allocation to this fraction was not as great in the shoot of 12mM nitrate-fed wheat, the increase may be attributable to carbon-bound amino compounds translocated from the root. The

overall ^{14}C allocation to nitrogen compounds (i.e. soluble + insoluble nitrogen) was significantly different ($p < 0.05$) between 12mM ammonium- and nitrate-fed wheat shoots (29% for ammonium- and 37% for nitrate-fed plants : Figure 8). To accommodate this increase in ^{14}C allocation to insoluble nitrogen compounds, ^{14}C allocation to the non-structural carbohydrate fractions (soluble plus storage) had decreased (43.5% for ammonium- and 33% for nitrate-fed wheat shoots). For both nitrate- and ammonium-fed wheat, ^{14}C allocation to structural material in the shoot was also reduced at the 12mM nitrogen feeding level.

4.6.2.2. Maize

4.6.2.2.1. ^{14}C Distribution within the Maize Root

In the 4mM treatment, the roots of maize showed a very high ^{14}C allocation ratio to carbohydrate compounds relative to nitrogenous compounds in comparison to the 12mM N treatment (Figure 9). This allocation ratio was higher in the case of nitrate-fed plants (13.1 : 1) than in ammonium-fed plants (11.6 : 1) reflecting a greater carbon requirement in nitrogenous compounds for the assimilation of ammonium in the roots. The relative allocation of ^{14}C to nitrogen- and carbohydrate compounds in the plants supplied 12mM N differed greatly from that observed in the 4mM N treatment, with far more ^{14}C in the root having been allocated to nitrogen compounds. Here, there was a significant difference ($p < 0.05$) in the proportion of ^{14}C allocated to carbohydrate compounds relative to nitrogen compounds in the ammonium-fed roots (4.5 : 1) in comparison to the nitrate-fed roots (5.7 : 1) (Figure 9).

In maize roots, the 4mM N treatment showed a 7-8% allocation of ^{14}C to nitrogen compounds. There was a large allocation of ^{14}C to soluble and storage carbohydrate reserves (49%) and a ^{14}C allocation to structural material of 44% (Figure 10). At the 4mM N feeding level there was very little difference between ammonium- and nitrate-fed maize in terms of percentage ^{14}C allocation to the various pools.

When the N concentration supplied to maize was increased to 12mM, a significantly larger proportion ($p < 0.05$) of ^{14}C was allocated to nitrogen-compounds, relative to the 4mM treatment, for both ammonium- and nitrate-fed plants. The 12mM ammonium-fed roots allocated a significantly greater proportion ($p < 0.05$) to these fractions (24.5%) than did the 12mM nitrate-fed roots (17.9%) (Figure 10). This increase was mainly in the soluble nitrogen compounds, and was accompanied by a reduction in the proportional ^{14}C allocation to soluble + storage carbohydrate compounds (to 35.9% of total ^{14}C in 12mM ammonium- and to 51.8% of total ^{14}C in the 12mM nitrate-fed plant roots). In the case of the ammonium-fed plants, ^{14}C allocation to the soluble carbohydrate compounds was reduced, while in the nitrate-fed plants, allocation to the storage and structural carbohydrate was reduced. Allocation to structural material was reduced when compared to 4mM N-fed maize, especially in the nitrate-fed treatment. 12mM nitrate-fed maize showed a significant difference ($p < 0.05$) in ^{14}C allocation to structural material relative to 4mM nitrate-fed maize, and to 12mM ammonium-fed maize (39.6% of total ^{14}C for 12mM ammonium- and 30.4% of total ^{14}C for 12mM nitrate-fed plants was allocated to structural material).

4.6.2.2.2. ^{14}C Distribution within the Maize Shoot

In shoots of maize, the ^{14}C allocation ratio to carbohydrate- compared to nitrogen compounds was relatively high in 4mM N-fed maize in comparison to 12mM N-fed maize (Figure 9). Shoots of 4mM nitrate-fed maize appeared to allocate more ^{14}C to nitrogen compounds relative to non-nitrogen compounds (presumably for nitrate assimilation) than shoots of those fed ammonium (ratio of ^{14}C allocation to carbohydrate : nitrogen compounds 12.6 : 1 for nitrate- and 14.4 : 1 for ammonium-fed plants). When the N concentration was increased to 12mM, a significantly different proportion ($p < 0.05$) of ^{14}C was allocated to nitrogen compounds in the shoot in comparison to the 4mM N-fed maize shoot, with the ratio of ^{14}C allocation to carbohydrate- : nitrogen compounds being 5.6 : 1 for ammonium- and 4.6 : 1 for nitrate-fed maize (Figure 9). These ratios also reflect an increase in allocation of ^{14}C to nitrogen compounds in the shoots of nitrate-fed plants relative to ammonium-fed plants probably as a result of nitrate assimilation occurring predominantly in the shoot.

In the shoots of maize fed 4mM N, the nitrate-fed plants appeared to have more ^{14}C allocated to storage carbohydrate than the ammonium-fed plants, at the expense of structural material. Allocation to the nitrogen pools was ca. 7%, to the carbohydrate pools ca. 44%, and to structural carbohydrate ca. 49% (Figure 10).

At the 12mM N level, a significantly greater ($p < 0.05$) proportion of ^{14}C was allocated to the shoot nitrogen pools when compared to the 4mM N level. A higher proportion was allocated to the nitrogen pools in the nitrate-fed shoots (18.6%) than in the ammonium-fed shoots (14.6%), indicating shoot assimilation of nitrate.

Allocation of ^{14}C to soluble and storage carbohydrate combined was similar in the shoots of ammonium- and nitrate-fed maize (ca. 53%) although the nitrate-fed shoot had a higher proportion of this allocated to soluble carbohydrate compared to storage carbohydrate than ammonium-fed plants. Allocation of ^{14}C to structural carbohydrate was slightly reduced in 12mM nitrate-fed maize shoots when compared to 12mM ammonium-fed maize shoots. The average allocation to structural material in both ammonium- and nitrate-fed plants (ca. 29%) was reduced in comparison to that in the shoots of 4mM N-fed maize (ca. 49%).

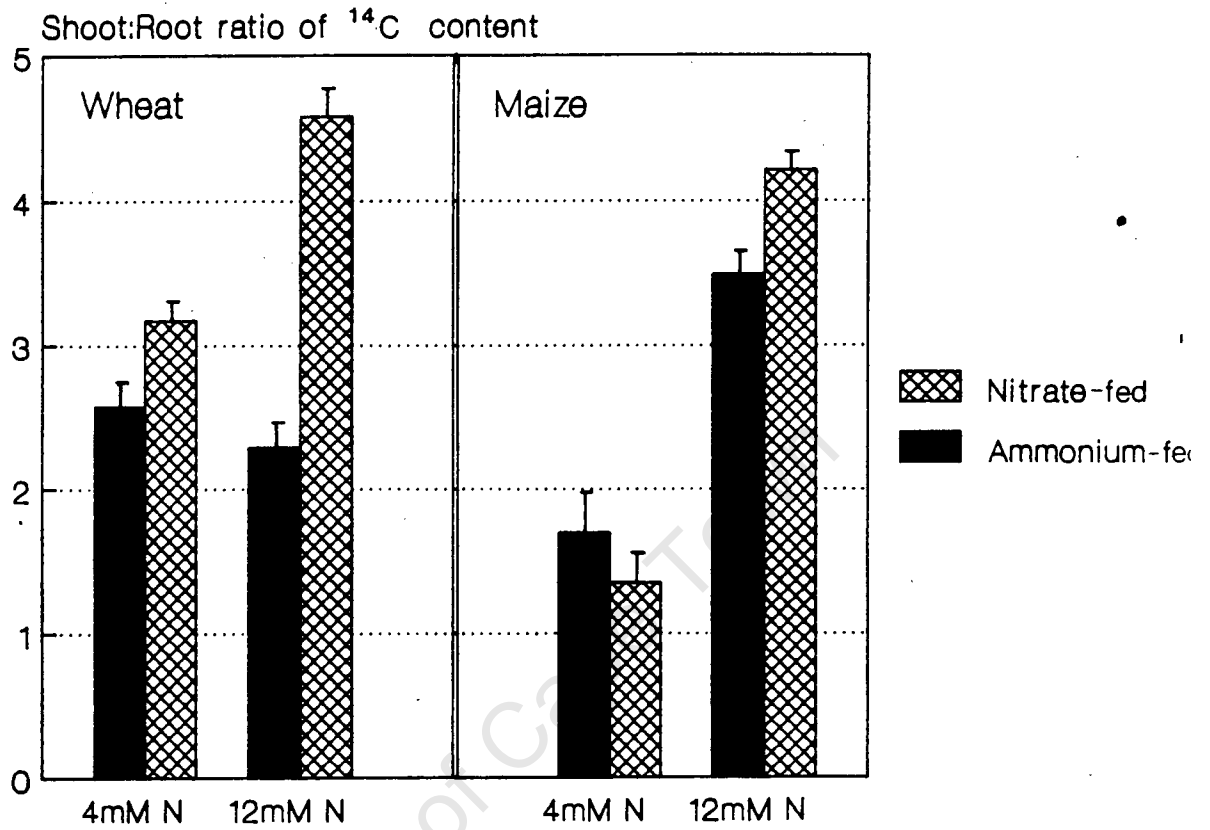


Figure 6: Ratio of shoot : root ^{14}C allocation in wheat and maize fed ammonium or nitrate at 4mM or 12mM concentrations. (Means of 3 replicates of 10 plants each for wheat and 2 plants each for maize. Bar heights represent S.E. Means and S.E. calculated after arcsin square root transformation).

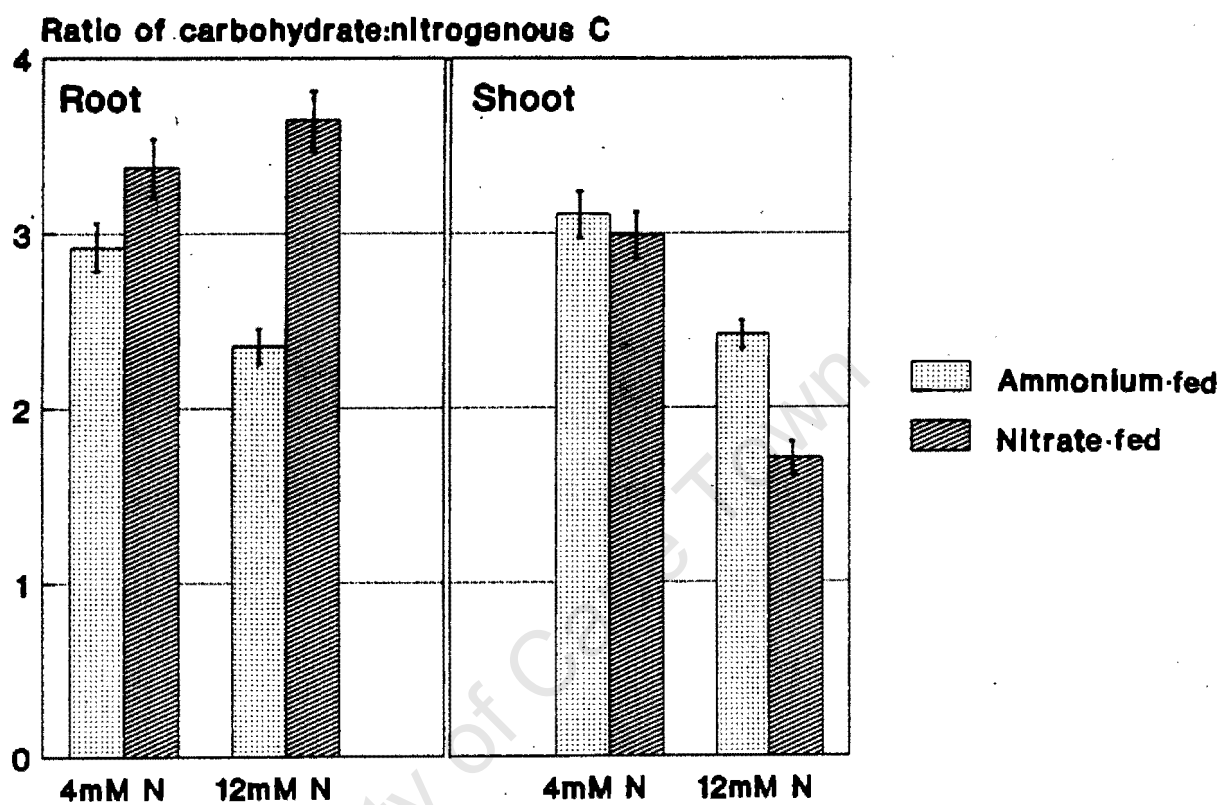


Figure 7: Ratio of ^{14}C allocation to carbohydrate : nitrogenous compounds in the roots and shoots of wheat fed ammonium or nitrate at 4mM or 12mM concentrations. (Means of 3 replicates of 10 plants each. Bar heights represent S.E. Means and S.E. calculated after arcsin square root transformation).

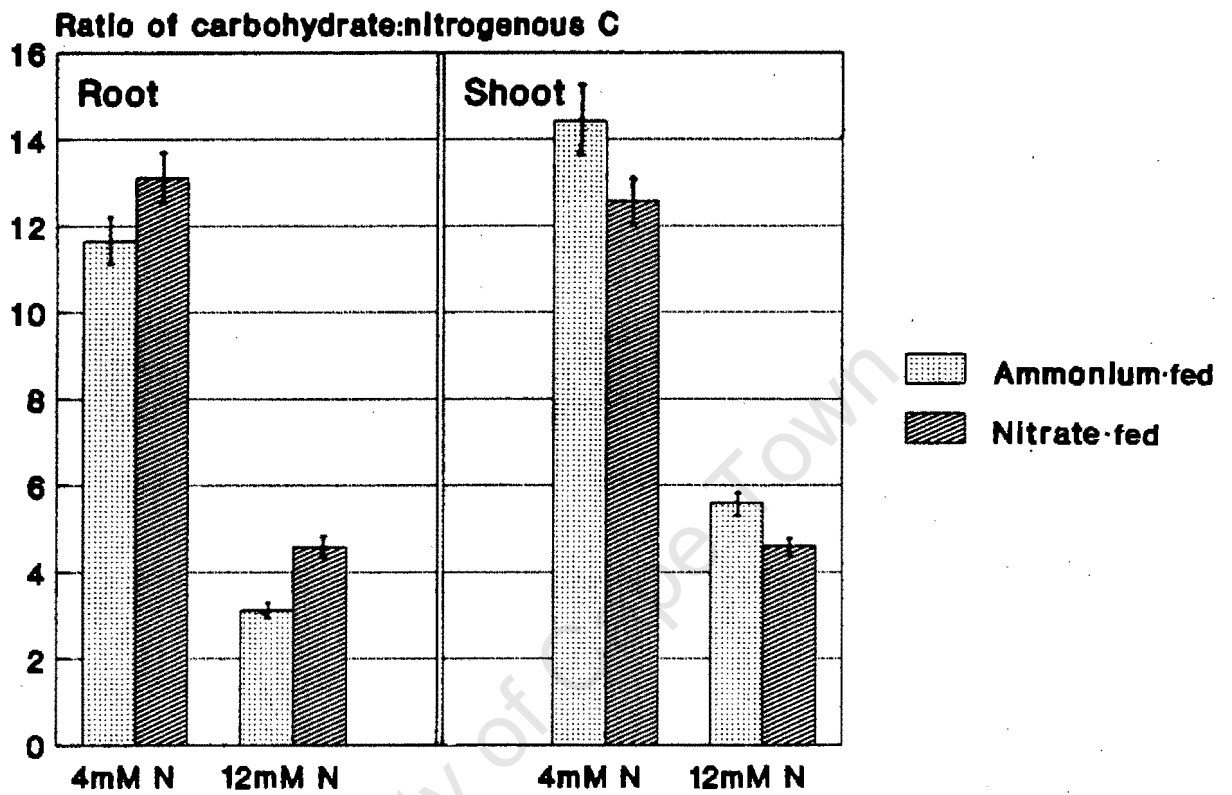


Figure 9: Ratio of ^{14}C allocation to carbohydrate : nitrogenous compounds in the roots and shoots of maize fed ammonium or nitrate at 4mM or 12mM concentrations. (Means of 3 replicates of 2 plants each. Bar heights represent S.E. Means and S.E. calculated after arcsin square root transformation).

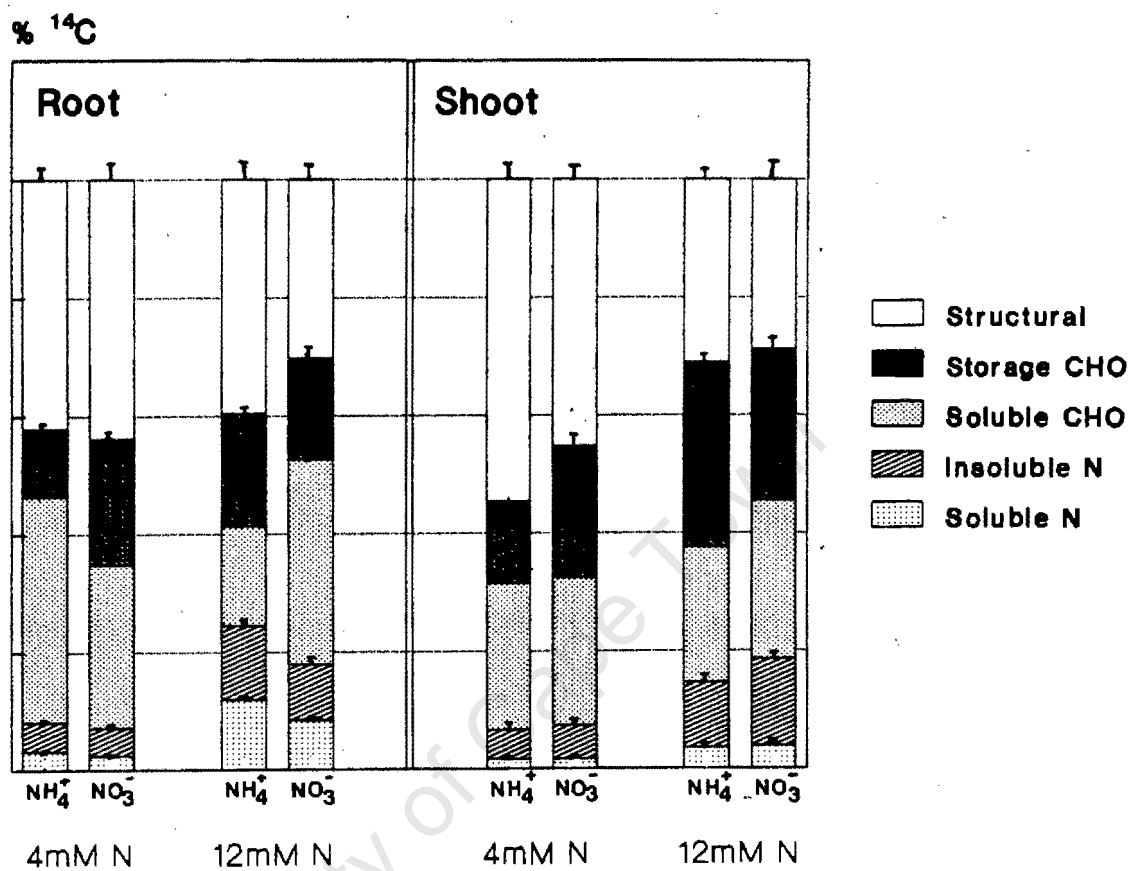


Figure 10: Ratio of ^{14}C allocation to soluble and insoluble nitrogenous- (N) and carbohydrate (CHO) fractions within the root and shoot of maize supplied ammonium or nitrate at 4mM or 12mM concentrations. (Means of 3 replicates of 2 plants each. Bar heights represent S.E. of the fractions below them. Means and S.E. calculated after arcsin square root transformation).

4.6.3. ^{14}C Allocation to Carbohydrate and Nitrogenous Compounds on a Whole Plant Basis

Percentage ^{14}C allocation to various fractions within roots or shoots based on within organ carbon content (Section 4.5.2.) is useful in that it may highlight changes occurring in ^{14}C allocation to various fractions once the ^{14}C is contained in either the root or shoot. It must be borne in mind however, that these carbon pools are not isolated in the root or shoot, and that all carbon pools except structural material, may be translocated from shoot to root or *vice versa*.

For this reason, it is of interest to consider ^{14}C allocation to various pools as a percentage of total ^{14}C in the plant. This allows for comparison of the relative sizes of the pools in roots and shoots in wheat (Table 6) and maize (Table 7).

4.6.3.1. ^{14}C Allocation in Wheat on a Whole Plant Basis

In 4mM ammonium-fed wheat, total ^{14}C allocation to nitrogen-compounds (soluble plus storage) was 24% where 7% was present in roots and 17% in shoots. In comparison, 4mM nitrate-fed wheat also showed an allocation of 24% to the same fraction, where 5% was present in the roots and 19% in the shoots (Table 6). Ammonium-fed plants appeared to allocate slightly more ^{14}C to the soluble nitrogen fraction, while nitrate-fed plants appeared to allocate slightly more to the insoluble nitrogen fraction on a whole plant basis.

In the 12mM N treatments, ammonium-fed wheat showed a ^{14}C allocation to nitrogen compounds of 29% (9% from the roots and 20% from the shoots) whereas nitrate-fed wheat allocated 34% (4% from the roots and 30% from the shoots : Table 6). Again, the nitrate-fed plants allocated more ^{14}C to the insoluble N compounds (28.4%) than to the soluble N compounds (5.7%) which was also true for ammonium-fed plants (allocation to soluble N compounds 12.1% and to insoluble N compounds 17.3%: Table 6).

However, on a whole plant basis, ^{14}C allocation to the nitrogen fractions (soluble plus storage) was not significantly different between ammonium- and nitrate-fed plants in either 4mM or 12mM nitrogen concentrations.

Allocation of ^{14}C to the soluble plus storage carbohydrate fractions at the 4mM N level was 31% in ammonium-fed plants (9% in the roots and 22% in the shoots) and 34% in nitrate-fed plants (9% in the roots and 25% in the shoots).

In 12mM N-fed wheat, ^{14}C allocation to the soluble plus storage carbohydrate fractions was 41.08% for ammonium- (10.81% in the roots and 30.27% in the shoots) and 34.61% for nitrate-fed plants (7.53% in the roots and 27.08% in the shoots : Table 6).

On a whole plant basis, ^{14}C allocation to non-structural carbohydrate fractions showed no significant difference between the ammonium- and nitrate-fed wheat at the 4mM N level. At the 12mM N level, there appeared to be a greater proportion of ^{14}C allocated to carbohydrates in ammonium-fed wheat.

The ^{14}C allocation to structural material on a whole plant basis was interesting, in that for both the 4mM and 12mM N levels, the ammonium-fed plants appeared to allocate proportionately more carbon to this fraction, especially in the root, when compared to the nitrate-fed plants. The percentage ^{14}C allocated to root structural carbohydrate was 11.16% for ammonium- and 9.41% for nitrate-fed wheat in the 4mM treatment, and 10.49% for ammonium- and 6.52% for nitrate-fed wheat in the 12mM treatment. A significant difference ($p < 0.05$) existed in the ^{14}C allocation to structural material between 12mM ammonium- and nitrate-fed wheat.

4.6.3.2. ^{14}C Allocation in Maize on a Whole Plant Basis

In 4mM ammonium- and nitrate-fed maize, total ^{14}C allocation to nitrogen compounds (soluble + storage) was 7%, where 3% was present in the roots and 4% in the shoots (Table 7).

In the 12mM N treatments, ammonium-fed maize showed a ^{14}C allocation to nitrogen compounds of 17%, where 6% was present in the roots and 11% in the shoots. 12mM nitrate-fed plants showed a similar ^{14}C distribution, where root nitrogen compounds contributed 4% and the shoot- 14% of the total plant ^{14}C allocation (18%) to nitrogen compounds (Table 7).

Proportional allocation of ^{14}C to nitrogen compounds in 4mM N-fed maize was similar (ca. 7%) for ammonium- and nitrate-fed treatments (Table 7) therefore. This allocation was significantly different ($p < 0.05$) in the 12mM N-fed plants (ca. 17%) when compared to the 4mM N-fed plants, but there was again no significant difference between maize from ammonium and nitrate treatments.

Allocation of ^{14}C to the soluble plus storage carbohydrate fractions at the 4mM N level was 43% for ammonium-fed maize (18% in the roots and 25% in the shoots : Table 7) and 48% for nitrate-fed maize (21% in the roots and 27% in the shoots : Table 7). In the 12mM N treatment, ^{14}C allocation to these combined fractions was slightly higher, with ^{14}C allocation to the soluble + storage carbohydrate fractions at 50% for ammonium-fed (8% in roots and 42% in shoots : Table 7) and 52% for nitrate-fed maize (10% in roots and 42% in shoots : Table 7).

Proportional allocation of ^{14}C to the soluble and storage carbohydrate fractions was high in both 4mM and 12mM ammonium- and nitrate-fed plants (ca. 50%) therefore. Again, there were no significant differences between maize fed ammonium or nitrate in this respect.

Allocation of ^{14}C to structural carbon in the 4mM N treatment was 50% for ammonium-fed maize (16% in roots and 34% in shoots : Table 7) and 45% in nitrate-fed maize (19% in roots and 26% in shoots : Table 7). In the 12mM N treatment, ^{14}C allocation to structural material was lower than that in the 4mM N treatment, being 33% in ammonium-fed maize (9% in the root and 24% in the shoot) and 29% in nitrate-fed maize (6% in the root and 23% in the shoot).

Table 6: Allocation of ^{14}C on a whole-plant percentage basis, to the carbon fractions of wheat fed either ammonium or nitrate at both 4mM and 12mM concentrations. (Means of 3 replicates of 10 plants each \pm S.E.).

C Fraction & location (Root (Rt), Shoot (Sht), or Total Plant (Plt))		4mM		12mM	
		Ammonium	Nitrate	Ammonium	Nitrate
Soluble N	Rt	2.57 \pm 0.21	1.21 \pm 0.08	4.91 \pm 0.33	1.32 \pm 0.17
	Sht	2.90 \pm 0.18	2.66 \pm 0.14	7.20 \pm 0.13	4.40 \pm 0.39
	Plt	5.48 \pm 0.44	3.87 \pm 0.34	12.11 \pm 0.31	5.72 \pm 0.32
Insoluble N	Rt	4.58 \pm 0.27	4.35 \pm 0.20	4.15 \pm 0.11	2.52 \pm 0.11
	Sht	14.61 \pm 0.31	16.40 \pm 0.64	13.16 \pm 0.64	25.90 \pm 0.75
	Plt	19.19 \pm 0.34	20.75 \pm 0.45	17.31 \pm 0.77	28.42 \pm 0.59
Soluble Carbo- hydrates	Rt	4.51 \pm 0.43	2.74 \pm 0.22	6.32 \pm 0.47	3.47 \pm 0.33
	Sht	10.88 \pm 0.61	12.88 \pm 0.63	16.61 \pm 1.54	14.07 \pm 1.50
	Plt	15.39 \pm 1.27	15.62 \pm 1.52	22.93 \pm 2.09	17.54 \pm 1.63
Storage Carbo- hydrates	Rt	5.14 \pm 0.55	6.23 \pm 0.09	4.49 \pm 0.13	4.06 \pm 0.17
	Sht	11.01 \pm 0.38	12.26 \pm 0.12	13.66 \pm 0.39	13.01 \pm 1.19
	Plt	16.15 \pm 0.77	18.49 \pm 0.13	18.15 \pm 0.51	17.07 \pm 1.67
Structural Carbon	Rt	11.16 \pm 0.75	9.41 \pm 0.97	10.49 \pm 0.91	6.52 \pm 0.09
	Sht	32.64 \pm 0.89	31.82 \pm 0.42	19.05 \pm 0.69	24.73 \pm 1.19
	Plt	43.80 \pm 1.42	41.23 \pm 2.26	29.54 \pm 1.79	31.25 \pm 1.40

Table 7: Allocation of ^{14}C on a whole-plant percentage basis, to the carbon fractions of maize fed either ammonium or nitrate at both 4mM and 12mM concentrations. (Means of 3 replicates of 2 plants each \pm S.E.).

C Fraction & location (Root (Rt), Shoot (Sht), or Total Plant (Plt))		4mM N		12mM N	
		Ammonium	Nitrate	Ammonium	Nitrate
Soluble N	Rt	1.17 \pm 0.07	1.00 \pm 0.18	2.67 \pm 0.15	1.62 \pm 0.12
	Sht	1.01 \pm 0.08	0.91 \pm 0.09	2.74 \pm 0.32	3.06 \pm 0.24
	Plt	2.18 \pm 0.15	1.91 \pm 0.26	5.41 \pm 0.37	4.68 \pm 0.16
Insoluble N	Rt	1.82 \pm 0.11	2.03 \pm 0.09	2.78 \pm 0.10	1.81 \pm 0.19
	Sht	3.10 \pm 0.13	3.30 \pm 0.35	8.64 \pm 0.36	11.25 \pm 0.57
	Plt	4.92 \pm 0.12	5.33 \pm 0.52	11.42 \pm 0.43	13.76 \pm 0.76
Soluble Carbo- hydrates	Rt	14.21 \pm 0.39	11.76 \pm 1.50	3.74 \pm 0.23	6.67 \pm 0.49
	Sht	15.76 \pm 0.21	14.44 \pm 0.69	17.82 \pm 1.72	21.76 \pm 2.42
	Plt	29.97 \pm 0.66	26.20 \pm 2.17	21.56 \pm 1.97	28.43 \pm 2.72
Storage Carbo- hydrates	Rt	4.24 \pm 0.58	9.07 \pm 0.92	4.26 \pm 0.37	3.27 \pm 0.31
	Sht	8.69 \pm 0.85	12.78 \pm 1.62	24.32 \pm 1.85	20.75 \pm 1.81
	Plt	12.93 \pm 1.21	21.85 \pm 2.15	28.58 \pm 1.97	24.02 \pm 1.92
Structural Carbon	Rt	15.60 \pm 1.67	18.66 \pm 1.48	8.82 \pm 0.60	5.81 \pm 0.06
	Sht	34.40 \pm 3.21	26.05 \pm 0.33	24.21 \pm 1.04	23.30 \pm 1.63
	Plt	50.00 \pm 2.59	44.71 \pm 1.81	33.04 \pm 1.10	29.11 \pm 1.66

4.7. Total N and ^{15}N Distribution in Maize

Total nitrogen content and ^{15}N content of maize plants fed 12mM N are shown in Table 8. In ammonium-fed maize, total nitrogen content of roots (2.66 mg N/g) and shoots (4.52 mg N/g) was higher than in roots (1.78 mg N/g) and shoots (3.41 mg N/g) of nitrate-fed plants respectively, but this was not a significant difference. The ^{15}N content was significantly higher ($p < 0.05$) in ammonium-fed plants (582 $\mu\text{g/g}$ in roots and 817 $\mu\text{g/g}$ in shoots respectively) when compared to nitrate-fed plants (265 $\mu\text{g/g}$ in roots and 300.54 $\mu\text{g/g}$ in shoots). This indicates faster uptake of nitrogen in ammonium-fed plants in comparison to nitrate-fed plants. It is interesting to note however, that this higher level of ^{15}N assimilation in ammonium-fed plants, compared to nitrate-fed plants, was not as evident in the measure of total nitrogen content. The ratio of ammonium-fed plant ^{15}N content : nitrate-fed plant ^{15}N content was 2.2 : 1 for roots and 2.7 : 1 for shoots, while the ratio ammonium-fed plant total nitrogen content : nitrate-fed plant total nitrogen content was 1.5 : 1 for roots and 1.3 : 1 for shoots (Table 8).

Table 8: Total nitrogen (mg N g^{-1} fw), ^{15}N enrichment (A%E) and ^{15}N concentration ($\mu\text{g N g}^{-1}$ fw) in roots and shoots of maize supplied either ammonium as $12\text{mM } 99\text{A}\% \text{E } ^{15}\text{N NH}_4\text{Cl}$, or nitrate as $12\text{mM } 99\text{A}\% \text{E } ^{15}\text{N KNO}_3$. (Means of three replicates of 2 plants each, \pm S.E.).

N-Fraction	Nitrogen Treatment	ROOT			SHOOT		
		N conc. mgN/g fw	^{15}N A&E	^{15}N conc. $\mu\text{gN/g fw}$	N conc. mgN/g fw	^{15}N A&E	^{15}N conc. $\mu\text{gN/g fw}$
Soluble	12mM ammonium	1.59 ± 0.05	28.15 ± 1.13	447.80 ± 22.17	2.99 ± 0.09	22.48 ± 0.84	686.01 ± 46.07
	12mM nitrate	1.29 ± 0.05	18.79 ± 0.25	245.47 ± 11.32	2.76 ± 0.14	10.10 ± 0.50	279.98 ± 10.16
Bound	12mM ammonium	1.07 ± 0.06	12.53 ± 0.28	134.36 ± 7.39	1.53 ± 0.43	8.67 ± 0.14	131.68 ± 9.35
	12mM nitrate	0.49 ± 0.02	3.95 ± 0.14	19.48 ± 1.28	0.65 ± 0.07	3.14 ± 0.05	20.56 ± 2.42

CHAPTER 5 : DISCUSSION

5.1. Wheat

5.1.1. Plant Growth Responses

From the observed growth response of wheat to ammonium nutrition (section 4.1.), and from the shoot : root ratios obtained in this work (Figure 3), it is clear that even when the pH of the growth media was carefully controlled, stunting of wheat, especially of the roots, still occurred in response to ammonium nutrition. This is in agreement with previous reported results (Lewis and Chadwick, 1983; Lewis *et al.* 1987). Ammonium toxicity therefore appears not to be a direct effect of increased acidity, as has been suggested (Hewitt, 1966; Maynard and Barker, 1969).

5.1.2. Photosynthesis

Ammonium-fed wheat did not show a significant increase in photosynthetic rate compared to nitrate-fed plants as measured by CO₂ uptake m⁻²s⁻¹ (Figure 4). This was suggested to occur in ammonium-fed plants due to the linear correlation between nitrogen content and photosynthetic rate (Field and Mooney, 1986). Ammonium-fed wheat did not appear to have a significantly higher shoot nitrogen content relative to nitrate-fed wheat shoots, however (Table 1), which may account for the similarity in photosynthetic rate observed between treatments.

5.1.3. Root Respiration

Due to the necessary assimilation of ammonium in the root, it has been suggested that more carbon may be lost via root respiration in ammonium-fed plants than in nitrate-fed plants. This would render less carbon available for allocation to growth and lead to the stunting observed (especially of the roots) in plants supplied ammonium (reviewed in Givan, 1979). Measurements of root respiration occurring in ammonium- and nitrate-fed plants (Figure 5) showed no significant differences in root respiration (as measured by CO_2 evolution g^{-1} fw) between the two nitrogen feeding regimes, however.

Growth, maintenance of biomass and transport (ion uptake) are the three major energy-requiring processes occurring in the root. In nitrate-fed plants, root respiratory energy is required for the active uptake of the nitrate ion against a concentration gradient, while in ammonium-fed plants, uptake of nitrogen is largely passive. Ammonium-fed plants, however, are required to assimilate this nitrogen in the root immediately via the ATP and reduced NAD-requiring GS-GOGAT pathway, thus using respiratory energy, while nitrate-fed plants expend relatively little root respiratory energy in transporting nitrate to the shoot for assimilation. In nitrate-fed plants, some nitrate is reduced in the roots however, and there may also be considerable losses due to the bicarbonate exchange for nitrate in the root, and the energy requirement for the active uptake of this ion. From the data presented here, it appears that the total of the respective energy-requiring processes in the roots of ammonium- and nitrate-fed plants is very similar. The respective respiratory carbon losses in the roots of ammonium-fed plants are due to the ATP and reduced NAD requiring GS-GOGAT pathway, and in nitrate-fed plants are due

to energy required for active nitrate absorption and some nitrate reduction in the root. It thus appears that root respiration is not an important source of carbon loss occurring exclusively in ammonium-fed plants, which can be held accountable for the reduced growth observed in these plants compared to nitrate-fed plants.

5.1.4. Xylem Sap

Ammonium-fed wheat has been shown previously to contain higher levels of total nitrogen and newly assimilated nitrogen, than nitrate-fed wheat (Table 1, section 2.4.). Results reported here from xylem sap analysis showed that more amino compounds are transported from the root in ammonium-fed plants compared with nitrate-fed plants, and the C:N ratio of the xylem sap was lower. This implies that ammonium-fed plants transported more nitrogen relative to carbon from the roots to the shoots than nitrate-fed plants. However, total carbon content of the xylem sap from ammonium-fed wheat was higher than that from nitrate-fed wheat, but not significantly so (Table 4). It therefore appears that although ammonium-fed plants transported more nitrogen to the shoots, this was incorporated into amino compounds that have a low carbon content relative to nitrogen, for example the amides. This has been reported previously for barley (Lewis and Chadwick, 1983).

The amount of ammonium detected in xylem sap was low, and not significantly different for ammonium- or nitrate-fed plants. This implies that the toxic effect of ammonium was not due to the uncoupling of photo-phosphorylation in the shoot, as has been suggested (Mills and Jones, 1979).

5.1.5. Plant Water Content

A significant difference was found in plant water content between ammonium- and nitrate-fed wheat (Table 5). It is possible that this may contribute to the poor response to ammonium nutrition observed, and may explain the tendency of ammonium-fed plants to lodge. It has been suggested (Smirnov and Stewart, 1985; Salsac *et al.*, 1987) that nitrate nutrition plays an important role in the osmoregulation of plants. These authors propose that although a high concentration of nitrate in the vacuole may serve as a reserve nitrogen source, it may be equally important in regulating osmotic balance of the plant in response to fluctuating external water availability. The accumulation of ions serves as the least energy-expensive mechanism of osmoregulation and nitrate is the ion most commonly available to plants for this purpose. Plants fed ammonium might have low levels of organic anions (e.g. malate) if their formation is dependent on the presence or reduction of nitrate. These low levels in turn cause a decrease in uptake and translocation of mineral cations and decrease vacuolar solute accumulation. This would impair the ability of the plant to osmoregulate effectively. If this were the case, then the favourable response exhibited by wheat to combined ammonium and nitrate nutrition (Lewis *et al.* 1987) would be due to nitrate inhibiting the passive flow of ammonium into the plant (Deignan and Lewis, 1988) as well as its stimulation of malate production aiding osmoregulation.

5.1.6. ^{14}C Allocation to Nitrogenous Compounds

The roots of ammonium-fed wheat appeared to act as greater sinks for newly assimilated carbon than those of nitrate-fed wheat (Figure 6), suggesting an increased carbon requirement in the roots for ammonium assimilation. Of the newly assimilated carbon translocated from the shoot to the root, more was found in nitrogenous compounds relative to carbohydrate compounds in ammonium- than in nitrate-fed wheat (Figure 7) and this difference was mainly due to an increase in allocation to the soluble nitrogen pool (Figure 8). A larger proportion of newly assimilated carbon was also allocated to this pool within the shoots of ammonium-fed plants at the 12mM level, compared to 12mM nitrate-fed wheat shoots. This supports the concept that assimilated ammonium is transported to the shoots with carbon.

5.1.7. ^{14}C Allocation to Structural Material

On a within root ^{14}C allocation basis, there was a small, but not significant, reduction in allocation to structural material in ammonium-fed roots (Figure 8). Although there is a possibility that this may have a cumulative effect over time, it appears, from data of ^{14}C allocation ratios based on whole plant ^{14}C , that this is not likely to be the case. On a whole plant basis, ammonium-fed plants appeared to allocate more ^{14}C to the structural carbohydrate than nitrate-fed plants. The reason why this apparent contradiction occurs, is that although proportionately more ^{14}C within the ammonium-fed root was allocated to nitrogenous compounds relative to structural compounds when compared to nitrate-fed plants, a larger proportion of total plant ^{14}C had been diverted to the root in ammonium-fed

plants, which accommodated this increase. This becomes clear when proportional ^{14}C allocation is expressed as percentage of that present in the whole plant.

5.1.8. Summary of Wheat Response to Nitrogen Form

In the ammonium-fed plant, there is a greater initial carbon allocation to the root (compared to nitrate-fed plants) to accommodate the need for the assimilation of large amounts of ammonium in the root (Table 1). This is then cycled back to the shoot in the form of soluble N compounds. Allocation to structural material does not appear to be affected by the obligatory assimilation of nutrient ammonium in the root, and root respiratory loss is not significantly different between ammonium- and nitrate-fed plants.

In nitrate-fed wheat, less carbon is diverted to the root than in ammonium-fed plants. The active uptake of nitrate from the external medium causes considerable respiratory carbon loss, however, that is comparable to the respiratory loss due to ammonium assimilation in the roots of plants supplied ammonium (Figure 5). Nitrate assimilation appears to occur predominantly in the shoots, as indicated by the larger allocation of ^{14}C to nitrogenous compounds in the shoots of nitrate- compared to ammonium-fed wheat (Figure 8).

The poor response of wheat to ammonium nutrition does not, therefore, appear to be due to large root respiratory carbon losses in these plants compared to nitrate-fed plants, nor to a reduced allocation to structural material. The necessity for the transport of nitrogen to the shoot accompanied by carbon in ammonium-fed plants

appears to be compensated for by an initial increase in the proportion of ^{14}C diverted from shoot to root in these plants.

The ammonium-detoxifying mechanisms of the plant are known to be very efficient (reviewed in Givan, 1979). However, it is possible that large-scale ammonium influx may disrupt root functioning by interference with transmembrane proton gradients at high nitrogen feeding levels, due to its presence in the root not being spatially or temporally controlled. The concentration of ammonium and nitrate in soils is seldom higher than 1mM (Harmsen and Kolenbrander, 1965), and the concentration required to sustain most species is in the region of 20uM (Ingstad, 1982).

Investigation of the possible role of nitrate in osmoregulation, the interrelating effects of malate production and their impact on plant response to ammonium and nitrate nutrition was not within the scope of the research reported here. However, the results obtained showing the significantly lower water content in ammonium-fed compared to nitrate-fed wheat, suggest that differences in osmoregulation may contribute to the poor response of wheat to ammonium nutrition. Lower water use efficiency has been reported for wheat fed ammonium in comparison to wheat fed nitrate by Lips *et al.*, (1990).

5.2. Maize

5.2.1. Plant Growth Responses

Maize appeared to show an equally favourable growth response, regardless of nitrogen form supplied. No stunting of root growth in ammonium-fed plants was evident (Figure 3) and the shoots were erect.

5.2.2. Photosynthesis

Photosynthetic rate was predictably high in maize, due to the C-4 photosynthetic pathway coupled with the high light intensity and high temperature growth conditions (Figure 4). Although photosynthetic rate appeared to be slightly higher in maize fed ammonium, this was not significantly different from that of nitrate-fed plants. As with wheat, the predicted higher photosynthetic rate of ammonium-fed plants based on their predicted higher nitrogen content (Field and Mooney, 1986) was not found in maize plants, and this is likely to be due to the lack of significant differences in the total shoot nitrogen content between shoots of ammonium- and nitrate-fed maize (Table 7).

5.2.3. Root Respiration

A significantly higher root respiratory rate (as measured by CO_2 respired g^{-1} fw) was found in maize fed nitrate in comparison to maize fed ammonium. In explaining this, two factors should be considered. Firstly, uptake of nitrate may cause CO_2 loss via the active uptake mechanism required for this ion, and via the

bicarbonate/nitrate exchange during uptake. These losses may be greater than those in ammonium-fed plants at high nitrogen feeding levels. Secondly, a contributing factor to the higher respiration rate observed in nitrate-fed plants may be the supply of the iron source as ferric citrate. Ferric citrate is the form in which iron is transported in the plant, and is reduced when required by the enzyme nitrate reductase (Campbell and Redinbaugh, 1984). Although this occurs mainly in the leaves, iron is important for a number of cellular functions in the root. Under the conditions prevailing in these experiments, the roots may be provided with their iron requirement by direct assimilation of ferric citrate in the root since this iron source is readily available in the nutrient medium. Since nitrate reductase is an inducible enzyme, it is possible that the nitrate-fed plants, which would have larger quantities of the enzyme, may assimilate more of the ferric citrate and so result in having a higher root respiration rate. This higher rate did not appear to have any adverse effect (in terms of growth rate or appearance) on the nitrate-fed maize.

5.2.4. Xylem Sap

Ammonium-fed maize transported significantly more amino compounds to the shoot in comparison to nitrate-fed maize. This transport was accompanied by significantly more carbon (Table 4). This indicates rapid assimilation of ammonium in the maize root and rapid translocation of this assimilate to the shoot accompanied by carbon.

5.2.5. Plant Water Content

No difference in water content between ammonium- and nitrate-fed maize was found (Table 5). It has been suggested (Smirnoff and Stewart, 1885; Salsac *et al.*, 1987) that nitrate nutrition may play a role in osmoregulation, by maintaining osmotic balance through vacuolar storage of nitrate, and by stimulating the production of malate. Maize possesses a mechanism for the active synthesis of organic anions independently of nitrate assimilation, since it is a C-4 malate former. If poor osmoregulation is a cause of the poor response of some plants to ammonium nutrition, then the ammonium tolerance exhibited by maize may be explained by this independent pathway for organic anion synthesis. No significant difference in water content was found between maize supplied ammonium or nitrate.

5.2.6. ^{14}C Allocation to Nitrogenous Compounds

At the 4mM nitrogen feeding level, a large proportion of newly assimilated carbon was diverted from the shoots to the roots in both ammonium- and nitrate-fed treatments (Figure 6). This indicates a rapid translocation of newly assimilated carbon from shoot to root. A very small proportion of this was allocated to nitrogenous compounds (Figure 9), with most going into soluble and storage carbohydrate (Figure 10). This suggests that the root may serve as a storage organ for reserve carbohydrate. At the 12mM N feeding level, a smaller proportion of newly assimilated carbon was found in the root compared to the 4mM feeding level, although there was a significantly greater proportion in the roots of ammonium- compared to nitrate-fed maize. Of the ^{14}C within the root, a far greater proportion

was allocated to nitrogenous compounds in comparison to 4mM nitrogen-fed roots, and a larger proportion was in association with nitrogen in the shoot.

5.2.7. ^{14}C Allocation to Structural Material

Proportional allocation of ^{14}C to structural material on a whole plant ^{14}C content basis appeared to be slightly higher in ammonium-fed maize in comparison to nitrate-fed maize. This indicates that allocation to this fraction was not reduced in response to ammonium nutrition, to compensate for increased ^{14}C allocation to processes associated with ammonium assimilation.

5.2.8. ^{15}N and Total Nitrogen Content of Maize

It is of interest to note that although the levels of newly assimilated nitrogen (^{15}N) were higher in ammonium- with respect to nitrate-fed maize, the difference in total nitrogen content between the two treatments was much smaller (section 4.7). While the newly assimilated N content was more than two fold higher in the roots and in the shoots of ammonium-fed compared to nitrate-fed maize, the total N content was only 1.5- (roots) and 1.3- (shoots) fold higher. This suggests that maize may have the capacity to cycle ammonium out of the plant again, thus controlling the amount of assimilation of this ion required in the root.

5.2.9. Summary of Maize Response to Nitrogen Form

It therefore appears that in maize fed 4mM nitrogen, there is a greater availability of carbon, which is rapidly translocated to the root and is present in this organ mainly as soluble and storage carbohydrate. Nitrate-fed plants appeared to allocate more ^{14}C to the roots at this feeding level than ammonium-fed plants. A small proportion of newly assimilated carbon in the root was allocated to nitrogenous compounds, relative to carbohydrate compounds. This indicates that the carbon required for nitrogen assimilation at the 4mM N feeding level represents only a small proportion of the total carbon fixed by maize.

At the 12mM nitrogen feeding level, a smaller proportion of newly assimilated carbon was found in maize roots than at the 4mM nitrogen feeding level, but a larger proportion of this was associated with nitrogen. Of the ^{14}C in the shoot, there was also more associated with nitrogen at this feeding level. This suggests that in ammonium-fed plants, nitrogen is assimilated rapidly and transported to the shoots (since negligible amounts of ammonium were found in the xylem sap : Table 8), while in nitrate-fed plants, nitrate is taken up actively and transported to the shoot for assimilation, although some assimilation also occurred in the root, as is evidenced by the amino compound content of the xylem sap (Table 8).

5.3. Comparison of Wheat and Maize Response to Nitrogen Form

The most important factor determining the difference between wheat and maize in their response to ammonium and nitrate nutrition, appears to be the far larger carbon gaining capacity of maize. This species appears to have large carbon reserves that can be mobilized at high nitrogen levels for efficient assimilation and rapid translocation of nitrogen. In wheat, carbon allocation to the root was increased only in response to ammonium nutrition. The carbon budget therefore appears to be much more tightly controlled. Maize, on the other hand, had large amounts of reserve carbon, and allocated root reserves to nitrogen assimilation and rapid translocation, instead of diverting carbon from the shoot to the root in response to ammonium nutrition, as occurred in wheat.

In the root of ammonium-fed wheat, the increase in allocation to nitrogenous compounds compared to that in nitrate-fed wheat appeared to be compensated for by an increase in the initial diversion of ^{14}C to the root. It appears, however, that retranslocation of this carbon to the shoot was slow. There was no large difference in carbon content of xylem sap from ammonium- and nitrate-fed plants (Table 4). From previous work (Table 1), it appears that most of the newly-assimilated carbon that had been diverted to the root was still in the root 24h after ^{15}N feeding. In maize, however, rapid translocation of ^{14}C and ^{15}N occurred.

If the toxic effects of ammonium observed in wheat were due to abnormally high nitrogen levels imposed experimentally, one may expect maize to exhibit a similar response. This was not the case, however. It is possible that the high rate of carbon assimilation afforded by the C-4 photosynthetic pathway and the high light

intensities provided, allowed for sufficient carbon fixation to cope with the imposed nitrogen stress.

Finally, if stored nitrate, or the induction of malate production by nitrate is important in osmoregulation, then maize fed ammonium has an alternative pathway for malate production, while wheat does not.

5.4. Suggestions for Further Research

The experiments carried out for this work traced the fate of labelled carbon and nitrogen after 24h assimilation time. It would be interesting to carry out a set of serial harvests after, for example, 6, 12, 18, 24, 36 and 48 hours of labelled feeding, to gain more insight into the flux of C and N through the plant.

Plants in these experiments were supplied ^{15}N or ^{14}C after three weeks growth with either ammonium or nitrate, which resulted in the ammonium-fed wheat already showing symptoms of poor growth and root stunting. Experiments where all plants are provided with a nitrate plus ammonium mix for the first two weeks growth, and then provided ammonium or nitrate as the sole nitrogen source would circumvent this problem. This could be followed by ^{15}N and ^{14}C tracing after one week and two weeks growth on either nitrogen source, to elucidate more clearly what changes in carbon allocation occur in response to ammonium or nitrate nutrition.

It appears that the carbon allocation patterns in maize differ from that in wheat mainly because of the large differences between the two species in the amount of carbon fixed. It would therefore be of interest to see how maize would allocate carbon in response to ammonium and nitrate nutrition under low light growth conditions, where the rate of carbon fixation was comparable to that of wheat. It would be interesting to compare this with a C-4 aspartate former, and to include measurements of malate produced under the different nitrogen feeding regimes.

It is interesting to note that plants fed ammonium (particularly maize) do not appear to have total N contents appreciably higher than nitrate-fed plants, although their ^{15}N (newly assimilated N) content is significantly higher. This implies that nitrogen is cycled out of these plants again in some way. The mechanism by which this occurs requires further research, and is currently being addressed by workers here and at the Ben Gurion University of the Negev.

CONCLUSIONS

The poor response of some species, such as wheat, to ammonium nutrition has often been attributed to a diversion of carbon from structural compounds (for root extension) to processes associated with detoxification of ammonium in the root. These include the provision of carbon skeletons for the formation of amino compounds which would subsequently be lost to the root through translocation to the shoot, and the increased carbon loss via root respiration due to the necessary ammonium assimilation in the root away from the site of carbon fixation.

The work reported here shows that root respiration of ammonium-fed wheat is not significantly different from that of nitrate-fed wheat. This suggests that carbon loss due to root respiration for the active uptake of nitrate is comparable to that lost due to assimilation of ammonium in roots. Increased carbon loss via root respiration in wheat supplied ammonium does not appear to explain root stunting observed in these plants. ^{14}C allocation to structural material in roots of ammonium-fed plants did not appear to be reduced compared to nitrate-fed plants. On the basis of these results, stunted root growth of ammonium-fed plants can therefore also not be attributed to a reduction in carbon available for root extension.

Although translocation of nitrogen to the shoots of plants fed ammonium was in association with carbon, this appeared to be compensated for by a larger initial diversion of ^{14}C to the root than in nitrate-fed plants.

Maize had larger carbon reserves than wheat, which appeared to be allocated to the root, but could be mobilized for rapid assimilation and translocation of nitrogen in maize supplied high (12mM) concentrations.

Alteration of carbon allocation in response to nitrogen form does not appear to explain the poor response to ammonium nutrition observed in wheat. Investigation of the effects of nitrogen form on osmoregulation efficiency was not within the scope of this research. It is, however, interesting to note the difference in water content found between ammonium- and nitrate-fed wheat, which was not evident in maize. If nitrate nutrition has an osmoregulatory function, then maize may be able to compensate under conditions of ammonium nutrition via the independent (of nitrate) production of malate.

APPENDIX 1**Pilot Study of the Maintenance of pH in Sand Culture Experiments**

A pilot study was carried out to test the effectiveness of using calcium carbonate to buffer pH fluctuations in sand used for plant culture. Plants were grown two per pot for two weeks (maize), or ca. 10 per pot for three weeks (wheat) in calcium carbonate-treated sand ($\pm 7\text{g CaCO}_3$ mixed into the sand for each pot) and fed either nitrate or ammonium. After the growth period, sand pH was tested using the method of Schoffield and Taylor (1955). Ten grams of sand from each pot and 25ml 0.01M CaCl_2 were shaken together for 30 minutes and the pH of the suspension read using a Beckman pH meter fitted with a glass electrode. Two replicates were carried out for each pot. The results show that the method maintained pH at a favourable, constant level in both treatments for the growth period required (pH of sand for nitrate-fed plants 6.55 ± 0.05 S.E.; for ammonium-fed plants 6.58 ± 0.02 S.E.; number of replicate pots = 5).

APPENDIX 2**Pilot Studies optimizing Hydroponic Cultivation of Maize**

Four varieties of maize, PNR 394, PNR 95, Kudu 9046 and PNR 6528 (Starke Ayres (Pty) Ltd, Evans Ave, Epping 1, Box 304, Epping Ind. Est., Cape) were tested for their compatibility with hydroponic culture under greenhouse conditions. Six replicates of three plants each for each variety were planted out on shade nets suspended over 1.7dm³ pots containing standard Long Ashton solution with 4mM KNO₃. The solutions were aerated vigorously using polythene tubes connected to an air supply, to prevent anaerobic conditions from developing in the feeding solution. After two weeks, it was found that the variety PNR 394 appeared to respond best to hydroponic culture. However, a repetition of the experiment under high light conditions caused symptoms of chlorosis and striation to recur.

Two possible problems were identified.

- 1) Vigorous aeration of nutrient solutions may adversely affect growth by damaging the delicate root hairs. This was suggested by the more healthy appearance of plants in a pot where the aeration tube became blocked.
- 2) Yellowing of the leaves suggests an iron deficiency, which has also been reported for hydroponically grown maize by Murphy (1984).

In the light of this, the following modifications were made:

1) Plants were cultivated in large troughs where the aeration source was situated far from the roots and did not agitate the solution directly bathing the roots. This method improved growth, but striation still occurred.

2) The iron source fed to plants was manipulated in several ways.

a) The iron content of the Long Ashton solution (in the form of FeEDTA) was increased three-fold and five-fold.

b) Supplementary iron feeding in the form of foliar supplied ferric citrate was given daily.

c) The FeEDTA iron supplement in the Long Ashton Solution was supplied as Sequestron (Ciba Geigy).

At this time, co-workers investigating the same problem in our laboratory found that a modified Long Ashton solution containing ferric citrate as the iron source produced favourable growth without striation occurring. The growth methods and materials finally used in this work therefore used this iron supplement.

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