AN INVESTIGATION OF ASPECTS OF THE NITROGEN PHYSIOLOGY OF *HELIANTHUS ANNUUS* L.

by

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Stulen, I. (1986). Interactions between nitrogen and carbon metabolism in a whole plant context. In: Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants. Eds. H. Lambers, J.J. Neeteson and I. Stulen. pp 455-471. Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster.

CONTENTS

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ABBREVIATIONS

ALA	alanine
ARG	arginine
ASN	asparagine
ASP	aspartate
ATP	adenosine triphosphate
A%E	atom percent excess
CYS	cysteine
GAB	amino butaric acid
GLN	glutamine
GLU	glutamate
GLY	glycine
GS	glutamine synthetase
GSA	glutamine synthetase activity
HIS	histidine
ILE	isoleucine
LEU	leucine
LYS	lysine
MET	methionine
NH_3	ammonia
NR	nitrate reductase
NRA	nitrate reductase activity
N-SERVE	2-chloro-6-(trichloromethyl)pyridine
PAL	phenylalanine
PRO	proline

PVP	polyvinylpyrrolidone
SER	serine
THR	threonine
TYR	tyrosine
VAL	valine

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PREFACE

The main objective of this work was to investigate aspects of nitrogen metabolism in Helianthus annuus L. as related to different nitrogen sources.

The thesis is structured into chapters, each investigating different aspects of the topic. A general introduction to the thesis is given to provide the background to the work. Each chapter, however, contains an introduction on the specific topics for which the individual experiments were designed. There is a chapter which contains the general materials and methods used in the separate experiments with each chapter containing a specialised materials and methods section for that particular experiment. The thesis is concluded with a unifying chapter which sums up the major conclusions derived from the work.

ABSTRACT

Helianthus annuus L. plants were grown hydroponically in Long Ashton nutrient solutions containing either 2 mM ammonium, 2 mM nitrate or 1 mM ammonium + 1 mM nitrate as the nitrogen supply to determine the effect of these nutrients upon physiological processes within the plant.

Nitrate fed plants had a larger root mass than ammonium fed plants whereas ammonium fed plants had a larger shoot mass than those fed with nitrate. Ammonium + nitrate fed plants produced both large shoots and roots. The fruit mass of plants fed nitrate was greater than plants fed ammonium. Ammonium + nitrate fed plants produced a greater fruit mass than either of the other single nitrogen sources. Nutrient supplied at late stages of fruit filling was shown to be beneficial to the fruit.

Sunflower plants supplied with ammonium + nitrate made more efficient use of water than ammonium fed plants which produced 2.87g dry mass l^{-1} water consumed while the mixed feed plants produced 4.15g l^{-1} H_2O lost.

Plants supplied with ammonium nutrient showed a lower net leaf photosynthetic rate than nitrate or ammonium + nitrate fed plants. Root respiration expressed as CO_2 exchange per gram fresh mass was significantly higher in both nitrate and

ammonium + nitrate fed plants than those fed with ammonium only. This indicated a higher requirement for ATP for the transport of nitrate into the root as well as the assimilation of part of that nitrate in the root in comparison to those fed ammonium only which would only require ATP for ammonium assimilation via glutamine synthetase.

Analysis of the ^{15}N free amino (+ amido) content of the xylem sap showed that the products of $^{15}NH_4^+$ contributed 80% to the xylem sap of plants fed ammonium + nitrate while the products of $^{15}NO_3^-$ constituted 20%. Of the total nitrogen content of the xylem sap, nitrate constituted 80% in the nitrate-only, 50% in the ammonium + nitrate and 0% in the ammonium-only fed plants, whereas ammonium was no greater than 2% for any of the three nutrient sources. Glutamine was the principal free amino compound in the xylem sap for all three treatments. It would appear from these findings that sunflower plants fed ammonium + nitrate, assimilate ammonium primarily in the roots while nitrate is assimilated in the leaves.

Ammonium uptake was shown to be more rapid than that of nitrate, and it appeared to suppress nitrate uptake when the two nitrogen sources were combined. Nitrate appeared to aid in the translocation of the products of ammonium which was primarily assimilated in the root. The assimilation of

organic compounds by mixed feed plants was greater than that for plants fed either nitrogen source alone.

$^{15}NO_3^-$ vacuum infiltration of mature leaves on 10 week old plants showed that the products of nitrate assimilation were transported mainly to the capitulum. Even at this late stage of fruit filling, the root was shown still to be processing nitrogen as 26% of the bound ^{15}N in the plant was found in the root.

Leaf nitrate reductase activity was 8 times that of the root while glutamine synthetase activity was 7 times, which indicated that the leaf was the major site of nitrate reduction. It was demonstrated that the presence of both casein and polyvinylpyrrolidone indicated that both proteases and phenolic compounds inhibit *in vitro* NRA and GSA in sunflower plants.

Ammonium + nitrate nutrient was shown to be more beneficial to the growth and fruit production of *Helianthus annuus* L. plants than either nitrogen source singly.

CHAPTER 1

INTRODUCTION

To ensure adequate nitrogen supply for crops, quantities of costly nitrogen fertilizer larger than actually required, are often added to the soil. This may at times result in inefficient use of nitrogen fertilizer and have adverse effects on plant growth (Goyal and Huffaker, 1984). New ways of improving crop yield in relation to nitrogen fertilizer application are consequently being sought.

Yields can be improved through the use of improved cultivars, pest control, improved physical and chemical conditions of the soil, moisture control and good management practices. Perhaps the most critical element in promoting extremely high yields is supplying nutrients in sequence with crop demand without creating toxic conditions.

Nitrogen use efficiency can be increased by improving plant use of nitrogen and by reducing loss of plant-available nitrogen (such loss is either through nitrogen removal from the soil by ways other than plant uptake, movement away from the plant root system, or conversion to relatively unavailable forms in the soil). According to Hauck (1984), several approaches have been or are being taken to increase the efficiency of nitrogen use by crop plants. These include use of (i) slow-release nitrogen fertilizers;

(ii) chemicals that inhibit biological nitrogen transformations in soils; (iii) amendments to nitrogen fertilizers that alter their physical and/or chemical properties; and (iv) improved crop and soil management practices. These approaches are directed mainly toward reducing nitrogen losses or maintaining an adequate supply of plant-available nitrogen in the plant root zone.

The main forms in which nitrogen becomes available for absorption from the soil by plant roots is as ammonium or nitrate ions. In well-aerated, non-acidic soils the activity of the nitrifying soil bacteria ensures that most of the available nitrogen is present as nitrate, and it is probably true to say that for higher plants in general and crop plants in particular, nitrate is the main source of nitrogen.

It is recognised that all nutrient elements are required in optimum amounts to achieve high potential yields. Compared with nitrogen, however, other nutrients can be adjusted more easily to optimum concentrations in the soil because they are either held by exchange complexes or have low solubility and are not easily lost from the system (Stanford and Legg, 1984).

Substantial quantities of nitrogen in the soil may be immobilised in organic forms that are not readily available

to plants (Stanford and Legg, 1984). The bulk of available nitrogen in soils is prone to losses through such processes as leaching, denitrification and volatilisation (Hauck, 1984; Goyal and Huffaker, 1984). Other processes, the importance of which is yet to be established, involve the evolution of N_2O during nitrification, chemical decomposition of NO_2^- or its reaction with soil constituents (chemodenitrification), and NH_3 evolution from plant leaf canopies and from floodwater (Hauck, 1984).

1.1 Absorption of Soil Nitrate

Factors which have an important influence on the absorption of nitrate by plants are:

(a) the availability of energy-rich compounds to drive the active permease mechanism;
(b) soil temperature (nitrate absorption falls off markedly at low temperatures; and
(c) pH (the maximum absorption of nitrate occurs from acidic root growth media). The presence of ammonium ions in the root medium greatly inhibits the uptake of nitrate, but the reason for this is still uncertain.

1.2 Absorption of Soil Ammonium

Environmental factors which affect the absorption of ammonium by plants are:

(a) pH (many plants absorb ammonium maximally from a medium with a pH of approximately 8);
(b) temperature (ammonium absorption by roots falls off at low temperatures but the process is less sensitive to cold than is the absorption of nitrate).

Ammonium uptake rate is also greatly dependent on the availability of a good carbohydrate supply to the root, much more so than is the case in nitrate absorption. This is probably because carbon skeletons are immediately necessary for the production of organic amino molecules from ammonium which would otherwise build up to toxic levels in the root. Nitrate, being less toxic than ammonium, may be temporarily stored or translocated and need not be reduced until it is assimilated, thus the ready availability of carbon skeletons is less critical when nitrate is being absorbed. Usually, there is no spectacular concentration of ammonium in the xylem stream as in the case of nitrate nutrition, because most plants appear to assimilate absorbed ammonium in their roots and load preformed amino compounds onto the xylem, rather than the toxic ammonium ion. In most plants studied, the uptake of ammonium ions is far more rapid than that of nitrate ions.

1.3 Nitrate vs Ammonium Nutrition

1.3.1 Advantages of Nitrate as a Nitrogen Source

(a) The nitrate ion appears to be non-toxic to plants, and certain crop plants and vegetables (notably members of the Chenopodiaceae) can accumulate large concentrations in their tissues. Forethought should be given to the extravagant application of nitrate fertilizers since excessive nitrate in food and/or drinking water can lead to methemoglobinemia, especially in infants, once the nitrate is converted to nitrite either externally or internally in the gastro-intestinal tract (Vulsteke and Biston, 1978; Bolin and Arrhenius, 1977). Nitrate toxicity seems to be far more common in livestock than man as a result of excessive nitrate fertilization (Nelson, 1984). These toxic levels of nitrate are then passed on to human beings in the products from livestock i.e. milk and meat (van Diest, 1986).

(b) The absorption of cations, especially potassium, calcium and magnesium, is enhanced by nitrate nutrition. This effect is considered by many workers to be due to the rise in soil pH following the excretion of bicarbonate ions by the plants in exchange for nitrate, thus producing favourable conditions for cation uptake (Ben Zioni *et al*, 1970; 1971; Lips *et al*, 1987).

(c) Although most plants possess nitrate reductase in both root and leaves, crop plants such as maize (Murphy, 1985),

barley (Chadwick, 1985; Lewis et al, 1982b) and sunflower (Kaiser and Lewis, 1984) assimilate most of the absorbed nitrate in their leaves, especially under conditions of high nitrate availability. This arrangement brings the nitrogen assimilatory processes requiring energy and carbon skeletons into close proximity with the photosynthetic apparatus, obviating the complex translocatory pathway necessary in ammonium nutrition.

Nitrate uptake appears to require energy (Rao and Rains, 1976) although an anion ATPase has not been conclusively demonstrated for nitrate uptake in higher plants. The observed enhancement of nitrate uptake by light may be partly the result of an increased supply of energy (Rao and Rains, 1976) or an increased supply of assimilates from the shoot (Schrader and Thomas, 1981).

1.3.2 Disadvantages of Nitrate as a Nitrogen Source

(a) Before nitrate can be used by the plant, it has to be reduced to NH_4^+, an energy consuming process requiring 347kJ $mole^{-1}$ to perform. This represents a significant loss of energy from the plant's overall economy, energy which could otherwise have been used in increasing the plant's productivity.

(b) Perhaps the most serious disadvantage of the use of nitrate as a fertilizer is the ease with which it may be

leached from soils. This is due to the negative charge on the ion, which prevents it being retained by most soils whose particles also possess negative charges. This factor, together with the ease with which denitrification of NO_3^- may take place under anoxic conditions, is responsible for the major loss of nitrogen from fertilized fields.

(c) Nitrate absorption is an active, energy dependent process requiring ATP to drive the permeases responsible for the uptake of the ion from the soil. Anoxic conditions such as those induced by waterlogging of the soil can thus severely inhibit root absorption of nitrate through their inhibition of oxidative phosphorylation.

(d) Iron and certain trace element deficiencies can be induced by nitrate nutrition. This effect is probably due to the internal binding of the metals with organic acids which are produced in large quantities in root and stem as a result of nitrate feeding. The flux of negatively charged nitrate into the plant stimulates the production of positively charged hydrogen ions in the form of organic acids, apparently to maintain electrical neutrality.

1.3.3 Advantages of Ammonium as a Nitrogen Source

(a) Unlike nitrate, ammonium does not require reduction prior to utilization by the plant, thus resulting in considerable energy saving. A major agricultural advantage

enjoyed by ammonium feeding of plants over nitrate feeding is the more effective retention of this ion by the soil, because of attractive forces that exist between the positively charged soil ammonium ions and the negatively charged soil particles. Nitrate ions, being negatively charged, are easily leached from the soil by rain and consequently there is an enormous loss of combined nitrogen in this form from agricultural lands - in fact, it is widely considered that over 50% of the increasingly expensive nitrogen fertilizer applied by farmers to the land is simply washed out to sea, often causing serious river and dam pollution on the way. It is mainly for this reason that serious efforts are now being introduced to prevent or retard the conversion of ammonium fertilizer to nitrate in the soil by the application of chemicals such as nitrapyrin which selectively inhibit the activity of the nitrifying bacteria, Nitrosomonas.

(b) Ammonium has apparently a double absorption system, one energy dependent and one energy independent. Thus, metabolic inhibitors and anoxia of the root environment have relatively little effect on ammonium absorption compared with nitrate absorption.

(c) Ammonium nutrition enhances anion absorption, particularly phosphate. Enhanced phosphate absorption is due to a lowering of rhizosphere pH by H^+ excretion in

response to ammonium absorption, resulting in a conversion of $H_2PO_4^-$ to HPO_4^{2-} ions, which are absorbed several times faster than $H_2PO_4^-$.

Ammonium absorption is a passive process which generally occurs at rates greater than those observed for nitrate uptake (Higinbotham, 1973).

1.3.4 Disadvantages of Ammonium as a Nitrogen Source

(a) The ammonium ion can be toxic to plants. This toxicity appears to have two main causes:

(i) ammonium uncouples photophosphorylation at concentrations as low as 2 mM, thus severely restricting ATP production in leaves. It is probably for this reason that nutrient ammonium assimilation takes place in the root and that ammonium is only loaded onto the xylem supply to the leaf in small quantities;

(ii) ammonium absorption is electrically balanced by the excretion of H^+ by the root into the soil. Acidification of the root environment severely retards growth, resulting in stunted rooting systems and greatly impaired nutrient absorption. This effect can be largely overcome by the liming of soil or the addition of calcium carbonate to nutrient solutions.

In most plant tissues the enzyme glutamine synthetase is present which produces the amide glutamine from glutamic acid and ammonium. This enzyme acts as an ammonium "detoxifier" in tissues containing excessive quantities of the ion; this was considered to be its main function in the plant until recently.

(b) Because of the immediate need to combine ammonium organically after absorption to prevent toxicity, large quantities of carbohydrate are immobilized in the production of N compounds such as glutamine and asparagine. At high levels of ammonium nutrition, this can severely restrict the amount of material available for structural purposes, resulting in small, weak plants and slow growth.

(c) Ammonium nutrition suppresses the absorption of K, Ca, Mg and NO_3^-, probably because they share a common absorptive permease system.

(d) In most plants, absorbed nitrate is processed in both root and shoot, but nutrient ammonium appears to be assimilated exclusively in the root. Relatively little ammonium is loaded onto the xylem for translocation to the shoot; instead, large quantities of amides and amino acids are transported to the shoot to maintain its nitrogen supply. This exclusive assimilation of nutrient ammonium in

the plant root must inevitably place stress on the shoot-root translocation system of the plant which is obliged to provide the root with large quantities of carbon skeletons manufactured in the leaf to utilize the ammonium absorbed.

The precise mechanism of the toxic effect caused by ammonium is not known, but it is believed to inhibit respiration, restrict photosynthesis and uncouple cyclic phosphorylation (Reisenauer, 1978). Lewis et al, (1986) suggested from work done with barley plants, that the need for detoxification of ammonium causes the immobilisation of photosynthate resulting in smaller, weaker plants.

1.4 Response of Cultivated Plants to Nitrate-Ammonium Nutrition

1.4.1 Ammonium-Only Nitrogen Nutrition

Calcifuge plants that grow naturally in acid soils where little nitrification occurs utilize ammonium in preference to nitrate (Haynes and Goh, 1978). Grasses respond rather favourably to ammonium fertilization. Ammonium-only fertilization in most crop production, however is not generally practiced in view of the strongly acidifying effect of ammonium fertilizers (van Diest, 1986). Ammonium-only grown non-nodulated plants of black alder (Alnus glutinosa) with pH control of nutrient solutions had twice the dry mass as ammonium-only grown plants grown without pH control (Troelstra and Blacquiere, 1986).

1.4.2 Nitrate-Only Nitrogen Nutrition

Calcicole plants usually occur on neutral or alkaline soils where pH is conducive to the activity of the soil nitrifying bacteria which convert all or most of the available soil ammonium into nitrate. Tomato seedlings (Kirkby and Mengel, 1967), tomato plants (Pill and Lambert, 1977) and plants of pea and cucumber (Barker and Manyard, 1972) have been shown to produce more dry matter when grown on NO_3^- than when grown on NH_4^+. Chaillou et al, (1986) found that after 45 days growth, the fresh weight of Phaseolus vulgaris plants grown on ammonium-only was half that of nitrate-only grown plants. Nitrate nitrogen supplied to wheat plants at the shooting and heading stages can double the grain yield of plants supplied nutrient only at the seeding stage (Przemeck and Kucke, 1986). Dry weights of all plant parts of lima beans grown in solution culture were consistently lower when 25% or more of nitrogen was supplied as NH_4^+ (McElhannon and Mills, 1978).

1.4.3 Ammonium + Nitrate Nutrient and Nitrification Inhibitors

The major form of inorganic nitrogen available to higher plants in most soils is nitrate, because nitrogen fertilizer and ammonia from ammonification of organic matter are readily oxidized to nitrate through nitrification by Nitrosomonas and Nitrobacteria (Rao and Rains, 1976). In

well aerated soils above 5°C, NH_4^+ is converted by soil organisms (Nitrobacteriaceae) to NO_3^- and results in acidification of the soil (Hageman, 1984). Calcium carbonate, which is sometimes added to regulate pH of NH_4^+ type nutrient solutions, has been shown to enhance nitrification (Hewitt, 1966). Since most plants can utilize both the NH_4^+ and NO_3^- forms of nitrogen, a reduction in the nitrification rate should not reduce nitrogen availability as long as the nitrogen (either NH_4^+ and NO_3^-) is in the zone of maximum root activity (Hoeft, 1984). Nitrification inhibitors (nitrapyrin, carbon bisulphide and water soluble tri-thiocarbonates) that block the conversion of NH_4^+ to NO_3^- in the soil improve the maintenance of NH_3 in the soil (Hageman, 1984). The use of these nitrification inhibitors with a mixed (nitrate + ammonium) nitrogen source can lead to more efficient utilization of nitrogen fertilizers.

Many reports show that NH_4^+ provided along with NO_3^- gives more beneficial effects than either form alone. Mohanty and Fletcher (1976) reported that 'Paul's Scarlet' rose cells in suspension culture grew twice as much when 0.91 mM NH_4^+ was included in medium containing 25 mM NO_3^-. Yields of wheat were 50% higher when grown in NH_4^+ plus NO_3^- than when grown in either source alone in a continuous-flow culture system (Cox & Reisenauer, 1973). Weissman (1964) reported that dry weights, total protein content, protein concentration and percent protein of total nitrogen were all higher in leaves

from sunflowers grown on NH_4^+ plus NO_3^- than either source alone. Schrader et al, (1972) reported that fresh weights of corn plant parts were higher when plants were supplied 100 mg/kg of nitrogen as a NO_3^-/NH_4^+ mixture (25:75, 50:50, or 72:25) than when 100 mg/kg of either NO_3^- (100:0) or NH_4^+ (0:100) were provided. Higher yields of wheat and dry matter and protein production by sunflower (Helianthus annuus L.) were obtained with NH_4^+ plus NO_3^- than with either source alone (Cox and Reisenauer, 1971). Lewis et al, (1986) found that the dry mass of barley plants grown in ammonium + nitrate were 12% greater than nitrate-only grown and 22% greater than ammonium-only grown plants.

1.5 The Assimilation of Nitrate

Ammonium and/or nitrate are assimilated by plants and go to make up the amino acids and proteins in plants. Only nitrogen in the fully reduced state can be assimilated into organic compounds. The enzymes catalyzing the reduction of nitrate to nitrite and then to ammonia are nitrate reductase (reduced nicotinamide adenine dinucleotide nitrate oxidoreductase, E.C.1.6.6.1.) (Notton and Hewitt, 1977) and nitrite reductase (reduced benzylviologen nitrite oxidoreductase, E.C.1.7.7.1) (Hucklesby, Crammack and Hewitt, 1977).

Nitrate reductase is a substrate inducible enzyme which, in leaf tissue, may also have the further requirement of light

to produce the energy necessary for induction and/or synthesis of the enzyme (Lawlor, 1987; Lips, 1972; Beevers *et al*, 1965; Canotilho Watt and Cresswell, 1986). Nitrite may be a direct inducer of nitrate reductase in bean seed cotyledons (Lips *et al*, 1973). When cultured spinach cells were transferred to fresh nutrient medium the observed change in NR activity was caused by the synthesis and gradual degradation of NR protein and not by activation of an inactive protein and inactivation of the active enzyme (Maki *et al*, 1986). Maeck and Tischner (1986) concluded that nitrate reductase synthesized in the early stage of sugar-beet seedling development was a constitutive enzyme and not dependent on nitrate uptake.

Nitrate may be reduced in roots or other non-green tissues and in leaves. Nitrate reductase activity (NRA) has been demonstrated in both the root and shoot of many plants (Dirr *et al*, 1973). *In vitro* NRA has been shown to be 7 times greater in the shoot than in the root of *Helianthus annuus* (Kaiser and Lewis, 1984).

1.6 The Assimilation of Ammonium

The generally accepted pathway of incorporation of inorganic nitrogen in the form of ammonium into plant amino acid metabolism is via the GS(EC.6.3.1.2L-Glutamate: ammonia ligase (ADP)) - GOGAT(EC.2.6.1.53., L-glutamine 2-

oxoglutarate aminotransferase (NADPH oxidizing)) pathway proposed by Lea and Miflin (1974).

Ammonium ions which are toxic to plant tissues, are "detoxified" upon entering the roots by their assimilation into amino acids and amides which are then transported through the xylem to the leaves, where organic nitrogen is accumulated mainly as protein (Schrader et al, 1972). The assimilation of ammonium ions require adequate supplies of carbohydrates from the shoot. This demand for carbon skeletons for the assimilation of ammonium ions can be so overwhelming that little of the assimilates imported from the leaf may remain available to invest in root growth. An alternative explanation for the limited development of roots of plants growing in the presence of ammonium could be the extensive uncoupling of respiration by ammonium, limiting the metabolic energy available for growth. No evidence for this explanation has been observed so far (Lips et al, 1987).

Researchers working with various crop plants such as wheat (Cox and Reisenauer, 1973); corn (Schrader et al, 1972); tomato (Ikeda et al, 1974); maize (Murphy, 1985); and barley (Chadwick, 1985) have shown that protein and mineral composition can be modified by regulating the nitrogen source supplied to the plant. Sunflower plants (Weissman, 1964) and young apple trees (Tromp and Ovaa, 1979) grown in

NH_4^+ invariably contained higher levels of free NH_4^+ and amide nitrogen than those grown in NO_3^-.

1.7 Translocation of Nitrogen

Much of the reduced nitrogen in a plant must be transported to other cells at least once during the plant's life cycle. The site of nitrate reduction influences the transport of reduced nitrogen (Schrader and Thomas, 1981). According to Pate (1980), nitrogen solutes frequently comprise the major component of xylem sap, and are second only to carbohydrates in the phloem sap. Amide nitrogen contributed 71% of the total nitrogen in xylem exudates of sunflower plants grown in NH_4^+ (Weissman, 1964) and 19% in NO_3^- (Weissman, 1964; Kaiser and Lewis, 1980). Weissman (1964) also reported that NH_4^+ nutrition favoured the presence of alanine, arginine, leucine, serine, and valine in xylem exudates, whereas NO_3^- nutrition favoured γ-aminobutyric acid, aspartic acid, glutamic acid and lysine.

1.8 Effect of Nitrate and Ammonium Nutrition on CO_2 Fixation

It has been shown that NH_4^+ increased photosynthetic CO_2 fixation in isolated cells of spinach (Woo and Canvin, 1980a, 1980b) and Papaver somniferum (Paul et al, 1978) and intact spinach chloroplasts (Bennedetti et al, 1976). The stimulation of photosynthesis by NH_4^+ was described as an

activation of RuBP carboxylase (Bennedetti *et al*, 1976). Results using intact *Anacystis nidulans* cells show that no competition for assimilatory power exists at light saturation between NO_3^- assimilation and CO_2 fixation, the photosynthetic apparatus being able to generate enough assimilatory power for the simultaneous assimilation of the two bio-elements. However, at below light saturating conditions there is competition for the assimilatory power for CO_2 fixation and NO_3^- assimilation resulting in a depression of CO_2 fixation. Ammonium appears to enhance the CO_2 fixation rate at and below light saturating conditions (Romero and Lara, 1987). Morot-Gaudry *et al*, (1986) concluded that the slight enhancement of the photosynthetic rate in $NH_4^+ + NO_3^-$ fed maize seedlings could be attributed to energy saving from lower nitrate reduction than in NO_3^--only grown plants. Romero and Lara (1987) go so far as to say that CO_2 fixation rate at any light intensity is in fact the balanced result of the negative effect of nitrate and the positive effect of NH_4^+ nutrition.

1.9 Sunflowers in Agriculture

In South Africa the sunflower is probably the most important oil-seed crop. It is used not only for culinary purposes (cooking oil and margarine) but as a fuel for diesel engines with the remaining high-protein oil cake used as a valuable stock feed (Billett, 1981).

Sunflower plants can be grown under irrigation or dry land conditions. The hybrid sunflower is an alternative to maize on high potential marginal soils in summer grain producing areas. The sunflower is more drought tolerant than maize and performs better in areas with erratic rainfall or low soil potential. It is also the best rotation crop for maize (Bondesio, 1985) and wheat (Billett, 1986) as it is not a member of the grass family, and is most effective as a counter to the build-up of maize and wheat diseases in the soil. Sunflower is a good catch crop which efficiently uses fertilizer applied, but not used, by the previous crop (Bondesio, 1985).

The response of sunflowers to nitrogen fertilizer depends largely on the nitrogen supplying power of the soil as well as the yields. In general 30 kg/ha N is required to produce 1.5 t/ha seed while 60 kg/ha will probably be required to produce over 2.0 t/ha seed. Heavier rates may be needed to achieve yields of the order of 3 t/ha seed under conditions that make such high yields possible (Birch, 1982).

1.10 Aims of this Work

The aims of this work were to investigate the assimilation and utilisation of nitrogen in *Helianthus annuus* at both the vegetative and fruiting stages as influenced by nitrogen source in the form of nitrate, ammonium or ammonium + nitrate.

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant Material

Seeds of Helianthus annuus L. var. Dwarf Sungold Sunflower were germinated in a Conviron Seed Germinator, Model G30 (Controlled Environments Ltd., Winnipeg, Manitoba, Canada), at 25°C and approximately 100% relative humidity on stainless steel trays containing No. 3 grade vermiculite. When the cotyledons of each plant were about five centimeters high and before the first leaves appeared, the vermiculite was carefully removed from the root which was then placed through the lid of a two or five litre jar containing one of the Long Ashton hydroponic feeding solutions described in Tables 2.1 and 2.2. Included with the micronutrients (Table 2.2) was 5 mg per litre nitrapyrin (2-chloro-6 (trichloromethyl) pyridine) (The Dow Chemical Company, Agricultural Products Department, Midland, Michigan 48640, USA). Every jar was aerated through a Festo manifold system (Festo (Pty) Ltd., Cape Town, South Africa) which supplied equalized air flow to all jars. Solutions were changed twice per week in the five-litre jars (Figure 2.1) and three times per week in the two-litre jars. The volume of solution used between changes was calculated by subtracting the final volume from the original volume

FIGURE 2.1
Sunflower plants growing in nutrient solution containing either 2mM nitrate, 2mM ammonium or 1mM ammonium + 1mM nitrate in five-litre containers.

introduced at the previous change and subtracting from that value the water lost by jars without plants but similarly aerated.

TABLE 2.1

Macronutrients, in grams, used to make up 100l of nutrient solution with different nitrogen sources (Hewitt, 1966)

Macronutrient	2mM Ammonium	2mM Nitrate	1mM Ammonium 1mM Nitrate
$MgSO_4.7H_2O$	36.8	36.8	36.8
K_2SO_4	47.8	47.8	47.8
$CaCl_2.2H_2O$	58.8	58.8	58.8
$Na_2HPO_4.12H_2O$	47.8	47.8	47.8
$NaH_2PO_4.2H_2O$	20.8	20.8	20.8
KNO_3		20.2	10.1
NH_4Cl	10.7		5.35

TABLE 2.2

Micronutrients, in milligrams, used to make up 100l of nutrient solution. Micronutrients were kept at the same concentration for all nutrient solutions.

Micronutrient	mg 100^{-1}
$MnSO_4.4H_2O$	223.0
$CuSO_4.5H_2O$	25.0
$ZnSO_4.7H_2O$	29.0
H_3BO_3	310.0
NaCl	590.0
$Na_2MoO_4.2H_2O$	12.1
FeNaEDTA	4.222×10^3
Nitrapyrin	500.0

Plants were grown from seedling stage to harvesting in a Conviron Model E15 Growth Chamber under the following conditions: day temperature 22°C, night temperature 18°C, relative humidity 70%, photoperiod 14 hours, with illumination supplied by Sylvania (Canada), cool white high intensity fluorescent lamps (irradiance at the leaf surface 400 $\mu Em^{-2}sec^{-1}$), supplemented with 60W incandescent lamps (irradiance at the leaf surface 32 $\mu Em^{-2}sec^{-1}$).

Due to the marked effect of temperature (Yoneyama *et al*, 1977) and irradiance (Tyschen, 1976) on amino acid metabolism, it was considered desirable to raise the plants

and to perform the experiments under the strictly controlled conditions of the growth chamber.

2.2 Methods of Isotope Feeding

2.2.1 Through the Transpiration Stream (Lewis and Pate, 1973).

Excised stems with leaf, bract and fruit intact were transferred to a feeding solution containing 15 mM $K^{15}NO_3$ and allowed to photosynthesize for 24 hours in the growth chamber.

2.2.2 Vacuum Infiltration of Leaves (Ito and Kumazawa, 1976).

Vacuum infiltration of leaves of Helianthus was carried out using 20 mM $K^{15}NO_3$. Leaves were completely immersed in the infiltration solution and subjected to a reduced pressure of $\pm$ 2 kpa for 3 minutes as shown in Figure 2.2. When the vacuum was released for the second time, penetration into the intracellular spaces could be easily determined visually. The plants were then returned to the growth chamber and allowed to carry out a normal 24 hour cycle prior to harvesting.

FIGURE 2.2
Apparatus used for the vacuum infiltration of sunflower leaves.

2.2.3 Hydroponic Feeding of ^{15}N to Roots.

The roots of the intact plants were transferred in the growth chamber to 2mM $K^{15}NO_3$, 2mM $^{15}NH_4Cl$ or 1mM $^{15}NH_4Cl$ + 1mM $K^{15}NO_3$ Long Ashton feeding solution, depending on the growth solution from which they had initially been taken.

2.3 Harvesting and Extraction

The fresh weights of roots, stem and petiole, leaves and stem apex or fruit were determined immediately on harvesting. The roots were washed in de-ionized water and blotted dry with absorbent tissue. Plant material was killed by immersion in liquid nitrogen to stop all enzyme activity, and then homogenized in cold 80% ethanol (1 g tissue per 50 ml ethanol) with an Ultra Turrax homogenizer (Janke and Kunkel KG, IKA Werk, Staufen i. Breisgau, BRD). Extraction of the soluble amino compounds was carried out at 0°C for 24 hours. The homogenate was then filtered through Whatman No. 4 filter paper in a Buchner funnel under vacuum and the ethanol extract evaporated under an airstream to a final volume of 5 ml to 1 gram fresh weight. These solutions were then kept frozen at -20°C until determinations were made. After the residue was oven dried at 80°C for 24 hours, dry weights of the residue were determined.

2.4 Sample Preparation

2.4.1 Bound Nitrogen Fraction

Duplicate aliquots of ± 100 mg dried samples were converted to ammonium sulphate by the Kjeldahl method. One mercury BDH catalyst tablet, (containing the equivalent of 0.1 g mercury and 1.0 g sodium sulphate) and 3 ml of nitrogen-free concentrated sulphuric acid were heated with the sample in a micro-Kjeldahl digestion flask. Once samples were digested they were dissolved in ammonia-free de-ionized water and made up to a final volume of 50 ml. The ammonia from 10 ml aliquots was distilled over in a Markham micro-distillation still after alkalization with 15 ml of 50% sodium hydroxide + 2.5% sodium thiosulphate (W/V). The ammonia was collected in 0.02N hydrochloric acid and titrated against standardized 0.005N sodium hydroxide using screened methyl red indicator (0.125 g methyl red + 0.083 g methylene blue, in 100 ml 100% ethanol). Samples were then acidified with a few drops of 0.02N hydrochloric acid to prevent loss of ammonia and concentrated down using an air stream and hot plate to a volume in which 0.2 ml contained 15-30 µg ammonia - N.

2.4.2 Soluble Nitrogen Fraction

2.4.2.1 Ammonium Fed Samples

The ammonia from duplicate aliquots of 5 ml were distilled over in a modified Markham micro-distillation apparatus after alkalization with 0.2 g AR MgO (previously heated to

600°C for 2 hours to drive off CO_2 which could interfere with titration results) and collected in 0.02N HCl. Samples were distilled for precisely 2 minutes to prevent any possible alkaline hydrolisis of the amides (Lewis et al, 1982a). The ammonia collected was treated as described under Section 2.4.1.

The remaining fraction was then filtered through Whatman No. 4 filter paper, to remove the undissolved MgO, and concentrated to ± 3 ml under an air stream. The sample was transferred to a micro-Kjeldahl digestion flask and the procedures for digestion and ammonia determination were carried out as described in Section 2.4.1. The samples were concentrated down to a level in which 0.2 ml contained 15-30 µg ammonia - nitrogen.

2.4.2.2 **Nitrate Fed Samples**

Duplicate aliquots of 5 ml were applied to 3 cm x 1 cm ion exchange columns of Dowex 50w - X8 standard H^+ resin, 100-200 mesh particle size (BDH Chemical Co., Poole, Dorset, UK) to separate the nitrate and organic plus ammonium nitrogen fractions (Atkins and Canvin, 1971). The water soluble fraction, including nitrate, was eluted with 50 ml de-ionized water and the remaining organic plus ammonium nitrogen was eluted with 100 ml 2N HCl.

The organic plus ammonium nitrogen fraction was then concentrated down with heat under an air-stream to ± 3 ml and after following the Kjeldahl procedure described in Section 2.4.2.1 the ammonia content was determined.

The nitrate fraction was concentrated down with heat under an airstream to ± 10 ml and distilled over as ammonia in a Modified Markham micro-distillation apparatus with 0.3 g Devarda's alloy (BDH) as a reductant and 0.2 g MgO. The ammonia was collected and treated as described under Section 2.4.1 and finally concentrated down to a volume in which 0.2 ml contained 15.30 µg ammonia - N.

2.4.2.3 Ammonium + Nitrate Fed Samples

Duplicate aliquots of 5 ml were distilled and the ammonia collected as described under Section 2.4.2.1. The remaining fraction, after filtration, neutralization and concentration, was treated as described under Section 2.4.2.2. to separate nitrate and the remaining nitrogen containing compounds. The resulting ammonia solutions were concentrated down to a level in which 0.2 ml contained 15-30 µg ammonia - N.

2.5 ^{15}N Analysis

2.5.1 N-Discharge Tube Preparation

Samples were prepared as described under Section 2.4. The method of N-discharge tube preparation used was that of

Faust (1967) using alkaline sodium hypobromite solution as an oxidant. The hypobromite reacted with the ammonium under reduced pressure to release nitrogen gas according to the reaction:

$$2NH_3 + 3NaOBr \rightarrow N_2 + 3H_2O + 3NaBr \text{ (Kamen, 1957).}$$

A pressure of 0.1 Pa was achieved by a mercury diffusion pump backed up by an Edwards rotary high vacuum pump operating at 0.1 kPa (Edwards High Vacuum, Crawley, England). The vapour pressure of the system was reduced by the use of liquid nitrogen in two cold traps. The nitrogen produced by the above reaction was sealed off in a discharge tube with final pressure of 40-70 kPa.

2.5.2 Determination of ^{15}N Enrichment

The sealed discharge tube was placed in a Packard N-15 Statron NOI-4 atomic emission spectrophotometer (AES) for analysis. The nitrogen gas enclosed in the discharge tube was excited by a high frequency discharge which produced a red/violet colour. A blue colour indicated contamination either by water vapour due to improper freezing at the final stage prior to sealing the tube, or bromine being liberated during the reaction between an over-acidified sample (final step in sample preparation) and sodium hypobromite. This method is based on the photoelectric recording of the intensity of the bandhead for the three molecules of

nitrogen at 315.9 nm for $^{14}N^{14}N$ at 316.2 nm for $^{14}N^{15}N$ and at 316.5 nm for $^{15}N^{15}N$. A typical trace for all three molecules with ^{15}N enrichments below 50% is shown in Figure 2.3. Enrichments were calculated using the formula:

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

where A and B are the bandheads of the $^{14}N^{14}N$ and $^{14}N^{15}N$ molecules respectively, and Va and Vb are the gain settings on the AES at which the bandheads A and B were recorded. Figure 2.4 shows a typical trace for all three molecules in which enrichments above 50% were obtained. Enrichments above 50% were calculated by the formula:

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

In both cases the average of three traces was used for calculations. All ^{15}N enrichments were corrected using the standard calibration curve shown in Figure 2.5 which was prepared using known standards ranging from 0.37% to 99% ^{15}N. The percentage enrichment (A%E) in excess of the natural abundance was obtained by subtracting a natural abundance of 0.37% from the corrected percentage enrichment.

The A%E value was multiplied by the total nitrogen content determined from the distillation procedure to give the mass ^{15}N present. This value was then multiplied by the mass of the plant part giving the final result as micrograms ^{15}N per plant part.

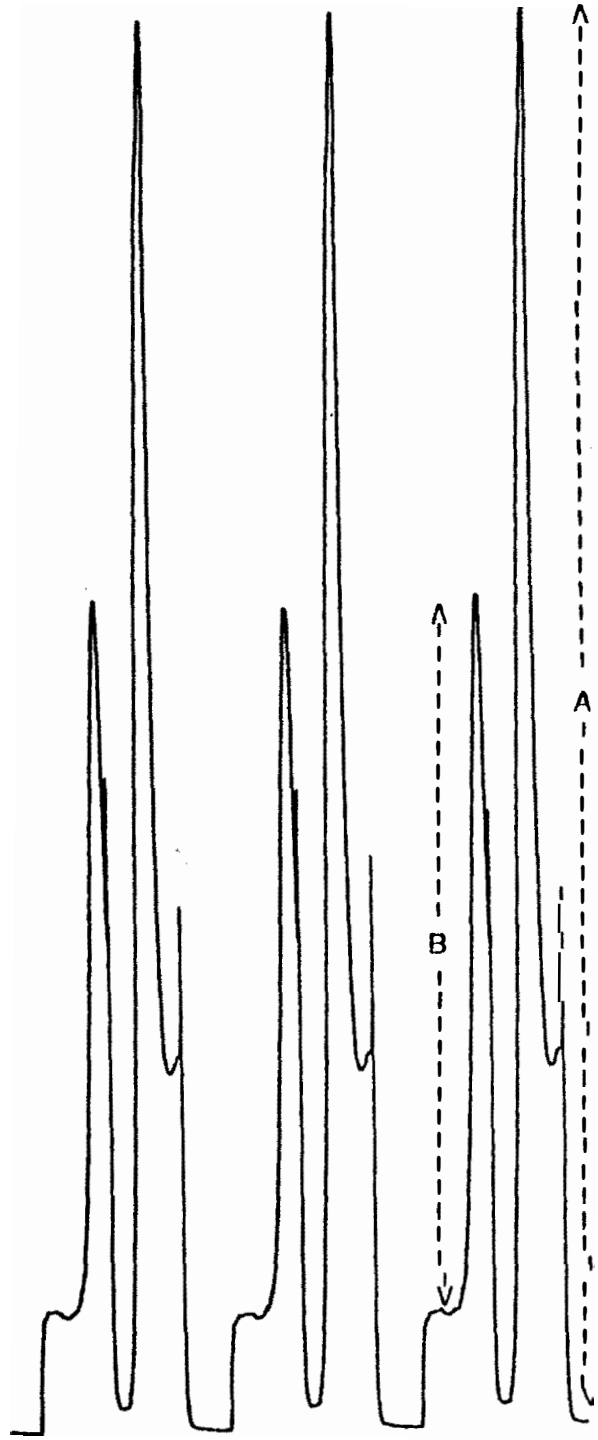


FIGURE 2.3
Typical traces for ^{15}N enrichments below 50%, showing good separation of the nitrogen molecules $^{14}N^{15}N$ and $^{14}N^{14}N$ where A and B represent the peak of the $^{14}N^{14}N$ and $^{14}N^{15}N$ bandheads respectively.

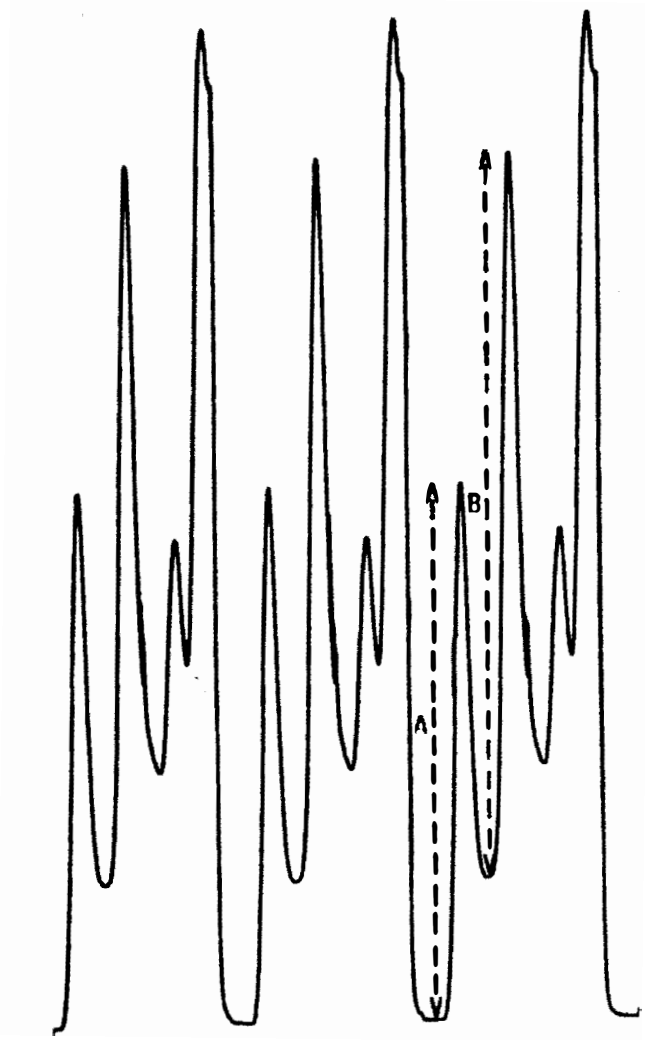


FIGURE 2.4
Typical traces for ^{15}N enrichments above 50%, showing good separation of the nitrogen molecules $^{15}N^{15}N$ and $^{14}N^{15}N$ where A and B represent the peak of the $^{15}N^{15}N$ and $^{14}N^{15}N$ bandheads respectively.

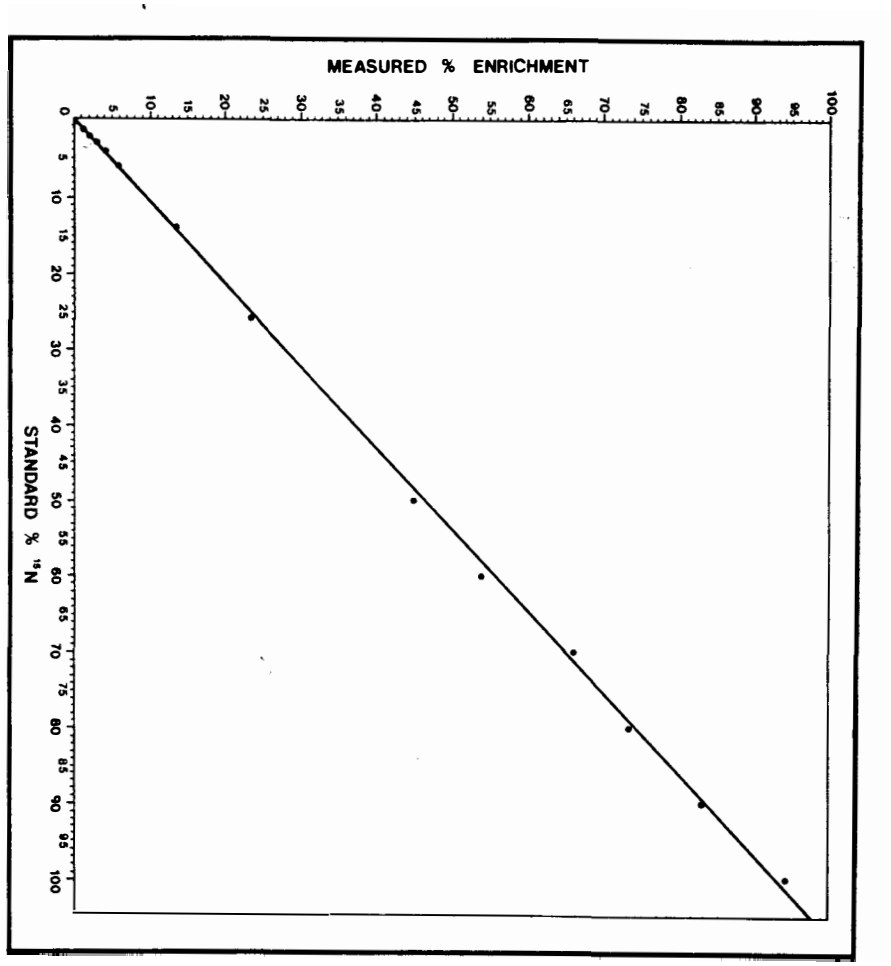


FIGURE 2.5
Standard curve for the correction of ^{15}N enrichments determined with the Statron molecular emission spectrometer.

2.6 Xylem Sap Analysis

2.6.1 Collection of Bleeding Sap

At the commencement of the experiment plants were removed from ^{14}N nutrient solutions, the roots washed under de-ionized water to remove any remaining solution and transferred to the appropriate Long Ashton nutrient solution containing $K^{15}NO_3$, $^{15}NH_4Cl$ or $^{15}NH_4{}^{15}NO_3$. At the assigned time interval, plants were excised just below the cotyledons, and a collar of Tygon tubing fitted onto the cut stem to act as a reservoir for the sap which was exuded through the action of root pressure. The liquid exuding from the cut surface is regarded as originating from the xylem vessels (Pate, 1962) which are believed to provide the main passage for the upward movement of dissolved nitrogenous compounds within the plant (Pate, 1973). Sap was collected in the growth cabinet over a period of one hour under conditions of constant temperature and humidity. The collected sap was stored in vials on ice and then kept frozen until analyzed for amino compound content (Section 2.7) and ^{15}N content (Section 2.5).

2.7 Amino Acid Analysis

2.7.1 Analytical Determinations of Soluble Amino Compounds

Analytical determinations of soluble amino compounds were carried out using 10-200 µl of xylem sap on a Beckman Model 120C Amino Acid Analyzer (Beckman Instruments, Inc., Fullterton, CA 92634, U.S.A.). Samples were applied to a 22

cm column of Beckman W2 spherical ion exchange resin and separated using three lithium citrate buffers (Kedenburg, 1971) in the following sequence:

for Acidics and Neutrals

Buffer A at pH 2.83, 0.2 N Li^+, 0.15 N citrate

Buffer B at pH 3.70, 0.2 N Li^+, 0.20 N citrate

for Basics

Buffer C at pH 3.75, 1.0 N Li^+, 0.20 N citrate

Once the amino compounds were separated they were combined with a ninhydrin reagent, incubated at 100°C and optical densities at 450 nm (for proline) and 540 nm (for all other amino compounds) and were recorded on a Honeywell Electronik 16 logarithmic recorder and a Beckman 125 digital integrator. An internal standard, nor-leucine, was used to supply a correction factor for the ninhydrin reagent which degrades with time. The pool sizes in µmole ml^{-1} for each amino compound were calculated using the digital integrator readings corrected by specific conversion constants for each amino compound, as determined from calibration runs.

2.7.2 Separation of Nitrogen Fractions and ^{15}N Analysis

The nitrate ammonium and organic fractions were separated as described under Section 2.4. Once separated, ^{15}N analysis was carried out as described under Section 2.5.

2.8 Nitrate and Ammonium Ion Determinations

2.8.1 Harvesting and Extraction

Plants which were grown in modified Long Ashton solutions (Tables 2.1 and 2.2) were divided into tip, leaves, roots and stem, and petiole. The roots were washed with de-ionized water to remove any ions from the root surface and excess $CaCO_3$. The fresh weights of the four fractions were taken and the material was oven dried at 80°C for 24 hours at which time dry weights were determined.

The dried material was then milled with a Wiley Mill Standard Model No. 3 (Arthur H. Thomas Co., Philadelphia, U.S.A.) fitted with a 0.15 mm mesh sieve to obtain homogenous samples. Aliquots of 100 mg were extracted in 25 ml de-ionized water for 30 minutes in a boiling water bath. The water extract was then analyzed for ammonium, nitrate and potassium. Additional aliquots of 100 mg were taken for Kjeldahl determinations of organic plus ammonium nitrogen.

2.8.2 Nitrate N Analysis

Nitrate determinations were made using Szechrome NAS (stabilized diphenylamine sulphonic acid chromogene, obtained from Yedetek Ltd., Rimon 10, Omer 84065, Israel) which was dissolved in a 1:1 mixture of ortho phosphoric and concentrated sulphuric acids. To 0.5 ml of sample 2.5 ml

Szechrome NAS reagent was added, shaken and allowed to stand for 20 minutes for colour development prior to measuring the optical density at 570 nm on a Beckman Model 42 spectrophotometer. Standard solutions of potassium nitrate were prepared to construct a calibration curve (Figure 2.6) and were run simultaneously with samples to check for degradation of the Szechrome NAS reagent. Triplicate results were averaged for each sample.

2.8.3 Ammonium N Analysis

Nessler-Reagent (AOAC, 1965) was used to determine the ammonium nitrogen content in the extracts. To 0.5 ml of diluted sample 2.5 ml of Nessler reagent was added, shaken and at exactly one minute after mixing the optical density was measured at 420 nm in a Beckman Model 42 Sepectrophotometer. Standard solutions of ammonium sulphate were used to prepare a calibration curve (Figure 2.7) each time samples were measured. Triplicate results were averaged for each sample.

2.9 Enzyme Assays

Analysis of nitrate reductase activity (NRA) and glutamine synthetase activity (GSA) were carried out on both leaves and roots of *Helianthus* using *in vitro* procedures (Lewis *et al*, 1982b). Plants 4-5 weeks old were used for these experiments and grown as described in Section 2.1.

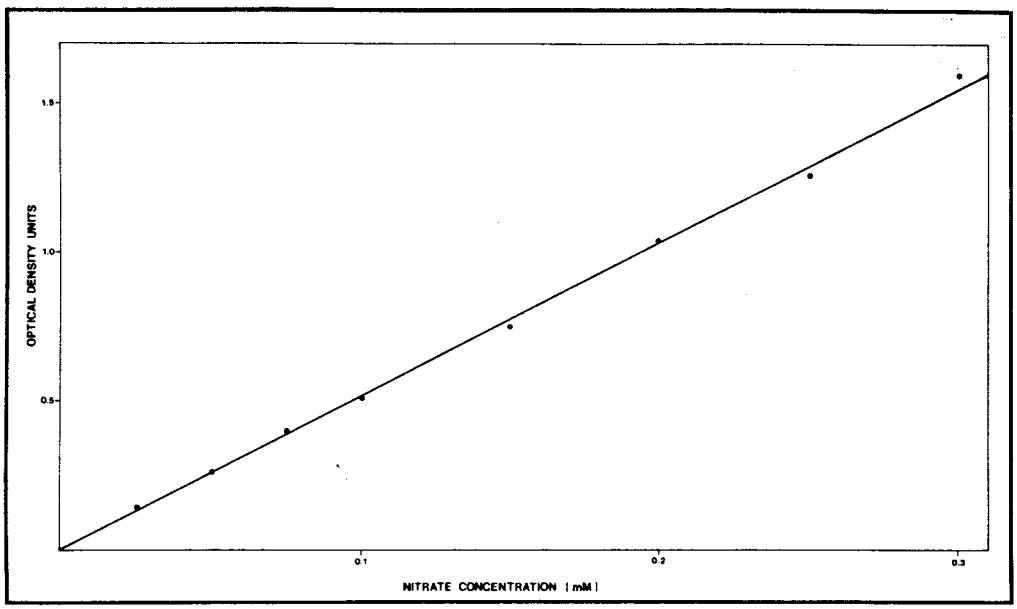


FIGURE 2.6
Standard curve for the colorimetric determination of nitrate concentration using Szechrome NAS reagent.

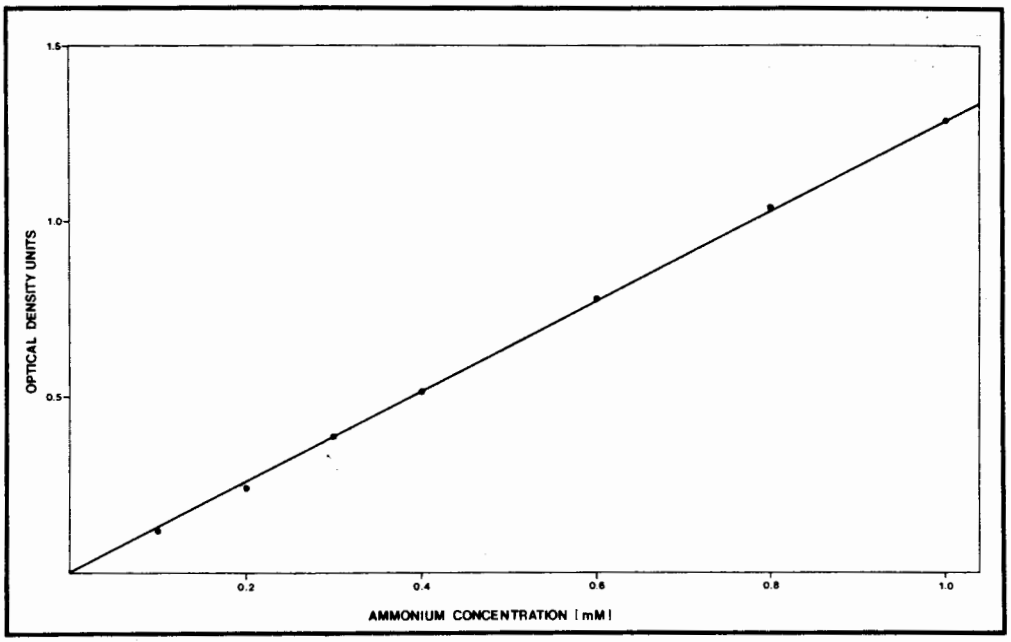


FIGURE 2.7
Standard curve for the colorimetric determination of ammonium concentration using Nessler's reagent.

2.9.1 Enzyme Extract Preparation

For the preparation of the crude enzyme extract 1 g root or leaf material was cut into small pieces (roots were washed thoroughly in distilled water and patted dry between layers of absorbent paper) and ground in a chilled mortar and pestle for 2 minutes at 4°C with 2.0 g acid washed sand and 12 ml of one of the following media chilled to 0°C:

Medium (1): 0.1 M phosphate buffer pH 7.5 1mM EDTA
2 mM dithiothreitol (Sigma, St Lousi, MO, U.S.A.) (Lewis et al, 1982b)

Medium (2): Medium (1) plus
1.5 g insoluble polyvinylpyrrolidone (PVP) (BDH Chemicals Ltd., Poole, U.K.) (Loomis and Battaile, 1966)

Medium (3): Medium (1) plus
2.5% soluble casein (BDH Chemicals) (Lewis et al, 1982b)

Medium (4): Medium (2) plus
2.5% soluble casein (Kaiser and Lewis, 1984)

The crude extract was filtered through two layers of cheese cloth and centrifuged at 2000G for 5 min at 2°C in a Beckman Model J21 centrifuge. The supernatant was stored on ice and assayed immediately.

2.9.2 Nitrate Reductase Activity Assay

Each assay mixture tube contained the following:

0.1 ml 1M potassium phosphate buffer pH 7.5

0.1 ml NADH (1 mg ml) (Sigma, St Louis, MO, U.S.A.)

0.2 ml 0.1M KNO_3

0.1 ml leaf extract or 0.2 ml root extract

together with water to make up a final volume of 2 ml (Lewis et al, 1982b). After a 15 minute incubation period at 28°C, the reaction was stopped by the addition of 1 ml of 1% (W/V) sulphanilamide (Sigma) in 1.5 N HCl and 1 ml of 0.02% (W/V) n-1-napthyl-ethylenediamine dihydrochloride solution (Sigma). All samples were centrifuged at 500G for 5 minutes to remove suspended matter. Nitrite was determined by measuring absorbance at 540 nm on a Beckman Model 42 spectrophotometer. Triplicate aliquots of crude extract were assayed in each experiment and nitrite produced was determined from a calibration curve (Figure 2.8).

2.9.3 Glutamine Synthetase Activity Assay

Each assay tube contained in a final volume of 2 ml of reaction mixture, 184 µmol L-glutamic acid (Sigma), 90 µmol $MgSO_4$, 12 µmol hydroxylamine (Sigma), 100 µmol imidazole (Sigma), 36 µmol ATP (Sigma), and 0.2 ml crude enzyme extract (Rhodes et al, 1975). The reaction tubes

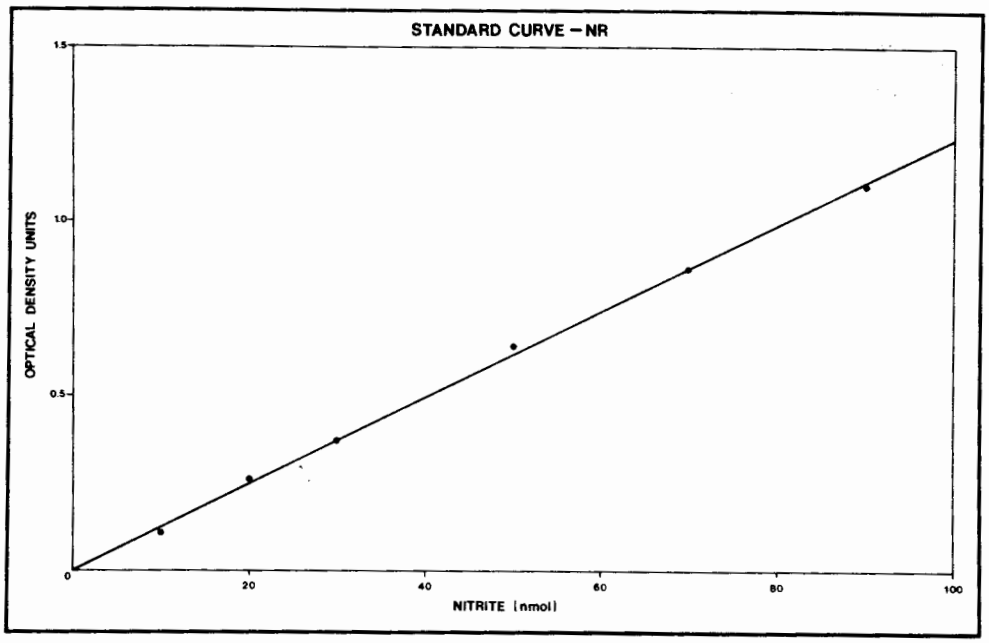


FIGURE 2.8
Standard curve for the determination of nitrite produced during the incubation period in the *in vitro* nitrate reductase assay.

were incubated for 15 minutes at 28°C, and the reaction stopped by the addition of 1 ml ferric chloride reagent (10 g trichloroacitic acid, 8 g anhydrous ferric chloride in 250 ml 0.5M HCl). Each tube was centrifuged at 500 g for 10 minutes to remove suspended matter and glutamyl hydroxamate (GMH) was determined by measuring absorbance at 500 nm on a Beckman Model 42 spectrophotometer. Each determination for GSA was carried out in triplicate and the GMH produced was measured from the calibration curve shown in Figure 2.9.

2.10 Carbon Exchange Determinations

Net photosynthesis, photorespiration, CO_2 compensation point and root respiration were carried out using an infra-red gas analyzer (IRGA) Model 225-MK3 (Analytical Development Company Ltd., Hoddesdon, Herts, England) with an open gas circuit system (Figure 2.10). Leaves of differing ages were used to give a better comparison between different plant feeding regimes. An entire attached leaf was contained in a clear perspex cuvette (Figure 2.11) through which an air stream of known CO_2 concentration with a flow rate of 0.200 l min^{-1} was passed. Temperature of the leaf chamber was kept constant at 25°C by means of a circulating water jacket both top and bottom connected to a Lauda (Messgerate - Werk Launda, Dr. R. Wobser KG, D-6970 Lauda - Konigshofen, Federal Republic of Germany) constant temperature circulating unit. The leaves were illuminated in the growth chamber with a photosynthetically active radiation (PAR) of

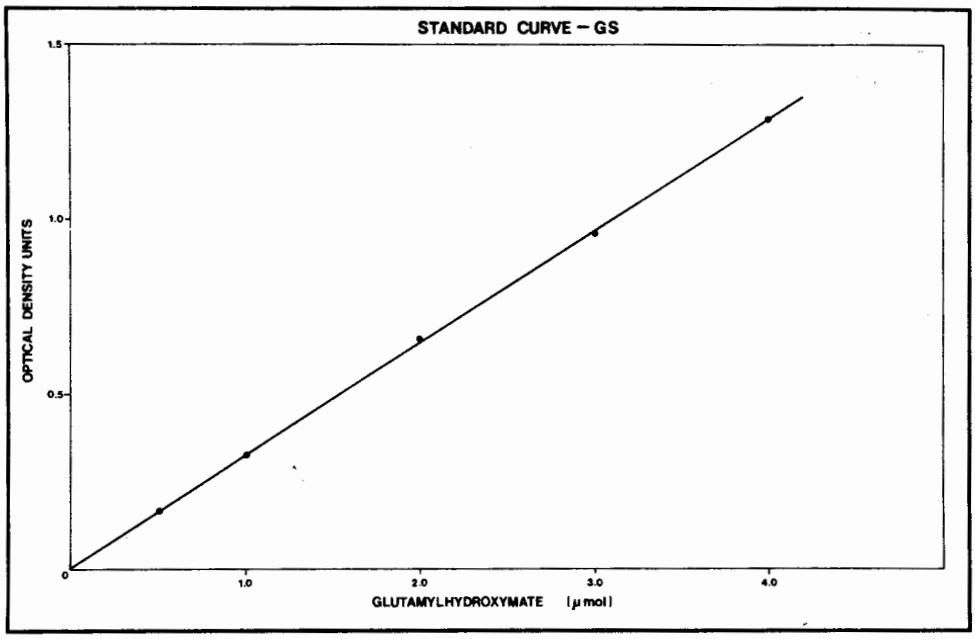


FIGURE 2.9
Standard curve for the determination of glutamylhydroximate accumulated during the *in vitro* glutamine synthetase assay.

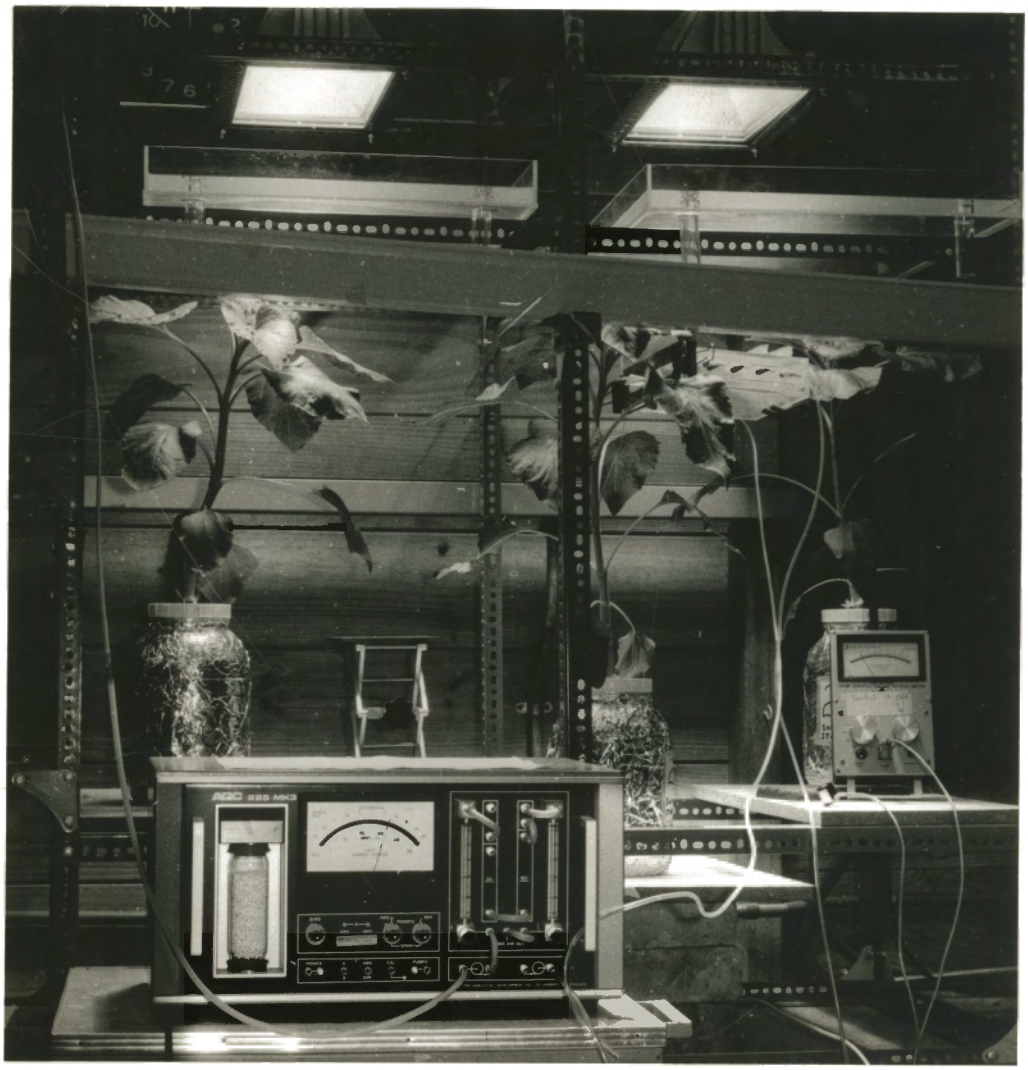


FIGURE 2.10
Open gas circuit system infra-red gas analyzer with perspex cuvette containing a single attached leaf from a sunflower plant growing in hydroponic solution. Separate quantum photometer is used with a quantum sensor next to the leaf being analyzed.

FIGURE 2.11
Perspex cuvette for leaf photosynthetic determinations fitted with water jacket to maintain constant temperature.

380 $\mu E\ m^{-2}\ s^{-1}$ which was measured with a Crump System 550 Quantum Photometer (Crump Scientific Products Ltd., Rayleigh, Essex, UK) using a Quantum Sensor Model 554. The CO_2 concentration of air from the outside was measured, passed through the leaf chamber and the remaining CO_2 was monitored until a steady state was reached. Carbon dioxide compensation point was determined by making a closed system by connecting the cuvette inlet airline to the IRGA outlet connection.

Using an ADC LCA portable measurement system IRGA (Figure 2.12) with a Parkinson Leaf Chamber, photosynthetic rates were determined for leaves of differing ages on each plant.

Dark respiration was determined by connecting the Model 225-MK3 IRGA as for photosynthetic measurements and switching the lights off in the growth chamber. Measurements were made after no further change in CO_2 concentration could be detected. Root respiration in photosynthetically active plants was determined by supplying air of known CO_2 concentration to the roots in nutrient solution and measuring the CO_2 concentration of the evolved air coming from the nutrient solution with the IRGA Model 225.

FIGURE 2.12
The ADC LCA portable measurement system IRGA with Parkinson Leaf Chamber connected to a leaf of a sunflower plant growing in a growth chamber.

CHAPTER 3

GROWTH AND CARBON DIOXIDE EXCHANGE DETERMINATIONS OF SUNFLOWER PLANTS GROWN WITH DIFFERENT NITROGEN NUTRIENT SOURCES

3.1 Introduction

Non-leguminous plants have shown variation in mass as a result of the form of inorganic nitrogen fertilizer used during the life of the plant. This may be in the vegetative stage producing a larger plant and/or in the reproductive stage resulting in larger or more fruit per plant. A greater plant mass was obtained through feeding nitrate than ammonium or urea for the following plants: tomato plants in liquid culture (Ganmore-Neumann and Kafkafi, 1980; Magalhaes and Wilcox, 1984); triticale, rye and wheat (Gashaw and Mugwira, 1981); wheat (Cox and Reisenauer, 1973; Lips *et al*, 1987); *Ricinus communis* (Allen *et al*, 1985); barley (Lewis and Chadwick, 1983); strawberry, melon, cucumber, watermelon, okra, egg plant, tomato, sweet pepper, soybean, pea and kidney bean with the nutrient solution kept at pH 5 and cucumber, watermelon, okra, egg plant, tomato, sweet pepper, soybean, pea and kidney bean with the nutrient solution kept at pH 7 (Ikeda and Osawa, 1979). Likewise, ammonium and urea have been shown to produce plants with a larger mass than those grown with nitrate: sweet corn with the nutrient solution kept at pH

5, sweet corn and strawberry with the nutrient solution kept at pH 7 (Ikeda and Osawa, 1979); tomato plants in peat (Magalhaes and Wilcox, 1984); maize (Dibb and Welch, 1976). A combination of ammonium and nitrate has been found to produce a larger plant mass than either of the single sources of nitrogen alone for triticale, rye and wheat (Gashaw and Mugwira, 1981); barley (Lewis and Chadwick, 1983); tomato plants in liquid culture (Ganmore-Neumann and Kafkafi, 1980); strawberry, melon, cucumber, watermelon, okra, egg plant, tomato, sweet pepper, soybean, pea and kidney bean (Ikeda and Osawa, 1979). Based upon all of these findings it was decided to grow one set of sunflower plants to a stage prior to the emergence of the apical bud and a second set to the fruit filling stage to determine the effect of nitrate, ammonium and ammonium + nitrate on plant mass.

One problem encountered in feeding the different nitrogen sources is that caused by bacteria in the soil which can cause nitrification of ammonium and urea (Huber et al, 1977; Haynes and Goh, 1978; Krishnapillai and Pethiyagoda, 1980) or denitrification of nitrate to ammonium (Caskey and Tiedje, 1980) or to N_2O and N_2 (Huber et al, 1977; McElhannon and Mills, 1981a and b). To prevent nitrification during hydroponic feeding experiments, frequent changing of the entire nutrient solution has been shown to be effective (Murphy, 1985). When nitrapyrin, a

nitrificaton inhibitor, was added to ammonium containing fertilizer, more nutrient nitrogen was made available to the plant in the ammonium form than without inhibitor addition (Dibb and Welch, 1976; Gomes and Loynachan, 1984; Tromp and Ovaa, 1979; Chancy and Kamprath, 1982). Notton, Watson and Hewitt (1979) found that at 5 ppm N-serve there was no nitrate found in NH_4^+ nutrient solution samples displaced 24 hours after passage through sand containing 58 day old radish plants. McElhannon and Mills (1981a and b) found that by preventing denitrification, through the use of nitrapyrin, the levels of nitrate increased in the soil. In view of the above results, nitrapyrin at a final concentration of 5 ppm was included in all of the nutrient feeding solutions (Chapter 2, Section 2.1) to eliminate the possibility of bacterial denitrification of the nitrate and nitrification of the ammonium, so that the contributions made by each of the two nitrogen forms could be identified and attributed to the specific ion.

Plants grown in a medium containing ammonium cause acidification of the soil (Cox and Reisenauer, 1973) and those grown with nitrate cause alkalinization of a nutrient solution (Warncke and Barber, 1973) when no pH correction is provided to the medium. A standard Hoagland nutrient solution recirculated through the roots of sunflower plants twice daily showed a pH decrease from pH 5.5 to pH 4.0 after eight days (Drakeford and Reid, 1984). Cox and Seeley

(1984) showed a pH drop from pH 6.1 to pH 3.1 in ten days using a Hoagland nutrient solution containing only ammonium nitrogen and a pH drop to pH 4.5 in a solution containing 25% ammonium and 75% nitrate with poinsettia plants. Tomato plants grown in liquid culture exhibited a pH increase to near neutrality irrespective of the initial pH when fed nitrate-N while ammonium-N solutions acidified the solution to approximately pH 3.5 irrespective of the initial pH (Pill and Lambert, 1977). In contrast to solution culture, where very large pH changes are attributable to the form of nitrogen absorbed, bulk soil pH relative to the nitrogen source appears to change very little through the addition of fertilizers (Dibb and Welch, 1976; Chancy and Kamprath, 1982; Bledsoe and Zasoski, 1983). Krishnapillai and Pethiyagoda (1980) were able to show a four-fold increase in leaf area and leaf dry mass production when calcium carbonate was included in an ammonium nutrient solution when compared to ammonium fertilized plants lacking calcium carbonate. Ammonium-only grown non-nodulated Alnus glutinosa plants with pH control of nutrient solutions had twice the dry mass as plants grown without pH control (Przemeck and Kucke, 1986). To eliminate the possible adverse effect of pH extremes in the present work, calcium carbonate was added to the liquid culture solutions combined with frequent solution changes (Chapter 2, Section 1).

Larsson, Olsson and Larsson (1985) have demonstrated that ammonium, as the only nitrogen source in cultures of Scenedesmus, caused some inhibition of CO_2 fixation whereas nitrate as the only nitrogen source enhanced CO_2 fixation under light saturation conditions. Lower leaves normally serve as the source of photosynthates for roots as shown by Pate (1966) in peas. The decrease in net photosynthesis shown by Campbell et al, (1986) using leaves of tomato plants, was considered to be the result of both decreased photosynthetic activity with average leaf age and increased shading of older leaves. They proposed that shading of the bottom leaves by the upper leaves might reduce the carbohydrate supply to the roots and impair nitrogen uptake unless the supply is met by the upper leaves. A low supply of carbohydrate and the strong nitrogen demands of growing organs appear to be partially responsible for the initiation of senescence in older leaves (Nova and Loomis, 1981).

The purpose of the experiment described in this chapter was to determine whether the growth, leaf net photosynthesis, dark respiration, photosynthetic compensation point and root respiration of sunflower plants vary between plants grown with differing nitrogen sources at the vegetative and fruit filling stages.

3.2 Experimental Methods

Ten replicate plants of Helianthus annuus L. were individually grown in a growth chamber at 400 $\mu Em^{-2}s^{-1}$ in two-litre jars in each of the three nitrogen sources, nitrate, ammonium and ammonium + nitrate, under the conditions described in Chapter 2, Section 2.1. After four weeks of strictly vegetative growth prior to the first appearance of an apical bud, plants were harvested into root and shoot. The roots were washed with de-ionized water and were then blotted dry with absorbent tissue prior to mass determinations. The fresh mass of the plant parts was ascertained and the material oven dried at 80°C for 24 hours. Prior to oven drying the leaves, leaf areas were determined by tracing the leaf outline onto 80 gram per m^2 bond paper immediately after the fresh mass was determined. The paper outlines of the leaves were then cut out and weighed to determine leaf areas using a conversion factor of 8 mg per cm^2. At the end of the drying period, the plant material was removed from the oven and allowed to cool to room temperature for 15 minutes at which time dry mass was determined.

A second set of 10 replicate plants for each N-source was individually grown in five-litre jars under the same conditions as described for the 4 week old plants above and harvested after 10 weeks of growth. These plants were at an advanced stage of seed filling but two additional weeks

would be required for complete fruit filling (Hocking and Steer, 1983). Fresh and dry mass for root, shoot and fruit were determined as described above. An additional set of 10 plants grown in nitrate-only nutrient solution minus nitrapyrin was included to determine whether the inclusion of nitrapyrin in the nutrient solution had any effect upon fresh and dry mass for root, shoot and fruit.

Plant transpiration for the entire growth period was determined by summing the water lost at each nutrient change (minus controls) as described in Chapter 2, Section 2.1 for both the 4 and 10 week old plants.

An additional experiment was carried out using the plants grown for the 10 week growth experiment. At the 4 week stage of growth, leaf photosynthetic rate, dark respiration rate and compensation point were determined using a portable ADC infra red gas analyser (IRGA). Root respiration was measured as the amount of carbon dioxide evolved from the intact root system into the air stream leading to an ADC Mk3 IRGA as described in Chapter 2, Section 2.10.

To determine if the photosynthetically active radiation (PAR) in the growth chambers was of sufficient intensity for this C3 pathway plant, light saturation curves were prepared as described in Chapter 2, Section 2.10. Mature fully expanded leaves near the top of the plant were selected.

High intensity mercury vapour lamps shown in Figure 2.10 (Chapter 2, Section 2.10) were used to provide an irradiance higher than that provided by the growth chambers to determine if the level of irradiance of the growth chambers was below or above the photosynthetic light saturation point for the plants.

At the 10 week stage only net leaf photosynthesis was determined. Carbon dioxide exchange determinations using the IRGA were made on mature, young and nearly mature leaves of the Helianthus plants immediately prior to harvesting for the 10 week growth experiment.

3.3 Results and Discussion

3.3.1 Plant Growth at 4 Weeks

From Table 3.1 and Figure 3.1 it would appear that plants receiving ammonium + nitrate were 34% heavier than the ammonium fed plants and 28% heavier than the nitrate fed plants on a fresh mass basis. The dry mass values (Table 3.1) show that the ammonium + nitrate fed plants had 40% more mass than the ammonium-only fed plants but only 14% more mass than the nitrate-only fed plants. These differences become more evident in Figure 3.1. The combination of the fresh and dry mass results would indicate that the ammonium fed plants contain a larger amount of water than do either the nitrate-only or the ammonium + nitrate fed plants, especially in the root. A comparison of

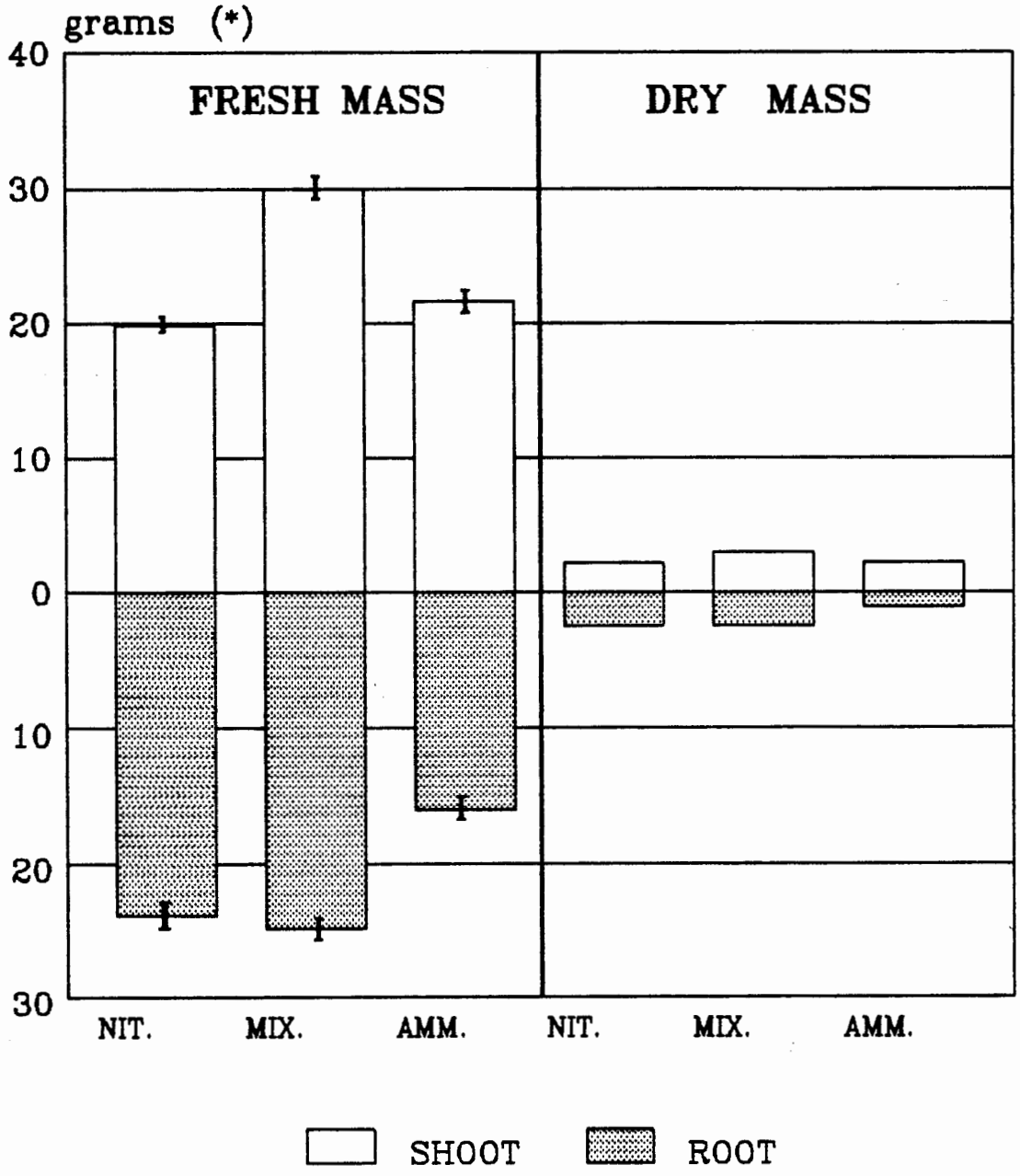
the results shown in Figure 3.1 and Table 3.1 show that the root dry mass to fresh mass ratio quotient was 0.1 for both the nitrate and mixed feed plants whereas the root dry mass to fresh mass ratio quotient for the ammonium fed plants was 0.06. This would indicate that the root of the ammonium fed plants contained much less solid material than did either the nitrate fed or the mixed feed plants. These results agree with those of Lewis and Chadwick (1983), working with barley, that nitrate fed plants produce a larger root mass than ammonium fed plants.

TABLE 3.1

Fresh and dry mass (g plant^{-1}) of 4 week old *Helianthus annuus* L. plants* and dry mass shoot to root ratio quotient (S/R) as affected by nitrogen source.

	Nitrogen Source					
	2 mM NO_3^-		1 mM NO_3^- + 1 mM NH_4^+		2 mM NH_4^+	
Leaf Area dm^2	6.71 ±0.39		8.64 ±0.36		7.09 ±0.37	
S/R	0.81 ±0.25		1.25 ±0.02		2.22 ±0.06	
	Fresh Mass	Dry Mass	Fresh Mass	Dry Mass	Fresh Mass	Dry Mass
Shoot	19.78 ±0.66	2.15 ±0.09	29.99 ±0.85	2.95 ±0.08	21.60 ±0.85	2.21 ±0.08
Root	23.94 ±1.10	2.42 ±0.08	24.88 ±0.78	2.36 ±0.08	16.00 ±0.71	1.00 ±0.06
Total	43.72 ±1.59	4.55 ±0.18	54.87 ±1.52	5.31 ±0.15	37.61 ±1.33	3.21 ±0.14

* Mean of 10 plants per feeding solution
± standard deviation



NIT.–2mM NO_3^- AMM.–2mM NH_4^+
MIX.–1mM NO_3^-+1mM NH_4^+
* means of 10 plants per treatment

FIGURE 3.1
Fresh and Dry mass of 4 week old *Helianthus annuus* plants fed different nitrogen sources.

The leaf area (Table 3.1) of the ammonium + nitrate fed plants was 18% larger than that of the ammonium fed plants and 23% larger than that of the nitrate fed plants. This would indicate that the ammonium + nitrate fed plants had a larger area for light interception needed for the production of photosynthate and consequently plant growth than did either the nitrate-only or ammonium-only fed plants.

The amount of water lost by the plants (Table 3.2) appears to be related to the assimilatory productivity. The mixed feed plants produced 1.5 times more dry mass per litre water transpired than the nitrate fed plants and 1.7 times more mass per litre water lost than the ammonium fed plants. This would indicate that ammonium + nitrate would produce larger plants than either ammonium or nitrate alone under agricultural conditions where water may be a limiting factor in crop production.

3.3.2 Light Saturation

Table 3.3 shows the correlation of different irradiance levels with photosynthetic rates of sunflower plants grown with the three different nitrogen nutrient sources. As can be seen from Figure 3.2 and Table 3.3 photosynthetic light saturation occurs at ca. 375 $\mu Em^{-2}s^{-1}$ for all three nitrogen sources. There is, however, a difference between the three

TABLE 3.2

Effect of nitrogen source on transpiration and water use efficiency of 4 week old plants of *Helianthus annuus*.*

N Source	Transpiration Rate			Water Use Efficiency
	ml H_2O lost $plant^{-1}$	ml H_2O lost $g\ dw^{-1}$	ml H_2O lost dm^{-2}	g dw gained $1\ H_2O^{-1}$ lost
2 mM NO_3^-	1238 ± 75	270.8 ± 7.1	184.6 ± 0.4	3.69 ±0.10
1 mM NO_3^+ plus 1 mM NH_4^+	1220 ± 49	229.8 ± 2.9	141.2 ± 0.7	4.35 ±0.05
2 mM NH_4^+	1246 ± 66	387.6 ± 6.1	175.7 ± 0.3	2.58 ±0.04

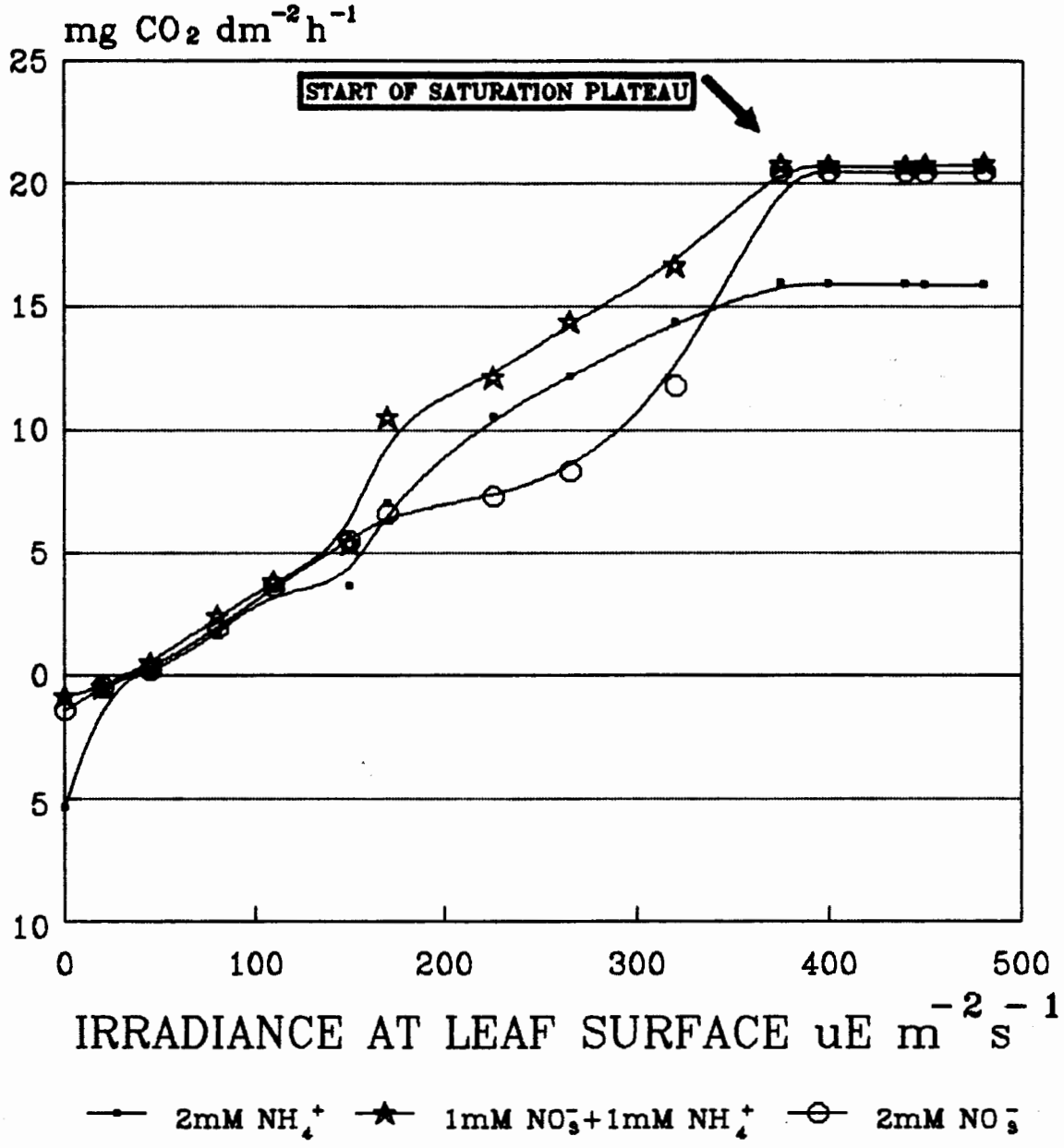
* Mean of 10 plants used per feeding treatment ± standard deviation

TABLE 3.3

Net photosynthetic rates of mature fully expanded leaves at different levels of irradiance at the leaf surface. Four week old plants of *Helianthus annuus* L.* were grown in nutrient solutions containing 2 mM nitrate, 2 mM ammonium or 1 mM nitrate + 1 mM ammonium as the nitrogen source.

Irradiance at leaf surface $\mu E\ m^{-2}s^{-1}$	Ammonium Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$	Ammonium + Nitrate Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$	Nitrate Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$
480	15.90 ± 0.83	20.77 ± 1.22	20.46 ± 1.20
450	15.91 ± 0.82	20.74 ± 1.67	20.46 ± 1.17
440	15.92 ± 0.78	20.73 ± 1.12	20.49 ± 1.13
400	15.92 ± 0.72	20.73 ± 1.11	20.47 ± 1.10
375	15.96 ± 0.65	20.75 ± 1.08	20.47 ± 1.08
320	14.36 ± 0.65	16.63 ± 0.96	11.75 ± 0.69
265	12.14 ± 0.59	14.33 ± 0.79	8.27 ± 0.46
225	10.52 ± 0.56	12.09 ± 0.80	7.28 ± 0.39
170	6.99 ± 0.29	10.47 ± 0.64	6.56 ± 0.35
150	3.62 ± 0.16	5.32 ± 0.64	5.45 ± 0.28
110	3.48 ± 0.11	3.75 ± 0.12	3.58 ± 0.18
80	1.72 ± 0.06	2.38 ± 0.06	1.91 ± 0.06
45	-0.03 ± 0.01	0.45 ± 0.04	0.19 ± 0.01
20	-0.41 ± 0.01	-0.51 ± 0.01	-0.48 ± 0.02
0	-5.38 ± 0.06	-0.88 ± 0.01	-1.42 ± 0.08

* Mean of 10 plants per nitrogen source for each level of irradiance ± standard deviation



MEAN READING FOR 10 PLANTS PER
NITROGEN SOURCE FOR EACH LEVEL
OF IRRADIANCE

FIGURE 3.2
Light saturation curve of Helianthus annuus plants fed different nitrogen sources, as determined in a growth chambers.

different feeding regimes in the photosynthetic rate at which that saturation occurs. From the mean values of 15.96 mg CO_2 $dm^{-2}h^{-1}$ for ammonium, 20.75 mg CO_2 $dm^{-2}h^{-1}$ for mixed and 20.47 mg CO_2 $dm^{-2}h^{-1}$ for nitrate it would appear that the ammonium fed plant shows the least photosynthetic activity of the three plants and that mixed feed and nitrate fed plants are closely related with respect to photosynthetic activity. An analysis of variance at this irradiance shows that the means are significantly different.

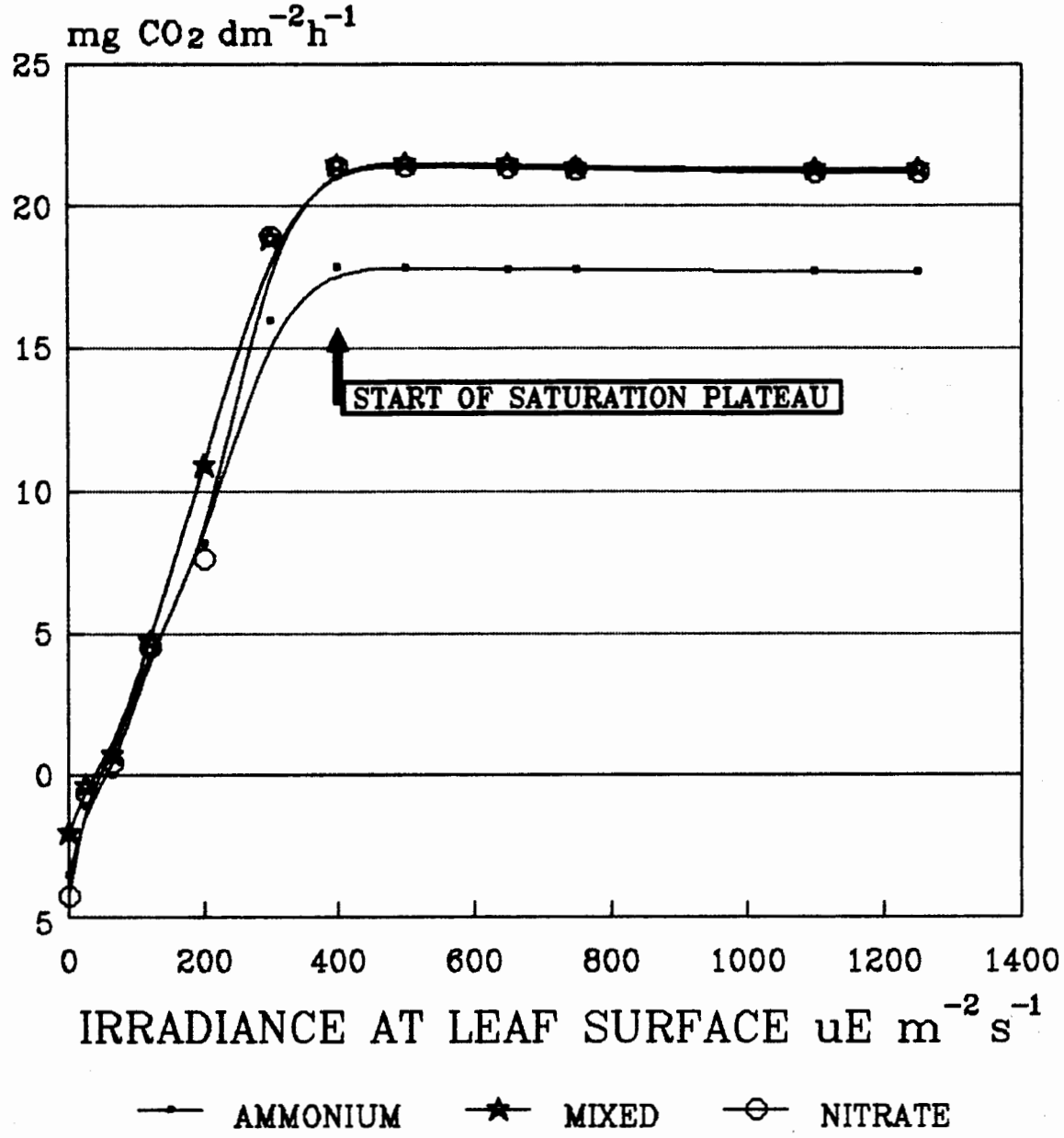
Table 3.4 shows a comparison of different irradiance levels to leaf net photosynthetic rates of plants grown on the three different nitrogen sources using the high intensity lamps (Chapter 2, Section 2.10, Figure 2.10). The same plants were used for these determinations as were used to provide data for Table 3.3. The values show the same pattern as shown in Table 3.3 with light saturation occurring at 400 μE $m^{-2}s^{-1}$ and the nitrate and mixed feed plants exhibiting a greater photosynthetic activity than the ammonium fed plants. This can be seen more clearly in Figure 3.3. An analysis of variance of the means showed that there was a significant difference between the ammonium fed plants and the other two treatments ($p=<10^{-6}$). These results agree with those shown by Marques et al, (1983) for non-nodulated bean plants.

TABLE 3.4

Net photosynthetic rates of mature fully expanded leaves at different levels of irradiance at the leaf surface. Four week old plants of *Helianthus annuus* L.* were grown in nutrient solutions containing 2 mM nitrate, 2 mM ammonium or 1 mM nitrate + 1 mM ammonium as the nitrogen source.

Irradiance at leaf surface $\mu E\ m^{-2}s^{-1}$	Ammonium Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$	Ammonium + Nitrate Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$	Nitrate Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$
1250	17.70 ± 0.67	21.28 ± 1.18	21.17 ± 1.21
1100	17.70 ± 0.64	21.29 ± 1.19	21.17 ± 1.21
750	17.77 ± 0.68	21.35 ± 1.18	21.26 ± 1.22
650	17.78 ± 0.74	21.45 ± 1.16	21.35 ± 1.18
500	17.82 ± 0.76	21.48 ± 1.14	21.38 ± 1.16
400	17.83 ± 0.74	21.44 ± 1.09	21.38 ± 1.14
300	15.98 ± 0.71	18.84 ± 1.18	18.95 ± 1.08
200	8.15 ± 0.38	10.91 ± 0.56	7.59 ± 0.34
120	4.21 ± 0.18	4.69 ± 0.23	4.51 ± 0.25
65	0.00 ± 0.01	0.73 ± 0.02	0.43 ± 0.02
25	-1.06 ± 0.05	-0.37 ± 0.01	-0.64 ± 0.02
0	-3.50 ± 0.11	-2.03 ± 0.07	-4.27 ± 0.20

* Mean of 10 plants per nitrogen source for each level of irradiance ± standard deviation



MEAN READING FOR 10 PLANTS PER
NITROGEN SOURCE FOR EACH LEVEL
OF IRRADIANCE

FIGURE 3.3
Light saturation curve of Helianthus annuus plants fed different nitrogen sources, as determined under high irradiance from mercury vapour lamps.

From the results in Tables 3.3 and 3.4 it would appear that ammonium fed plants have a lower rate of net photosynthesis than either the nitrate or the ammonium + nitrate fed plants. These results agree with Lips et al, (1987) who also found that the net photosynthetic rate for ammonium-only grown wheat plant leaves was lower than the rates exhibited by nitrate-only and ammonium + nitrate grown plants. The nitrate and ammonium + nitrate fed plants were photosynthetically more active than the ammonium fed plants, which could partially explain their overall higher plant mass shown in Table 3.1.

The net photosynthetic rates (Pn's) of the light saturation plateaus of the ammonium fed plants (Figures 3.2 and 3.3) were significantly different ($p=<10^{-6}$) to those of nitrate and ammonium + nitrate grown plants which were identical at the 95% confidence interval.

3.3.3 Leaf Net Photosynthesis, Dark Respiration and CO_2 Compensation Point

The results for the leaf CO_2 exchange determinations in 4 week old plants can be seen in Table 3.5. As can be seen the mature leaves exhibit a slightly higher photosynthetic rate than the young leaves of plants fed all three nitrogen sources. The ammonium fed plants exhibited a lower photosynthetic rate than either the nitrate or ammonium + nitrate fed plants for leaves at all three ages.

The rates of dark respiration were greater in mixed feed and nitrate fed plants as compared to the ammonium fed plants. These results do not agree with Marques et al, (1983) who found that the leaves of young bean plants exhibit an increase in dark respiration and CO_2 compensation point in ammonium compared to nitrate fed plants. In all three treatments the younger leaves showed a significantly lower compensation point ($p=<10^{-6}$) than did the mature leaves. The compensation point for ammonium + nitrate fed plants (80 ppm CO_2) was slightly (but significantly) different to that of both nitrate-only and ammonium-only fed plants (both 77 ppm CO_2). This difference could be nutrient related as was shown by Cresswell, Tew and Lewis (1977) who found that the CO_2 compensation point for vacuum infiltrated leaves of Panicum maximum more than doubled (36 - 80 ppm CO_2) with increased nitrate concentration.

3.3.4 Root Respiration

De Visser and Lambers (1983) found that the rate of root respiration in non-nodulated pea plants utilizing ammonium increases with the increasing requirements of the root for ATP. ATP in these plants is consumed at a higher rate during N-accumulation in the root compared to those utilizing nitrate. Nitrate uptake is an active process which requires ATP or some other source of energy (Eisele and Ullrich, 1977).

The results of the root respiration determinations in 4 week old H. annuus are shown in Table 3.6 and are expressed as mg CO_2 evolved by the entire root system per hour and CO_2 loss per g fresh root mass. As can be seen, the ammonium fed plant released the least amount of CO_2 per gram fresh mass from the roots whereas the nitrate fed plant respired the most. The nitrate fed plant was respiring CO_2 at a slightly greater rate than the mixed feed plant and nearly 3 times greater than the ammonium fed plant per fresh gram mass indicating a higher requirement for ATP in both the nitrate and ammonium + nitrate fed plants than in the ammonium-only fed plants. These results agree with those of Lips et al, (1987) who found that root respiration was higher from nitrate-only fed plants than from ammonium-only or ammonium + nitrate fed plants.

In the present experiment the shoot to root mass ratios of nitrate and ammonium + nitrate fed plants are less than those of ammonium-only fed plants (Table 3.1). This indicates an increased energy requirement for the root production of the nitrate and ammonium + nitrate fed plants than in the case of ammonium-only fed plants.

TABLE 3.5

Leaf net photosynthetic rate, dark respiration rate and photosynthetic compensation point in Helianthus annuus L. plants* grown on 2 mM nitrate, 2 mM ammonium or 1 mM nitrate + 1 mM ammonium for 4 weeks. Leaves measured were young (L12), old fully expanded (L3) and an intermediate (L8).

N Source	Leaf	Net Photosynthesis mg CO_2 $dm^{-2}h^{-1}$	Dark Respiration mg CO_2 $dm^{-2}h^{-1}$	Compensation Point ppm CO_2
2 mM NO_3^-	L3	20.00 ± 0.85	6.18 ± 0.29	79.8 ± 1.8
	L8	20.53 ± 0.77	6.85 ± 0.27	79.8 ± 1.8
	L12	19.14 ± 0.62	4.19 ± 0.15	71.8 ± 1.8
1 mM NO_3^- +	L3	21.50 ± 0.95	6.94 ± 0.18	79.8 ± 1.8
	L8	22.99 ± 0.87	7.20 ± 0.23	87.3 ± 2.8
1 mM NH_4^+	L12	20.48 ± 0.83	5.16 ± 0.16	73.8 ± 1.8
2 mM NH_4^+	L3	18.34 ± 0.64	5.81 ± 0.22	79.8 ± 1.8
	L8	18.86 ± 0.72	5.99 ± 0.17	79.8 ± 2.4
	L12	16.78 ± 0.64	3.58 ± 0.35	71.7 ± 2.3

* Mean of 10 plants per nitrogen source ± standard deviation

TABLE 3.6

Root respiration rates of *Helianthus annuus* L. plants* grown with 2 mM nitrate, 2 mM ammonium or 1 mM nitrate + 1 mM ammonium.

N Source	mg CO_2 lost $root^{-1}h^{-1}$	CO_2 lost $g^{-1}fm$
2 mM Nitrate	7.31 ± 0.27	0.31 ± 0.01
1 mM Nitrate + 1 mM Ammonium	5.62 ± 0.30	0.23 ± 0.01
2 mM Ammonium	1.73 ± 0.07	0.11 ± 0.00

* Mean of 10 plants per nitrogen source ± standard deviation

The root respiration (Table 3.6) of the nitrate fed plants, being higher than the ammonium and mixed feed plants, would indicate a high requirement of energy for the transport of nitrate into the root as well as assimilation of part of that nitrate in the root. This requirement of energy for nitrate uptake appears to be much greater than that of the ammonium plants which in the processing of N only need energy in the form of ATP for ammonium assimilation via glutamine synthetase.

3.3.5 Plant Growth at 10 Weeks

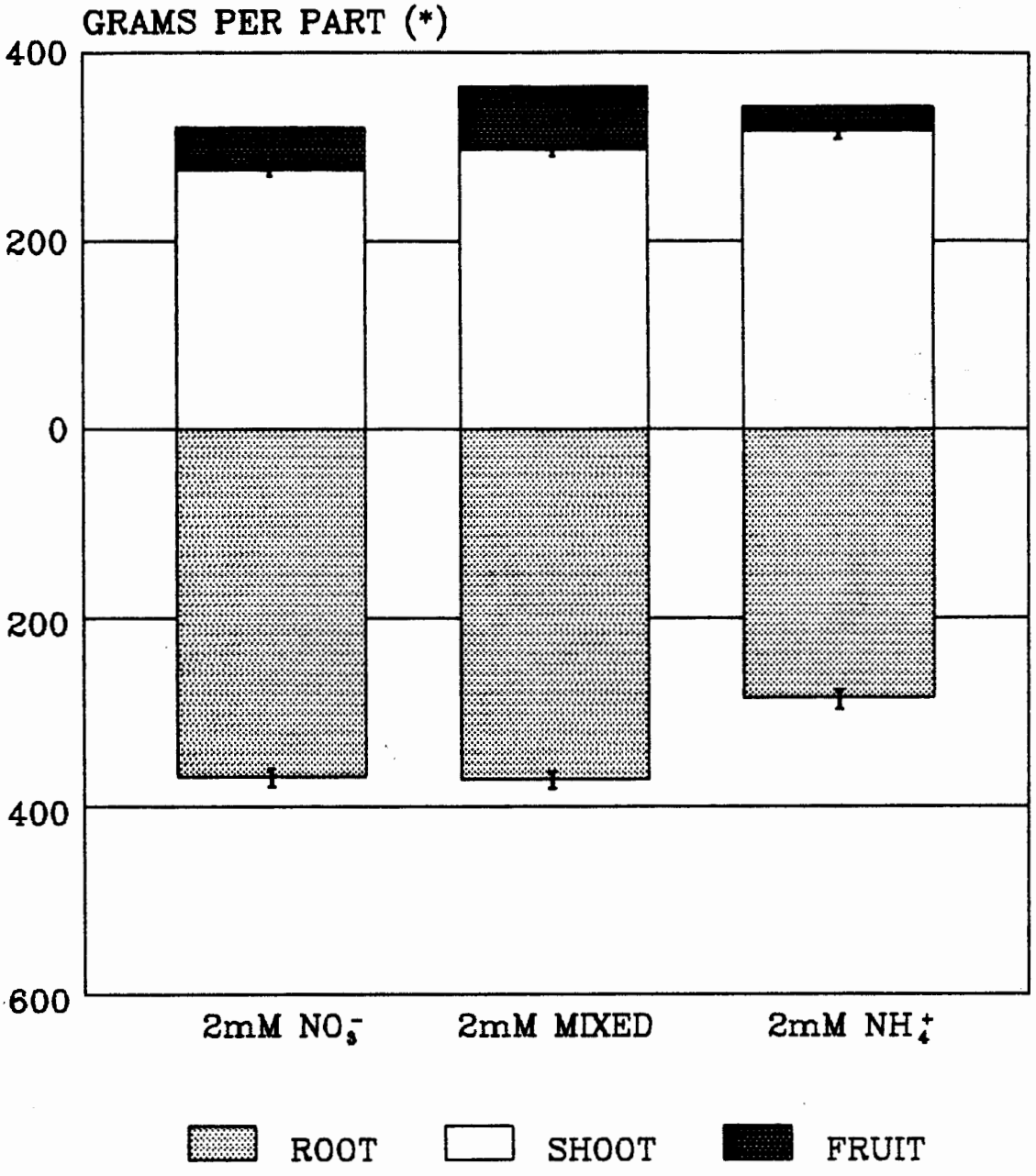
Table 3.7 shows the results of the 10 week growth experiment. The roots of both the ammonium + nitrate and nitrate fed plants exhibited a 1.3 times greater fresh and dry mass (Figures 3.4 and 3.5) than the ammonium-only fed plants. The shoot to root ratio quotients (Table 3.7) showed that the ammonium plants with a ratio quotient of 1.3 had a greater dry mass in the shoot than either the nitrate or ammonium + nitrate fed plants. The ammonium + nitrate fed plants produced double the fruit dry mass of the ammonium-only fed plants and 1.5 times more fruit dry mass than the nitrate-only fed plants. These results agree with those of Chadwick (1985) who showed that ammonium + nitrate produced a greater mass of fruit for barley than either the nitrate or ammonium fed plants.

TABLE 3.7

Fresh and dry mass (g plant^{-1}) of 10 week old Helianthus annuus L. plants* and dry mass shoot (including fruit) to root ratio quotient (S/R) as affected by nitrogen source.

	Nitrogen Source					
	2 mM NO_3^-		1 mM NO_3^- + 1 mM NH_4^+		2 mM NH_4^+	
S/R		1.0 ±0.0		1.0 ±0.0		1.3 ±0.0
	Fwt	dwt	Fwt	dwt	Fwt	dwt
Fruit	44.17 ±2.01	6.98 ±0.20	65.08 ±2.13	10.63 ±0.16	24.94 ±1.06	4.45 ±0.08
Shoot	277.31 ±6.18	25.94 ±1.02	298.66 ±7.09	26.54 ±0.77	318.17 ±10.50	29.09 ±0.81
Root	367.74 ±9.71	34.70 ±0.81	372.24 ±10.44	36.23 ±0.95	284.17 ±10.37	26.67 ±0.77
Total	689.22 ±17.73	67.62 ±1.98	735.98 ±19.34	73.40 ±1.88	627.28 ±21.84	60.21 ±1.67

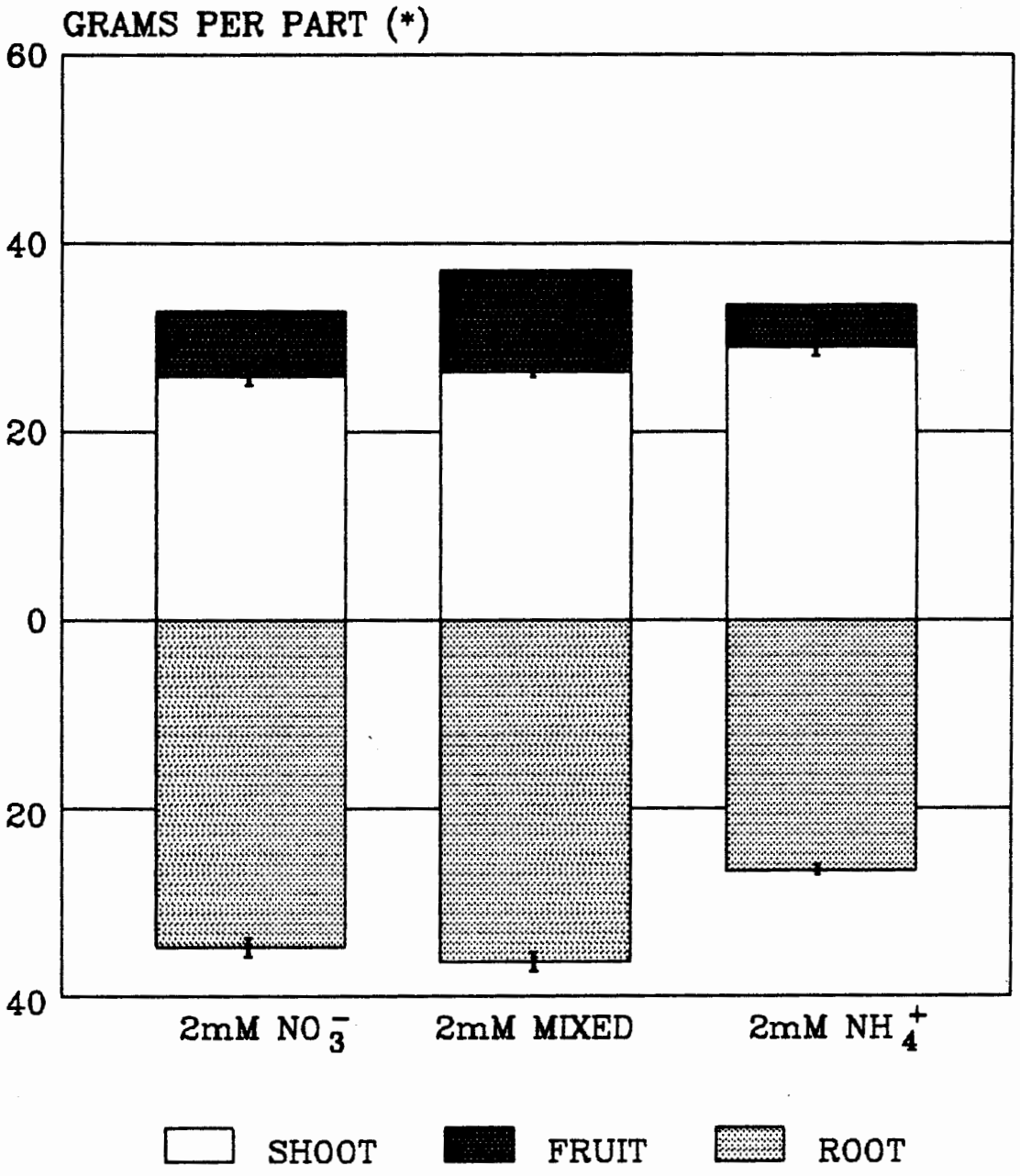
* Mean of 10 plants per nitrogen source ± standard deviation



MIXED- 1mM NO_3^- +1mM NH_4^+

(*) MEANS OF 10 PLANTS PER TREATMENT

FIGURE 3.4
Fresh mass of 10 week old plants of Helianthus annuus.



MIXED- 1mM NO_3^- + 1mM NH_4^+

(*) MEANS OF 10 PLANTS PER TREATMENT

FIGURE 3.5
Dry mass of 10 week old plants of Helianthus annuus.

The rate of water lost per plant and per plant unit mass (Table 3.8) from the ammonium-only fed plants was 1.2 times greater than the nitrate-only fed plants and 1.4 times greater than the ammonium + nitrate fed plants. The ammonium + nitrate plants were the most efficient in dry mass production per litre water lost with a production of 4.15 gl^{-1}. The mixed feed plants were only slightly more efficient than the nitrate fed plants in dry mass production but were 1.4 times more efficient than the ammonium-only fed plants at 2.87 gl^{-1}. The combination of the two nitrogen sources has been shown to produce a greater root mass than either of the nitrate-only or ammonium-only grown plants for the C_3 plant, barley (Lewis and Chadwick, 1983; Chadwick, 1985). Weissman (1964) demonstrated that sunflower leaves produce a greater dry mass with a mixed ammonium + nitrate (1:1) than either the nitrate or ammonium-only fed plants.

In the present work the whole shoot shows this same pattern indicating that not only the leaves but the stem and petioles also attain a greater mass in the mixed feed than the single source feed.

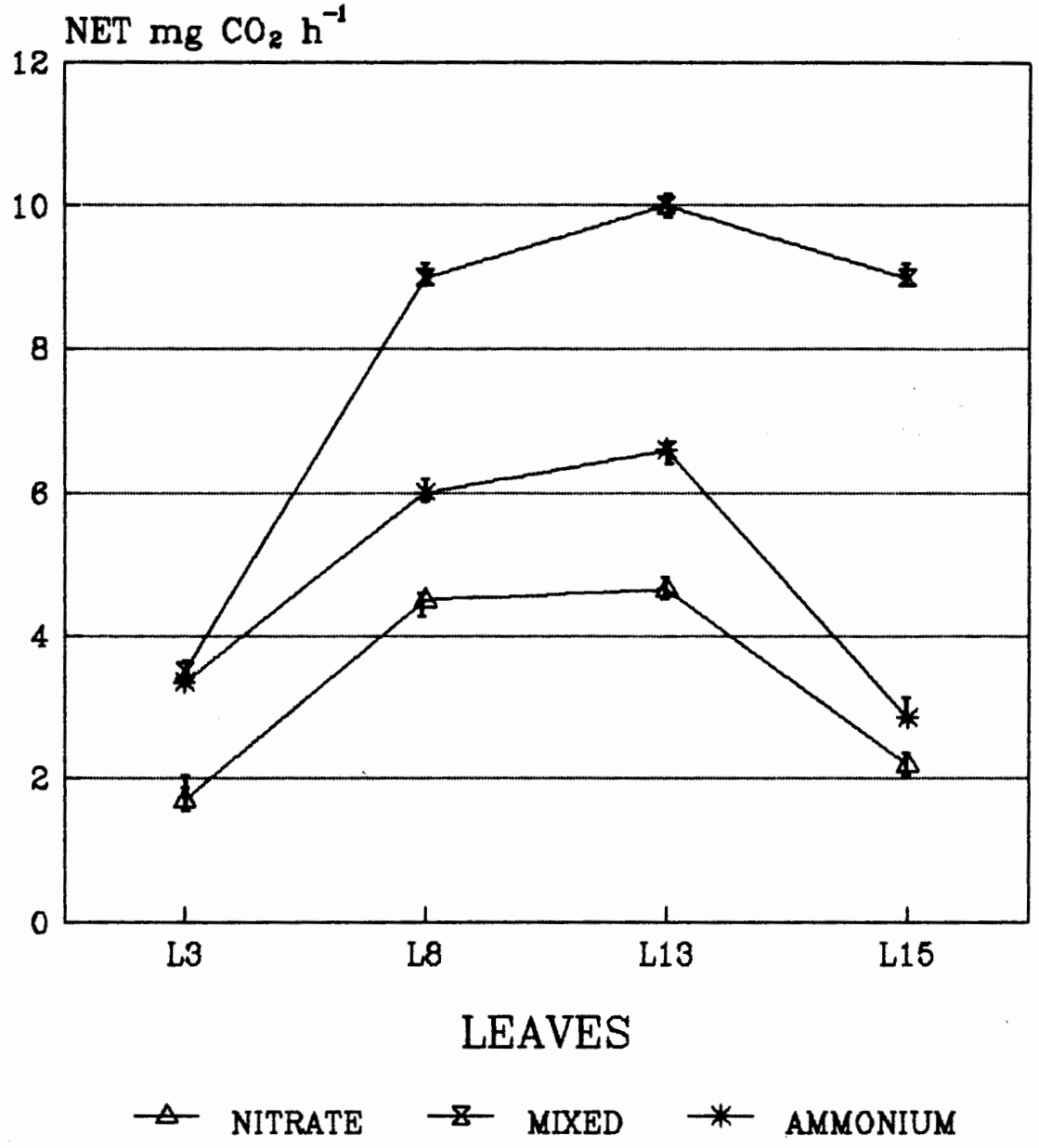
From Figure 3.6 it can be seen that photosynthetic rates were higher in mature leaves (L13 and L8) and lowest in the youngest (L15) and oldest leaves (L3). An exception to this was the young leaf (L15) on the ammonium + nitrate fed

TABLE 3.8

Effect of nitrogen source on transpiration and water use efficiency of 4 week old plants of *Helianthus annuus*.*

N Source	Transpiraton Rate		Water Use Efficiency
	ml H_2O lost plant^{-1}	ml H_2O lost g dw^{-1}	g dw gained 1 H_2O^{-1} lost
2 mM NO_3^-	17280 ± 201	257.0 ± 4.4	3.91 ±0.07
1 mM NO_3^- + 1 mM NH_4^+	17637 ± 225	242.2 ± 5.4	4.16 ±0.06
2 mM NH_4^+	20946 ± 307	347.8 ± 4.7	2.88 ±0.04

* Mean of 10 plants per nitrogen source ± standard deviation



L3 OLD LEAF
L8 AND L13 MATURE LEAVES
L15 YOUNG LEAF

FIGURE 3.6
Net photosynthetic rates of young, mature *Helianthus annuus* plants fed different nitrogen sources.

TABLE 3.9

Net photosynthetic rates of *Helianthus annuus* L. leaves* at an irradiance of 400 $\mu E\ m^{-2}s^{-1}$ grown with 2 mM nitrate, 2 mM ammonium or 1 mM nitrate + 1 mM ammonium for 10 weeks. Leaves measured were young (L15, L13), fully expanded mature (L8) and senescing (L3). Values are expressed as mg $CO_2\ dm^{-2}h^{-1}$.

Leaf	Nitrate Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$	Ammonium + Nitrate Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$	Ammonium Plant Photosynthesis mg $CO_2\ dm^{-2}h^{-1}$
L3	1.73±0.12	3.60±0.19	3.35±0.19
L8	4.53±0.22	8.95±0.33	6.07±0.34
L13	4.79±0.18	9.96±0.34	6.36±0.36
L15	2.22±0.12	9.36±0.51	2.82±0.14

* Mean of 10 plants per nitrogen source
± standard deviation

plants which showed an activity comparable to the mature leaves.

3.3.6 Fruiting Plant Net Photosynthesis

Table 3.9 shows the photosynthetic rates for plants at the fruit filling stage. It can be seen that the photosynthetic rates of specific leaves have decreased to one half for the mixed feed plants, one third for the ammonium fed plants and one fifth for the nitrate fed plants of the values of leaves in a similar location on the young plants (Table 3.5). The decrease noted for the photosynthetic rate from the young plant to the plant at fruit filling has been observed to occur with the flag leaves of wheat (Sinha and Rajagopal, 1980) and leaves of sunflower (Rawson and Constable, 1980).

3.4 General Discussion

It would appear that the mixed feed plants can combine the shoot development effect of ammonium with the root development of nitrate to produce plants which are larger and produce a greater mass of fruit than either nitrogen source alone. The increased fruit mass production of the mixed feed plants, combined with dry mass production per litre water lost for the whole plant, would indicate that the mixed feed nitrogen source would be more efficient than either of the single nitrogen sources agronomically.

CHAPTER 4

EFFECT OF NITROGEN SOURCE ON THE NITROGENOUS CONTENT OF XYLEM SAP

4.1 Introduction

The constituents of xylem sap vary with different species and also within the same species depending upon the form of nitrogen fed to the plant. Pate (1973) showed that only 7% of the nitrogen content in the xylem sap of Lupinus and Raphanus fed with 10 mM nitrate was nitrate. Several workers have shown, however, that most of the nitrate taken up by the roots is exported to the shoot, as can be seen by the percentage of nitrogen which is in the nitrate form in the xylem sap: 95% for Xanthium (Wallace and Pate, 1967), Cucumus (Olday et al, 1976) and Gossypium (Radin et al, 1975); 77-94% for Helianthus annuus (Kaiser and Lewis, 1980); 80% for Datura (Probyn, 1978); 80-90% for Hordium (Lewis et al, 1982a); 65% for maize (Ivanko and Ingversen, 1971).

Plants fed with ammonium as the sole nitrogen source transport nitrogen mainly as amino compounds and not as ammonium. The percentages of the total nitrogen transported via the xylem as ammonium for some of these plants are: 0.5% for maize (Ivanko and Ingversen, 1971); 0.6% for citrus

trees (Kato, 1981); 2.5% for sunflower (Weissman, 1964); 5.5% for barley (Lewis *et al*, 1982a).

When a combination of the two nitrogen forms is fed, the nitrate and ammonium contents as percentages of the total nitrogen in the xylem sap, have been shown to be approximately one half that of the nitrate-only fed plants and just slightly lower than that of the ammonium-only fed plants respectively (Lewis *et al*, 1982a; Weissman, 1964).

In order to assess the relative contribution made by nitrate and ammonium in the feeding solution to the nitrogen composition of *Helianthus annuus* xylem sap, ^{15}N-nitrate and ^{15}N-ammonium were used in the present set of experiments.

4.2 Experimental Methods

Plants of *Helianthus annuus* were grown as described in Chapter 2, Section 2.1 to an age of 4 weeks. At the commencement of the experiment whole plants were removed from the ^{14}N nutrient solutions, the roots washed under de-ionized water to remove any remaining solution and transferred to the appropriate Long Ashton nutrient solution containing $K^{15}NO_3$, $^{15}NH_4Cl$ or $^{15}NH_4{}^{15}NO_3$ (Chapter 2, Section 2.1). At intervals of 4 hours and 8 hours after the commencement of the experiment, plants were excised just below the cotyledons. The cut surface was washed with de-ionized water, blotted dry with tissue paper and a collar of

tygon tubing fitted onto the cut stem to act as a reservoir for the sap which was exuded through the action of root pressure. The liquid exuding from the cut surface is regarded as originating from the xylem vessels (Pate, 1962) which provide the main passage for the upward movement of dissolved nitrogenous compounds within the plant (Pate, 1973). Sap was collected from quadruplicate plants in the growth cabinet over a period of one hour under the conditions described in Chapter 2 Section 2.1. The collected sap from each of the four replicates was stored in separate vials on ice and then kept frozen until analysed for nitrogenous compounds. The four replicates were analysed separately and the results were then averaged. The nitrogenous fractions were separated as described in Chapter 2, Section 2.4.2 and analysed for ^{15}N content (Chapter 2, Section 2.5) and soluble amino compound composition (Chapter 2, Section 2.7).

4.3 Results and Discussion

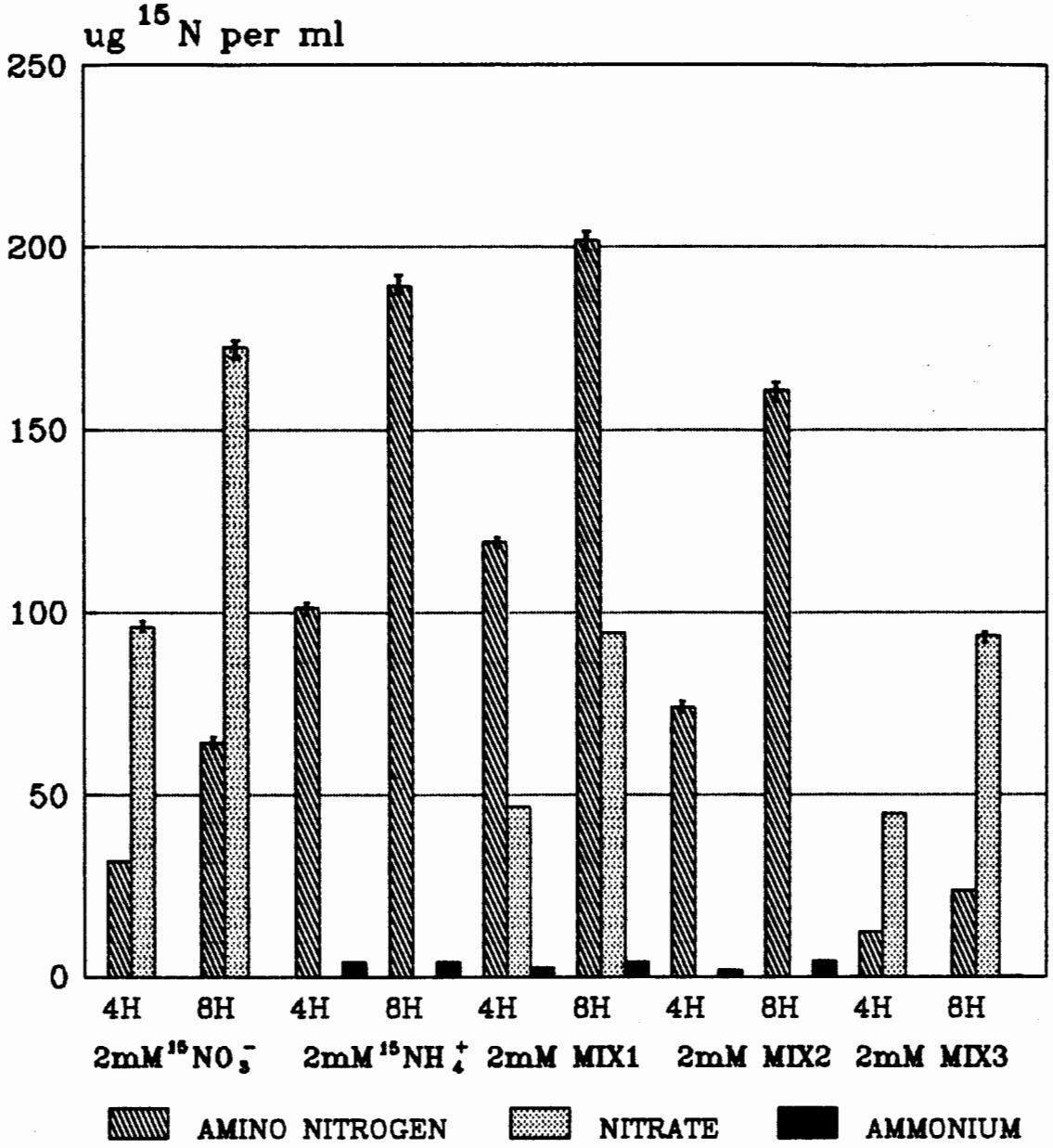
The nitrogen compound composition xylem sap obtained from sunflower plants fed with nitrate, ammonium and ammonium + nitrate are shown in Table 4.1 and Figures 4.1 and 4.2. As can be seen the major amino compound transported via the xylem stream regardless of nitrogen source, was glutamine. The ammonium-only fed plants (Table 4.1 and Figure 4.1) showed no nitrate present whereas the sap of the mixed feed plants contained 50% and the sap of the nitrate-only fed

TABLE 4.1

Soluble nitrogen composition of xylem sap in 4 week old sunflower plants as influenced by nitrogen source. Figures have been averaged from triplicate determinations of triplicate plants and are expressed as average ± range.

	N content of nitrate fed plants	N content of ammonium + nitrate fed plants	N content of ammonium fed plants
	μm N ml^{-1}	μm N ml^{-1}	μm N ml^{-1}
Aspartic Acid	0.238 ± 0.011	0.069 ± 0.006	0.044 ± 0.004
Threonine	0.125 ± 0.012	0.131 ± 0.009	0.151 ± 0.013
Serine	0.176 ± 0.012	0.207 ± 0.015	0.291 ± 0.021
Asparagine	0.146 ± 0.013	0.390 ± 0.027	0.496 ± 0.031
Glutamic Acid	0.371 ± 0.024	0.068 ± 0.006	0.245 ± 0.019
Glutamine	1.234 ± 0.089	4.230 ± 0.132	7.662 ± 0.378
Proline	T	T	T
Glycine	0.032 ± 0.002	0.011 ± 0.000	0.010 ± 0.000
Alanine	0.101 ± 0.007	0.591 ± 0.019	0.486 ± 0.022
Valine	0.049 ± 0.002	0.048 ± 0.002	0.078 ± 0.003
Cystine	0.023 ± 0.000	0.020 ± 0.000	0.043 ± 0.001
Methionine	0.003 ± 0.000	T	0.006 ± 0.000
Isoleucine	0.075 ± 0.004	0.052 ± 0.003	0.074 ± 0.003
Leucine	0.055 ± 0.002	0.046 ± 0.003	0.127 ± 0.008
Tyrosine	0.008 ± 0.000	T	0.032 ± 0.001
Phenylalanine	0.005 ± 0.000	T	0.006 ± 0.000
Ammonia	0.294 ± 0.011	0.270 ± 0.012	0.201 ± 0.010
Lysine	0.036 ± 0.002	0.035 ± 0.001	0.091 ± 0.004
Histidine	0.016 ± 0.000	0.032 ± 0.001	0.076 ± 0.003
Arginine	0.014 ± 0.001	T	T
γ Amino Butyric acid	0.150 ± 0.010	0.294 ± 0.016	0.185 ± 0.011
TOTAL N	3.156 ± 0.202	6.494 ± 0.292	10.304 ± 0.992
Nitrate	12.700 ± 0.978	6.500 ± 0.327	0.000 ± 0.000
% as Nitrate	80.1 ± 0.3	49.9 ± 0.4	0.0 ± 0.0

T - trace



MIX1 – 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX2 – 1mM $^{14}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX3 – 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$

FIGURE 4.1
Amino (+amido), nitrate and ammonium ^{15}N content of xylem sap of *Helianthus annuus* plants fed different nitrogen sources.

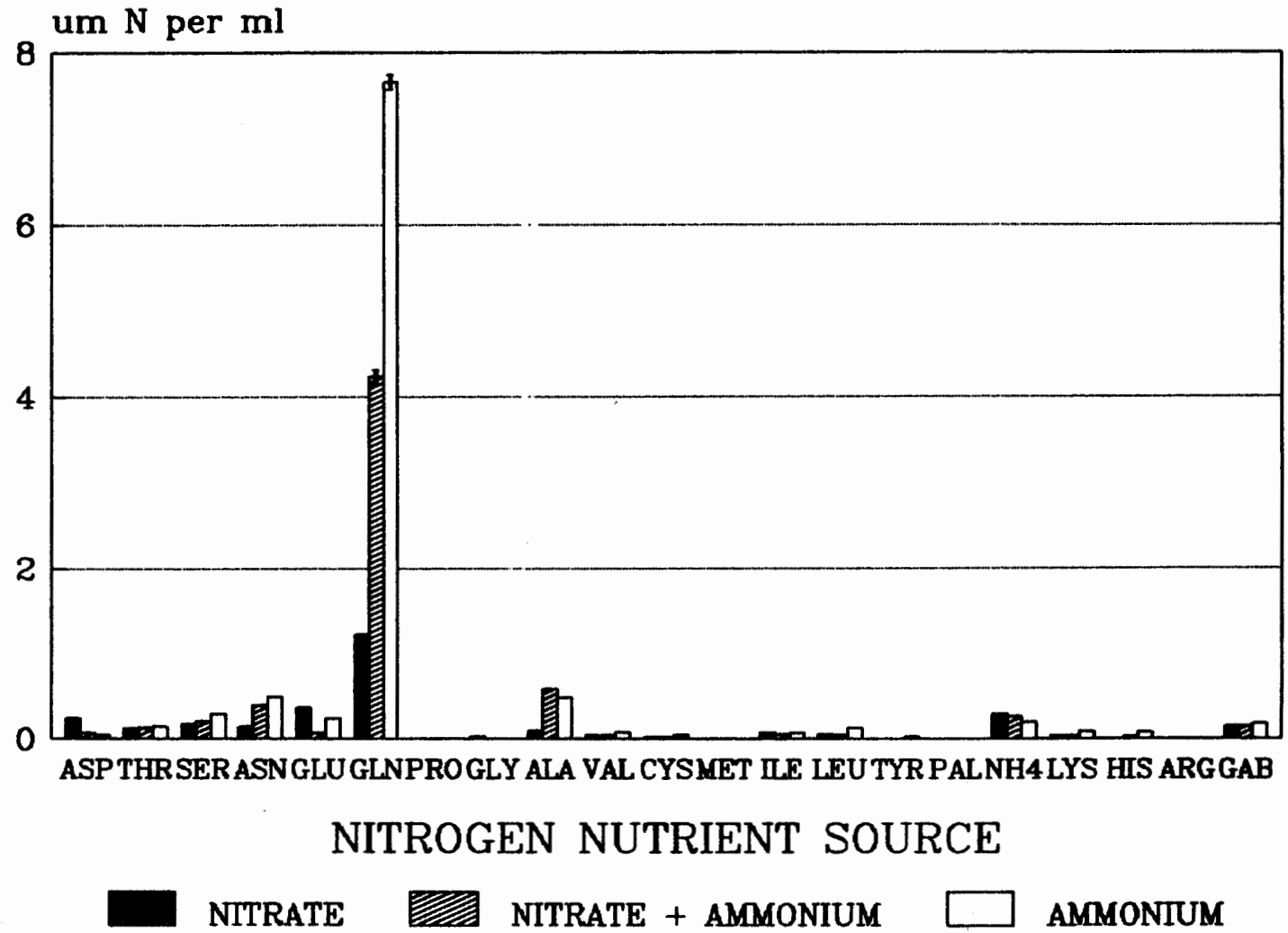


FIGURE 4.2
Free amino + amido nitrogen of the xylem sap of *Helianthus annuus* grown on different nitrogen sources.

plants contained 80% nitrogen as nitrate-N. A comparison of the nitrate levels from both Tables 4.1 and 4.2 show that the ^{15}N-nitrate had almost reached the level of total nitrate in the xylem sap at 8 hours for the nitrate fed and mixed feed plants, indicating that within the 8 hour period all of the xylem nitrate was from nitrate which was newly taken up by the roots. Although ammonium was contained in the nutrient solution for two of the three plants analysed, very little ammonium was found in the xylem sap.

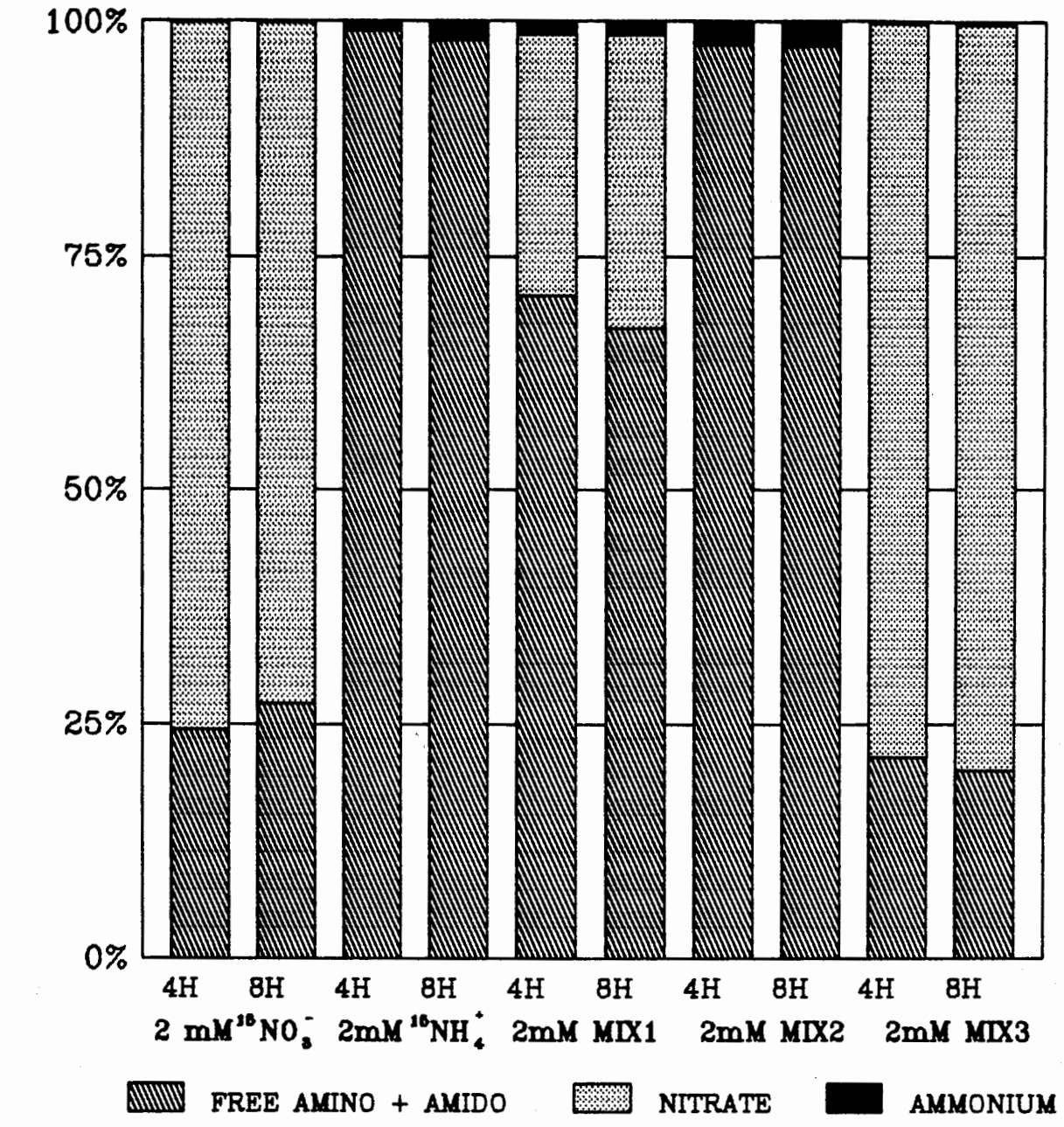
Figure 4.3 shows the comparison of ^{15}N content for nitrate, amino compounds and ammonium of sunflower xylem sap on a percentage basis. The nitrate-only fed plants contained 75% of the ^{15}N transported as nitrate and 25% as free amino compounds at the 4 hour harvest and 73% as nitrate and 27% free amino compounds at the 8 hour harvest with very little ^{15}N transported as ammonium at either harvest. The A%E increase from 54% at the 4 hour harvest to 90% at 8 the hour harvest (Table 4.2) would tend to indicate that 2 mM nitrate was not at a saturation level for nitrate feeding. Hydroponically grown plants of Helianthus annuus have been shown to possess the ability to accumulate nitrate ions from the external medium for loading onto the xylem stream when fed nitrate as the only nitrogen source (Kaiser and Lewis, 1980). It has also been demonstrated by Kaiser and Lewis (1980) that the roots of the sunflower have the ability to assimilate inorganic nitrogen for loading onto the xylem

stream and that this organic nitrogen is mainly in the form of glutamine. Many crop plants have been shown to transport high levels of both nitrate and organic nitrogen in the xylem sap (Pate, 1971). With ammonium as the sole nitrogen source (Figure 4.3), 99% at the 4 hour harvest and 98% at the 8 hour harvest of the ^{15}N content in the xylem sap was found in the free amino compound fraction. No detectable nitrate was found and only 1% at the 4 hour harvest and 2% at the 8 hour harvest of the ^{15}N was in the form of ammonium. The concentration of ammonium exported is well below the 2 mM concentration at which ammonia uncouples photosynthetic phosphorylation (Good, 1960). Maize (Murphy, 1985), barley (Lewis and Chadwick, 1983), Citrus trees (Kato, 1981) and rice (Muhammad and Kumazawa, 1974a) have been shown to export very little ammonia via the xylem stream when hydroponically fed ammonium as the solitary nitrogen source. This virtual lack of ammonium and very high concentration of free amino compounds in the sunflower xylem sap indicates that most of the absorbed ammonium is assimilated into organic nitrogenous compounds in the roots. The lack of nitrate in the xylem sap due to the presence of nitrifying bacteria could be attributed to the presence of the nitrification inhibitor nitrapyrin in the hydroponic solution (Chapter 2, Section 2.1), or due to the frequent changing of the hydroponic solutions as was shown for maize (Murphy, 1985). The free amino compound fraction of the

TABLE 4.2

^{15}N content of xylem sap components from sunflowers fed for 4 hours and 8 hours with ^{15}N-nitrate, ^{15}N-ammonium, ^{15}N-nitrate ^{15}N-ammonium, ^{15}N-ammonium ^{14}N-nitrate or ^{14}N-ammonium ^{15}N-nitrate. Values are the average from duplicate plants and are expressed as µg ^{15}N per ml ± range.

N Source	Harvest	Free Amino Nitrogen	NO_3	NH_4
2 mM ^{15}N-NO_3	4 h	31.0 ± 2.0	96.0 ± 3.0	0.1 ± 0.01
	8 h	64.5 ± 2.3	172.5 ± 3.7	0.1 ± 0.01
2 mM ^{15}N-NH_4	4 h	101.1 ± 4.1	0	1.0 ± 0.01
	8 h	189.3 ± 3.3	0	3.9 ± 0.03
Combined 1 mM ^{15}N-NO_3 1 mM ^{15}N-NH_4	4 h	119.2 ± 4.2	47.0 ± 2.1	2.2 ± 0.02
	8 h	201.9 ± 2.3	94.4 ± 3.0	4.1 ± 0.05
Combined 1 mM ^{15}N-NH_4 1 mM ^{14}N-NO_3	4 h	74.0 ± 4.3	0	1.9 ± 0.04
	8 h	161.0 ± 5.0	0	4.3 ± 0.10
Combined 1 mM ^{14}N-NH_4 1 mM ^{15}N-NO_3	4 h	12.3 ± 0.1	45.1 ± 1.8	0.1 ± 0.03
	8 h	23.7 ± 1.1	93.8 ± 2.7	0.3 ± 0.05



MIX1- 1mM $^{15}NO_3^-$ +1mM $^{15}NH_4^+$
MIX2- 1mM $^{14}NO_3^-$ +1mM $^{15}NH_4^+$
MIX3- 1mM $^{15}NO_3^-$ +1mM $^{14}NH_4^+$

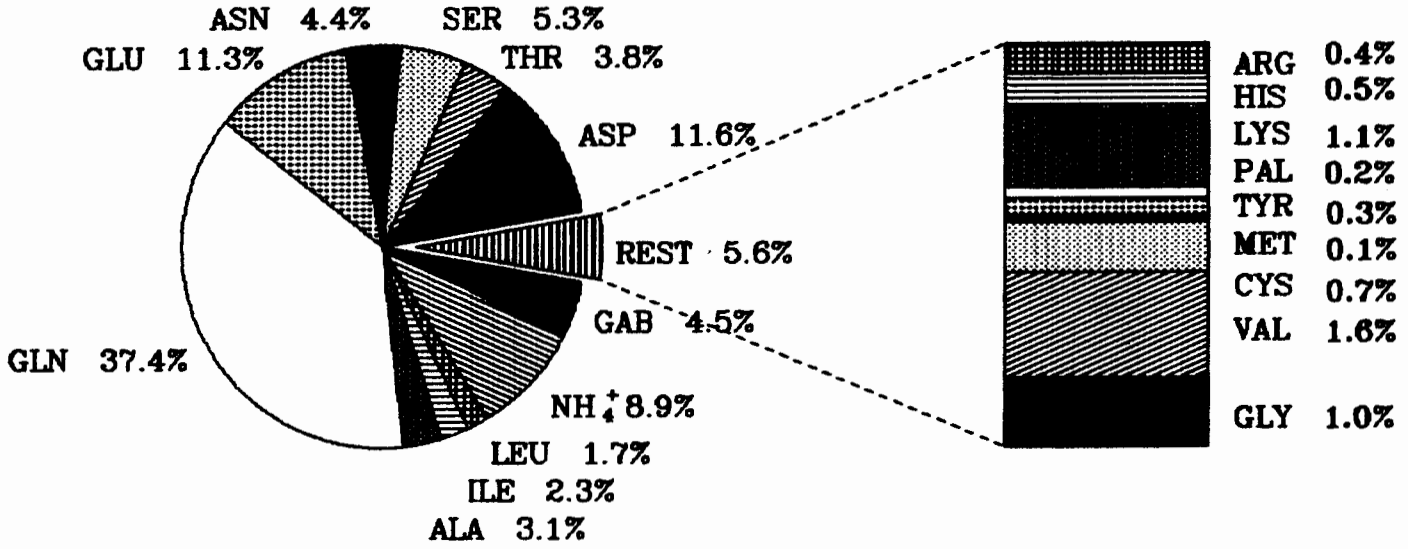
FIGURE 4.3
Percentage distribution ^{15}N between the major nitrogen fractions (free amino + amido, nitrate, ammonium) of the xylem sap of 4 week old *Helianthus annuus* plants fed different nitrogen sources.

ammonium-only fed plants (Figure 4.1) was approximately three times higher at both the 4 hour and the 8 hour harvests than that found in the xylem of the nitrate fed plants. Glutamine was the main organic form of nitrogen transported to the shoot in sunflower plants fed ammonium as the single nitrogen source (Figures 4.2, 4.4a, 4.4b and 4.4c).

The xylem sap of the combined ^{15}N-nitrate + ^{15}N-ammonium fed plants exhibited an approximate doubling of the ^{15}N label from the 4 hour harvest to the 8 hour harvest for all three fractions (Table 4.2). In spite of this, the relative amounts of the total ^{15}N transported remained the same from the 4 to 8 hour harvest for the three fractions with 1% as ammonium, 69% as free amino compounds and 30% as nitrate.

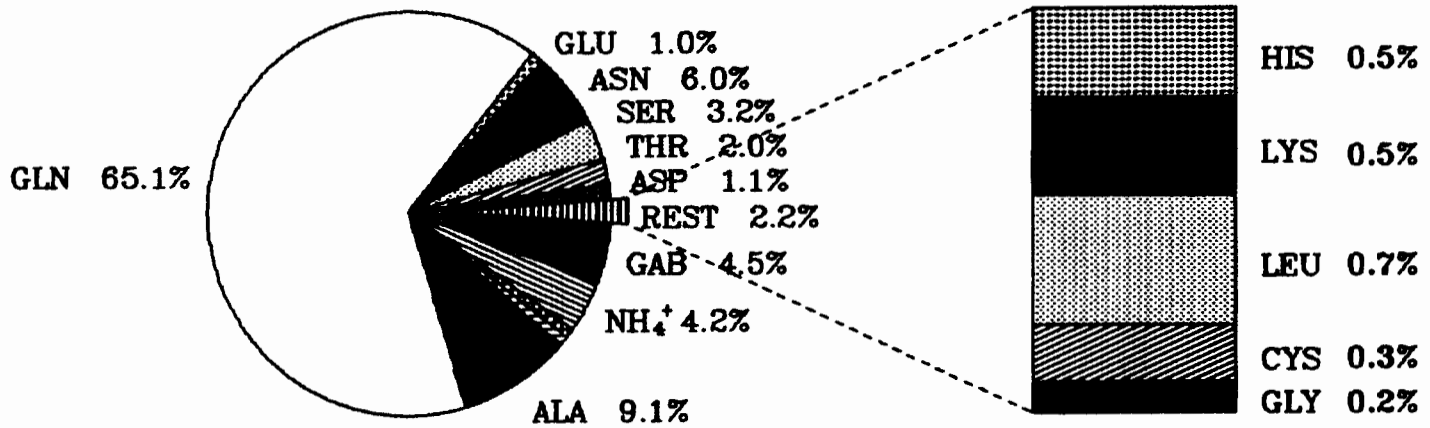
The labelling of only one nitrogen component of the ammonium nitrate with ^{15}N gave a better estimation of the contribution of each nitrogen ion from the mixed ammonium + nitrate nitrogen nutrient solution.

There was no detectable ^{15}N-nitrate in the combined 1 mM ^{15}N-ammonium plus 1 mM ^{14}N-nitrate fed plant sap at either the 4 hour harvest or the 8 hour harvest (Table 4.2). Although both the free amino compound and the ammonium



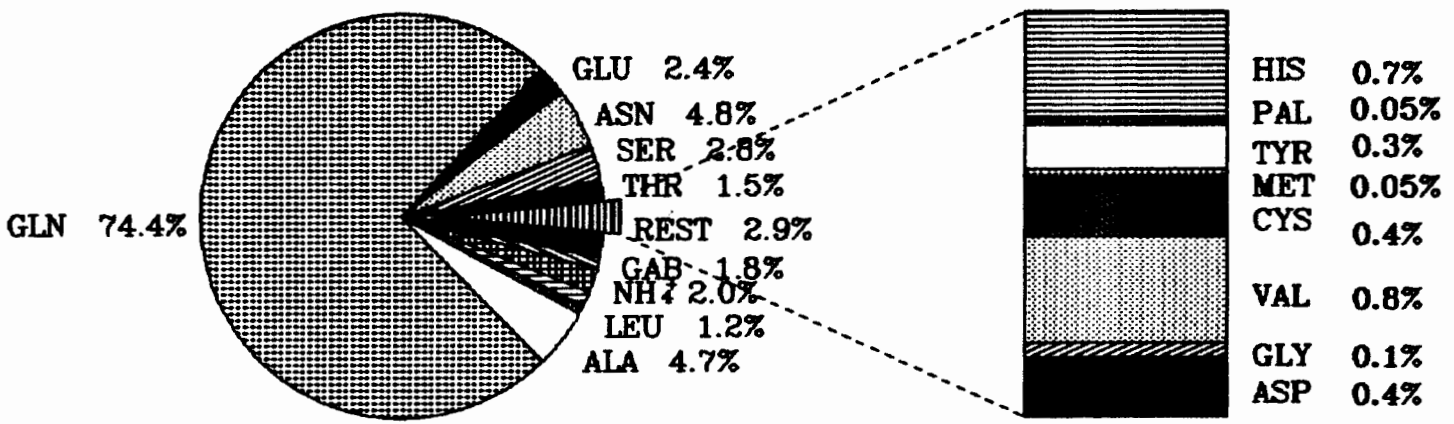
PRO WAS DETECTED ONLY IN TRACE AMOUNTS

FIGURE 4.4a
Spectrum of amino compounds found in the xylem sap of 4 week old *Helianthus annuus* plants fed 2 mM nitrate.



THE FOLLOWING WERE ONLY IN TRACE AMOUNTS
PRO, MET, TYR & PAL

FIGURE 4.4b
Spectrum of amino compounds found in the xylem sap of 4 week old *Helianthus annuus* plants fed 1 mM ammonium + 1 mM nitrate.



THE FOLLOWING WERE IN TRACE AMOUNTS ONLY
PRO & ARG

FIGURE 4.4c
Spectrum of amino compounds found in the xylem sap of 4 week old *Helianthus annuus* plants fed 2 mM ammonium.

fractions displayed a doubling of ^{15}N from the 4 hour harvest to the 8 hour harvest, the percentage of the total ^{15}N transported as free amino compounds and as ammonium, remained constant at 97% and 3% respectively (Figure 4.3).

The ^{15}N content of the ammonium fraction from the 1 mM ^{15}N-nitrate plus 1 mM ^{14}N-ammonium combined nitrogen fed plants trebled from the 4 hour to 8 hour harvest (Table 4.2). This, however, represented less than 1% of the total ^{15}N content in the xylem sap at either the 4 hour or the 8 hour harvest. The free amino compound and nitrate ^{15}N percentage concentration (Figure 4.3) remained constant from the 4 hour harvest to the 8 hour harvest at 20% and 79% respectively.

Sunflowers fed 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$ (Figure 4.1) exhibited 3 to 4 times greater levels of free amino compounds in the xylem sap than the nitrate-only fed plants. This indicates that the ammonium portion of the mixed nitrogen source was the major contributor to the free amino compounds in the xylem sap. This has been shown to an even greater extent with maize xylem sap where the ammonium contribution to the free amino compounds was 9 times that of the nitrate (Murphy, 1985).

The implications of these findings are: (a) nitrate is primarily exported to the shoot for assimilation, (b) nitrate produces plants with larger root and fruit mass than ammonium-only fed plants, (c) ammonium, newly taken up by the roots, is almost exclusively assimilated in the roots, (d) ammonium produces plants with larger shoots than nitrate-only fed plants, (e) ammonium + nitrate fed plants appear to combine features (a) and (c) assimilating ammonium in the root and nitrate in the shoot, and features (b) and (d) producing plants with larger root, shoot and fruit mass than either nitrogen source alone.

CHAPTER 5

THE INFLUENCE OF NITROGEN SOURCE ON THE DISTRIBUTION OF NITROGEN IN 4 WEEK OLD SUNFLOWER PLANTS.

5.1 Introduction

Most plants are able to utilize both ammonium and nitrate as nitrogen sources, with assimilation taking place in either or both the leaf and root. The relative contribution of roots and leaves to the assimilation of nitrogen in intact plants varies between plant species (Oghoghorie and Pate, 1972; Pate, 1973) and between nitrogen sources for a single species (Lewis and Chadwick, 1983; Kato, 1980; Ikeda and Yamada, 1978; Cox and Reisenauer, 1973). Ammonium has been shown to produce plants with a larger shoot and smaller root system than nitrate fed plants whereas a combination of the two nitrogen sources produces plants with a larger root system and shoot than either of the single nitrogen sources (Chapter 3, Section 3.3.1). Nitrogen concentration has been found to be greater in the leaves of sunflower plants fed NO_3^- + NH_4^+ than in plants fed either of the nitrogen sources singly (Weissman, 1964). With NO_3^- + NH_4^+ feeding, there appears to be an ammonium-induced inhibition of nitrate utilization/uptake which decreases as the concentration of ammonium in solution decreases (Fuggi *et al*, 1981) (Chapter 4, Section 4.3).

The present work examines the incorporation of $^{15}NO_3^-$, $^{15}NH_4^+$ and $^{15}NO_3^-$ + $^{15}NH_4^+$ into the root, stem plus petiole, leaf and shoot apex of young sunflower plants to ascertain where in the plant the different nitrogen sources are being assimilated and utilized.

5.2 Experimental Methods

Five sets of four plants each were fed with 2mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$, 1 mM $^{15}NO_3^-$ + 1 mM $^{15}NH_4^+$, 1 mM $^{15}NO_3^-$ + 1 mM $^{14}NH_4^+$ or 1 mM $^{14}NO_3^-$ + 1 mM $^{15}NH_4^+$ respectively and harvested after 4 and 8 hours as described in Chapter 2, Sections 2.2 and 2.3. The extraction of free amino nitrogen, bound nitrogen, nitrate and/or ammonium fractions was carried out as described in Chapter 2, Section 2.3 and prepared for ^{15}N determinations as described in Chapter 2, Section 2.4. Analyses were performed to determine the ^{15}N content of the following separate plant components: root, stem plus petiole, leaf and shoot apex. Ammonium was not determined separately for the nitrate-only fed plants or for the $^{15}NO_3^-$ + $^{14}NH_4^+$ fed plants since it was considered that labelled ammonium would have originated from the reduction of nitrate and should be considered as a component of the free amino fraction. Tests for ammonium contamination of nitrate-only feeding solutions were carried out on both new and old solutions at each nutrient solution change using Nessler's Reagent as described in Chapter 2, Section 2.8.3. Nitrate determinations for each plant fraction and the hydroponic

feeding solutions of the ammonium-only fed plants were carried out using Szechrome NAS Reagent as described in Chapter 2, Section 2.8.2. Since no nitrate was detected, no further nitrate determinations were performed for the ammonium-only fed plants.

5.3 Results and Discussion

5.3.1 2 mM $^{15}NO_3^-$ Nutrient Feeding

As can be seen from Table 5.1 the assimilated ^{15}N enrichment (A%E) values for plant material from plants fed $^{15}NO_3^-$ range from 1.1 A%E in the bound fractions of both stem plus petiole and leaf at the 4 hour harvest, to 22.3 A%E in the free amino fraction of the leaf at the 8 hour harvest. The ^{15}N enrichment of nitrate and free amino fractions at the 8 hour harvest were double the 4 hour harvest values indicating that the assimilatory nitrogen pools did not become saturated with ^{15}N within the time range of the experiment. Although the root exhibited the highest enrichment value of 46.7 A%E for $^{15}NO_3^-$ for the 8 hour harvest, Table 5.1 shows that the $^{15}NO_3^-$ content was greater in the shoot (1675 µg $^{15}NO_3^-$ at 4 hours; 5444 µg $^{15}NO_3^-$ at 8 hours) than in the root (1520 µg $^{15}NO_3^-$ at 4 hours; 3205 µg $^{15}NO_3^-$ at 8 hours) in the nitrate-only fed plants. This shows that although the $^{15}NO_3^-$ pool of the root doubled from the 4 hour harvest to the 8 hour harvest, much more of the $^{15}NO_3^-$ was transported to the shoot than was retained in the root over the time interval.

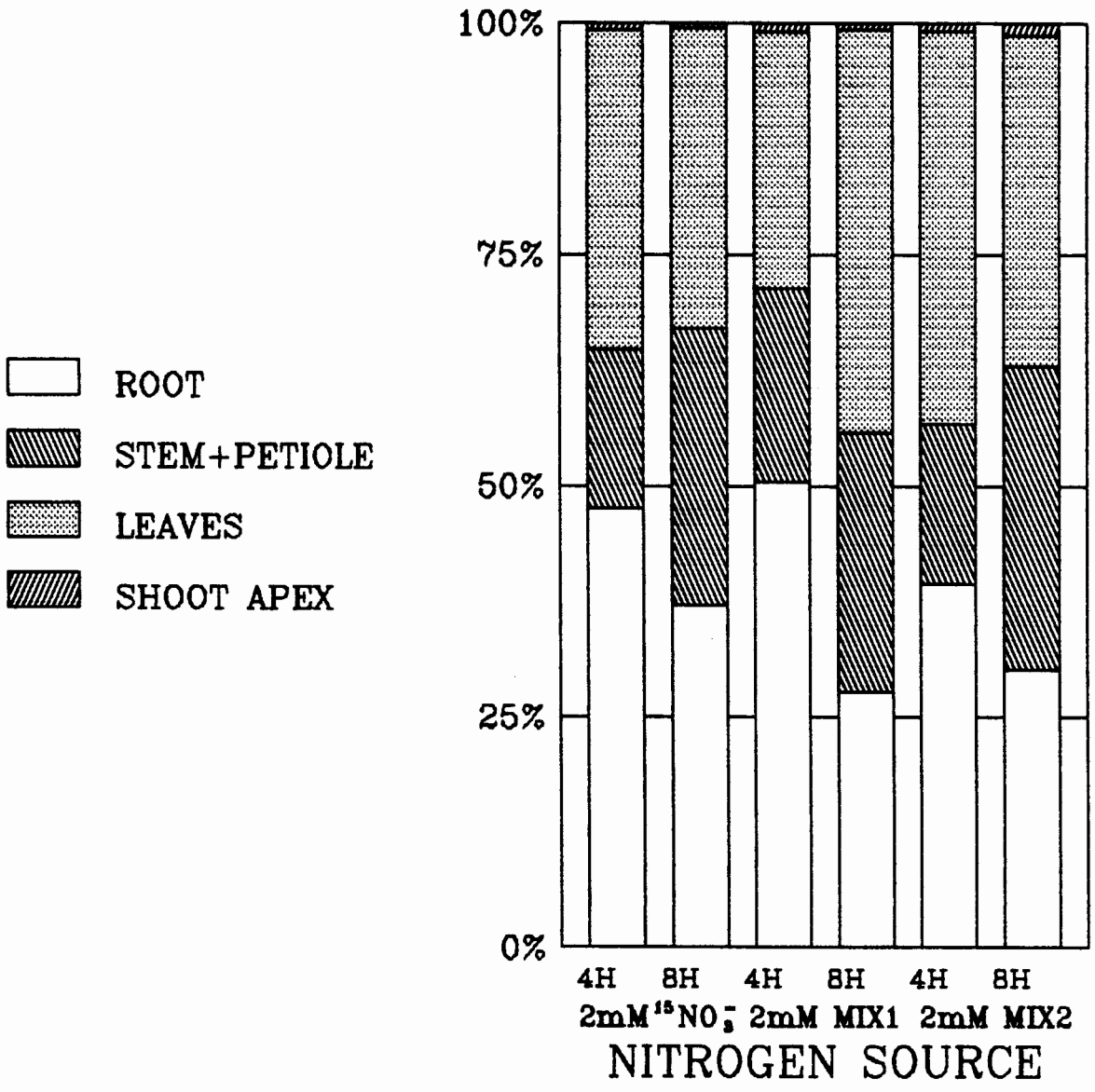
TABLE 5.1

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of nitrate-N, free amino compound-N and bound-N fractions of root, stem + petiole, leaf and shoot apex of 4 week old sunflower plants fed 2 mM $^{15}NO_3^-$ and harvested 4 h and 8 h after commencement of ^{15}N feeding. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

N Fraction	Harvest	Root			Stem + Petiole			Leaf			Shoot Apex			Total for Shoot
		ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug ^{15}N Plant^{-1}
Nitrate	4 h	6762.3 ±270.5	22.3 ±0.0	1519.6 ± 48.7	7913.9 ±387.8	6.9 ±0.0	546.1 ±26.7	10340.1 ± 341.2	10.7 ±0.1	1106.4 ± 36.9	193.8 ±13.6	11.4 ±0.1	22.1 ±1.6	1674.6 ± 65.2
	8 h	6862.9 ±240.2	46.7 ±0.3	3205.0 ±133.5	7783.1 ±358.0	33.2 ±0.4	2584.0 ±120.3	11126.5 ± 333.8	25.3 ±0.2	2815.0 ± 85.1	199.1 ±11.9	27.0 ±0.3	45.2 ±3.3	5444.2 ±208.7
Free Amino Compounds	4 h	19840.5 ± 396.8	8.4 ±0.0	1666.7 ± 33.2	11620.4 ± 267.3	10.8 ±0.0	1255.1 ± 28.9	30630.6 ± 673.9	10.1 ±0.1	3093.7 ± 68.7	1588.2 ± 44.5	6.8 ±0.0	108.0 ± 3.0	4456.7 ±100.6
	8 h	20685.9 ± 372.3	15.6 ±0.1	3227.1 ± 79.1	10394.2 ± 343.0	20.8 ±0.1	2162.0 ± 71.7	31116.6 ± 653.4	22.3 ±0.2	6939.0 ±147.0	1570.3 ± 45.5	12.8 ±0.1	201.0 ± 5.9	9302.0 ±224.6
Bound Nitrogen	4 h	80200.9 ±2406.0	2.0 ±0.0	1604.0 ± 48.1	16984.7 ±1036.1	1.1 ±0.0	168.8 ±11.4	93597.2 ±3275.9	1.1 ±0.0	1029.6 ± 36.0	3298.1 ±197.9	1.5 ±0.0	49.5 ±3.0	1247.9 ± 50.4
	8 h	84536.6 ±2620.6	4.1 ±0.0	3466.1 ±107.4	17481.5 ±1048.9	2.7 ±0.0	472.0 ±28.3	95875.0 ±2780.4	2.4 ±0.0	2301.0 ± 66.7	3371.4 ±134.9	3.5 ±0.0	118.0 ± 4.7	2891.0 ± 99.7

The shoot apex received very little $^{15}NO_3^-$ at either the 4 hour or 8 hour harvest (Table 5.1). The percentage distribution of $^{15}NO_3^-$ (Figure 5.1) in the root decreased from the 4 hour harvest to the 8 hour harvest, but increased in the shoot over the same time interval, indicating a movement of $^{15}NO_3^-$ from the root to the shoot which is the site where nitrate reductase is most active in Helianthus annuus (Kaiser and Lewis, 1984). These results agree with those of Muhammad and Kumazawa (1974b) who showed that the majority of the nitrate nitrogen is reduced in the leaves of rice plants.

Table 5.1 shows that the ^{15}N content of the free amino pool of the shoot was 2.5 times greater than that of the root at the 4 hour harvest and 2.9 times greater at the 8 hour harvest. For all of the different plant parts harvested the ^{15}N concentration in the free amino fraction was highest in the leaf (6939 µg ^{15}N) for the 8 hour harvest. These results indicate that the shoot, and in particular the leaf, was the major site of nitrate reduction. The bound ^{15}N content shows a slightly greater incorporation of ^{15}N in the root than the shoot at both the 4 hour harvest and the 8 hour harvest indicating that the root was active in nitrogen storage.



MIX1- 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$

MIX2- 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$

FIGURE 5.1
Percentage distribution of $^{15}NO_3^-$ in Helianthus annuus plants fed 2 mM $^{15}NO_3^-$, 1mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$ and 1 mM $^{15}NO_3^-$ + 1 mM $^{14}NH_4^+$ for 4 h and 8 h.

The results in Table 5.6 show that the rate of ^{15}N assimilation into organic compounds from $^{15}NO_3^-$ increased only slightly from the 4 hour to the 8 hour harvest. This indicates that the nutrient supply to the plants was not depleted during the time course of the experiment.

5.3.2 2 mM $^{15}NH_4^+$ Nutrient Feeding

The ^{15}N enrichment values for the ammonium of the root (Table 5.2) indicates a very rapid uptake of ammonium compared to nitrate (Table 5.1) with an apparent saturation of the assimilatory ammonium pool with ^{15}N at the 4 hour harvest as evidenced by the lack of increase of ^{15}N enrichment of this pool from the 4 hour to the 8 hour harvest.

Although the S:R quotient of the free amino ^{15}N only increased from 1 to 1.4 from the 4 hour harvest to the 8 hour harvest, the free amino ^{15}N content of the shoot increased 1.7 times between harvests, indicating an increasing diversion of newly assimilated ^{15}N to the shoot with time. The bound ^{15}N S:R quotient changed from 1.6 at the 4 hour harvest to 2.6 at the 8 hour harvest, indicating a greater accumulation and storage of newly incorporated nitrogen in the shoot than the root in the ammonium-only fed plants.

TABLE 5.2

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of ammonium-N, free amino compound-N and bound-N fractions of root, stem + petiole, leaf and shoot apex of 4 week old sunflower plants fed 2 mM $^{15}NH_4^+$ and harvested 4 h and 8 h after commencement of ^{15}N feeding. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

N Fraction	Harvest	Root			Stem + Petiole			Leaf			Shoot Apex			Total for Shoot
		ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug ^{15}N Plant^{-1}
Ammonium	4 h	8573.2 ±523.0	69.8 ±3.1	5984.1 ±377.1	1479.0 ± 90.2	31.0 ±1.0	458.5 ±28.9	2847.4 ±170.8	29.3 ±2.0	834.3 ±53.5	514.0 ±32.4	15.7 ±1.0	80.7 ±5.4	1373.5 ± 87.8
	8 h	8710.4 ±513.9	51.8 ±2.4	4512.0 ±278.5	1332.0 ± 81.3	33.7 ±1.1	448.9 ±28.3	2937.6 ±176.3	39.6 ±1.8	1163.3 ± 73.0	464.8 ±28.8	18.7 ±0.9	86.9 ±5.6	1699.1 ±106.9
Free Amino Compounds	4 h	16798.1 ± 520.7	28.8 ±1.1	4837.9 ±155.7	14123.6 ± 423.6	20.6 ±1.1	2909.5 ± 91.9	7413.2 ±173.0	26.6 ±0.9	1971.9 ± 47.6	1052.9 ± 32.5	5.1 ±0.4	53.7 ±1.8	4935.1 ±141.3
	8 h	16365.6 ± 545.5	37.2 ±1.3	6088.0 ±210.0	13845.1 ± 427.8	41.0 ±1.3	5676.5 ±180.9	7308.8 ±170.5	35.3 ±1.3	2580.0 ± 62.4	1119.0 ± 26.1	14.7 ±0.3	164.5 ± 3.9	8421.0 ±247.2
Bound Nitrogen	4 h	90391.3 ±3615.7	2.3 ±0.1	2079.0 ± 86.8	89746.2 ±3141.1	2.4 ±0.0	2153.9 ± 75.4	54916.3 ±2141.7	1.7 ±0.0	933.6 ±36.4	8913.7 ±356.5	2.7 ±0.0	240.7 ± 9.6	3328.2 ±121.4
	8 h	86171.4 ±3533.0	3.5 ±0.2	3016.0 ±130.7	90000.0 ±3519.1	4.7 ±0.2	4230.0 ±172.4	56159.1 ±2134.0	4.4 ±0.2	2471.0 ± 98.2	9058.1 ±362.3	8.6 ±0.2	779.0 ±31.9	7480.0 ±302.5

From the results shown in Table 5.6 it can be seen that the rate of $^{15}NH_4^+$ assimilation into organic compounds decreased from the 4 hour harvest to the 8 hour harvest indicating that the ammonium nutrient supply at the 8 hour harvest was becoming depleted. The ammonium-only fed plants assimilated nitrogen nearly twice as fast as the nitrate-only fed plants, based on the respective ^{15}N contents of the plants at the 4 hour harvest.

5.3.3 1 mM $^{15}NO_3^-$ + 1 mM $^{14}NH_4^+$, 1 mM $^{15}NO_3^-$ + 1 mM $^{15}NH_4^+$ and 1 mM $^{14}NO_3^-$ + 1 mM $^{15}NH_4^+$ Feeding

The results shown in Tables 5.3 ($^{15}NO_3^-$ + $^{15}NH_4^+$ fed plants) and 5.4 ($^{15}NO_3^-$ + $^{14}NH_4^+$ fed plants) show that the ^{15}N enrichment of both the root and shoot nitrate pools doubled from the 4 hour harvest to the 8 hour harvest, indicating, as was shown for the nitrate-only fed plants (Table 5.1), that the $^{15}NO_3^-$ assimilatory pools of both the root and the shoot were not at saturation levels in either feeding experiment.

From Tables 5.3 ($^{15}NO_3^-$ + $^{15}NH_4^+$ fed plants), 5.4 ($^{15}NO_3^-$ + $^{14}NH_4^+$ fed plants) and 5.5 ($^{14}NO_3^-$ + $^{15}NH_4^+$ fed plants) it can be seen that the major contributor to the free amino ^{15}N content in the root was the ammonium ion at both the 4 and 8 hour harvest. A comparison of the tables show that a major

TABLE 5.3

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of nitrate, ammonium-N, free amino compound-N and bound-N fractions of root, stem + petiole, leaf and shoot apex of 4 week old sunflower plants fed 1 mM $^{15}NO_3^-$ + 1 mM $^{15}NH_4^+$ and harvested 4 h and 8 h after commencement of ^{15}N feeding. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

N Fraction	Harvest	Root			Stem + Petiole			Leaf			Shoot Apex			Total for Shoot
		ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug ^{15}N Plant^{-1}
Nitrate	4 h	3824.1 ±233.7	21.6 ±0.9	826.0 ±52.6	6071.4 ±370.3	5.6 ±0.5	340.0 ±22.6	3864.4 ±189.7	11.8 ±0.9	456.0 ±24.1	135.9 ± 6.8	10.3 ±0.8	14.0 ±0.8	810.0 ±47.5
	8 h	3561.5 ±213.7	44.7 ±1.4	1592.0 ± 98.5	6777.3 ±384.0	23.8 ±1.7	1613.0 ± 98.7	3506.9 ±207.2	21.7 ±1.0	2497.0 ± 47.0	128.0 ± 6.9	25.4 ±1.2	32.5 ±1.8	3842.0 ±147.5
Ammonium	4 h	11302.1 ± 691.6	87.3 ±3.1	9866.7 ±625.2	1821.0 ±105.8	22.9 ±1.1	188.0 ±25.4	3871.4 ±231.9	14.0 ±0.8	542.0 ±34.3	235.3 ±11.7	6.8 ±0.4	16.0 ±0.8	1046.0 ± 60.5
	8 h	10971.8 ± 638.4	85.1 ±2.7	9337.0 ±560.6	1617.6 ± 98.7	27.2 ±1.0	440.0 ±27.8	4157.5 ±207.0	36.2 ±1.8	1505.0 ± 78.7	260.9 ±15.7	23.0 ±0.9	60.0 ±3.7	2005.0 ±110.2
Free Amino Compounds	4 h	15096.2 ± 357.6	34.2 ±1.0	5162.9 ±125.9	16748.4 ± 484.0	15.5 ±0.5	2596.0 ± 77.4	36173.5 ±1128.6	31.0 ±1.0	11213.8 ± 361.2	1417.1 ± 43.9	17.5 ±1.0	248.0 ± 8.1	14057.8 ± 446.7
	8 h	16099.7 ± 497.5	41.1 ±0.9	6616.9 ±208.9	15543.1 ± 439.9	34.6 ±0.7	5377.9 ±155.3	35758.8 ± 961.9	39.8 ±0.9	14232.0 ± 391.5	1772.7 ± 46.3	30.8 ±0.9	546.0 ±14.7	20155.9 ± 561.4
Bound Nitrogen	4 h	94230.8 ±3298.1	2.9 ±0.1	2732.7 ±98.9	19866.7 ±1433.0	2.1 ±0.0	523.0 ±30.1	143158.1 ± 5707.7	2.4 ±0.1	2434.7 ±142.7	8213.2 ±337.6	1.5 ±0.0	123.2 ± 5.1	3080.9 ±177.8
	8 h	96227.8 ±3233.3	7.8 ±0.3	7505.8 ±261.9	18918.9 ±1247.3	6.1 ±0.2	2008.1 ± 78.6	135142.9 ± 5120.6	5.6 ±0.2	7568.0 ±297.0	8095.4 ±249.3	9.7 ±0.4	785.3 ±25.2	10361.4 ± 400.8

TABLE 5.4

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of nitrate, free amino compound-N and bound-N fractions of root, stem + petiole, leaf and shoot apex of 4 week old sunflower plants fed 1 mM $^{15}NO_3^-$ + 1 mM $^{14}NH_4^+$ and harvested 4 h and 8 h after commencement of ^{15}N feeding. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

N Fraction	Harvest	Root			Stem + Petiole			Leaf			Shoot Apex			Total for Shoot
		ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug ^{15}N Plant^{-1}
Nitrate	4 h	3437.2 ±189.0	23.1 ±1.2	794.0 ±45.9	6960.8 ±417.6	5.0 ±0.2	348.0 ±21.7	7962.6 ±477.8	10.7 ±0.8	852.0 ±54.9	346.7 ±20.8	4.7 ±0.3	16.3 ±1.0	1216.3 ± 77.6
	8 h	3345.5 ±160.6	44.0 ±1.8	1472.0 ± 73.5	7148.0 ±285.9	22.3 ±0.9	1594.0 ± 66.3	8668.3 ±502.8	20.2 ±0.7	1751.0 ±105.1	384.6 ±22.3	15.6 ±0.4	60.1 ±3.6	3405.1 ±175.0
Free Amino Compounds	4 h	18031.7 ± 450.8	4.8 ±0.2	865.5 ±22.5	11701.9 ± 339.4	8.7 ±0.3	1018.1 ± 30.5	30171.4 ± 844.8	7.4 ±0.1	2232.7 ± 63.4	1853.8 ± 48.2	3.2 ±0.1	59.3 ±1.6	3310.1 ± 95.5
	8 h	19869.2 ± 417.3	9.9 ±0.3	1967.1 ± 42.6	12967.9 ± 259.4	18.7 ±0.5	2425.0 ± 49.8	35000.0 ± 770.0	16.7 ±0.2	5845.0 ±130.1	1926.6 ± 38.5	10.9 ±0.3	210.0 ± 4.3	8480.0 ±184.2
Bound Nitrogen	4 h	88461.6 ±1726.2	0.3 ±0.0	265.4 ± 5.2	18461.5 ± 174.0	0.5 ±0.0	92.3 ±0.9	126500.0 ± 4933.5	0.8 ±0.0	1012.0 ± 39.5	9615.4 ±471.2	0.3 ±0.0	28.8 ±1.4	1133.1 ± 41.8
	8 h	88125.0 ±1562.5	2.9 ±0.1	2555.6 ±135.0	17521.7 ± 473.3	2.3 ±0.1	403.0 ± 28.9	152714.3 ± 3054.3	1.4 ±0.1	2138.0 ± 45.8	9800.0 ±392.0	2.5 ±0.1	245.0 ±10.2	2786.0 ± 84.9

TABLE 5.5

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of ammonium, free amino compound-N and bound-N fractions of root, stem + petiole, leaf and shoot apex of 4 week old sunflower plants fed 1 mM $^{14}NO_3^-$ + 1 mM $^{15}NH_4^+$ and harvested 4 h and 8 h after commencement of ^{15}N feeding. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

N Fraction	Harvest	Root			Stem + Petiole			Leaf			Shoot Apex			Total for Shoot
		ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	ug ^{15}N Plant^{-1}	
Ammonium	4 h	10202.7 ± 816.2	84.6 ±1.1	8631.5 ±699.5	1442.7 ± 79.3	20.7 ±1.0	298.6 ±17.2	2987.1 ±119.5	13.2 ±0.9	394.3 ±16.8	139.0 ± 7.6	5.6 ±0.4	7.8 ±0.5	700.7 ±34.5
	8 h	10335.1 ± 723.5	81.4 ±0.9	8412.8 ±595.4	1495.8 ± 76.3	23.6 ±0.9	353.0 ±18.7	2936.5 ± 96.9	31.5 ±1.2	925.0 ±31.7	141.6 ± 7.1	11.3 ±0.7	16.0 ±0.8	1294.0 ± 51.2
Free Amino Compounds	4 h	11979.1 ± 335.4	32.1 ±0.8	3845.3 ±110.4	5829.3 ±163.2	16.4 ±0.7	956.0 ±27.9	26131.8 ± 784.9	21.3 ±1.1	5566.1 ±175.6	1023.3 ± 23.5	4.3 ±0.0	44.0 ±1.0	6566.1 ±204.5
	8 h	12342.9 ± 308.6	34.7 ±0.7	4283.0 ±109.2	5526.6 ±116.1	31.9 ±0.7	1763.0 ± 37.8	25473.1 ± 662.3	27.9 ±0.9	7107.0 ±190.7	1091.5 ± 21.8	14.2 ±0.1	155.0 ± 3.1	9025.0 ±231.6
Bound Nitrogen	4 h	86819.1 ±2170.5	2.7 ±0.0	2344.1 ± 58.6	18924.0 ± 757.0	1.7 ±0.0	321.7 ±12.9	155882.4 ± 7014.7	1.7 ±0.0	2650.0 ±119.3	8297.2 ±207.8	1.4 ±0.0	88.2 ±2.9	3059.9 ±135.1
	8 h	87791.7 ±2107.0	3.9 ±0.1	3423.9 ± 84.3	19384.6 ± 562.2	3.9 ±0.2	756.0 ±23.0	157513.1 ± 5197.9	3.4 ±0.0	5355.4 ±176.7	8372.1 ±159.3	6.8 ±0.2	433.3 ±11.2	6544.7 ±210.9

TABLE 5.6

Rates of ^{15}N assimilation into organic compounds by sunflower plants fed either 2mM $^{15}NO_3^-$, 2mM $^{15}NH_4^+$, 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$, 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$ or 1mM $^{14}NO_3^-$ + 1mM $^{15}NH_4^+$ and harvested at 4 and 8 hours. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

Nitrogen Source	μg ^{15}N plant^{-1} h^{-1} 4 h	8 h	Rate Change from 4h - 8h
2mM $^{15}NO_3^-$	2243.9 ± 58.1	2360.8 ± 63.8	1116.9 ± 5.7
2mM $^{15}NH_4^+$	3795.1 ±126.3	3034.1 ±111.3	-740.0 ±15.0
1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$	6258.6 ±212.3	5580.0 ±179.1	-659.6 ±33.2
1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$	1393.5 ± 41.3	1973.6 ± 55.8	599.1 ±14.5
1mM $^{14}NO_3^-$ + 1mM $^{15}NH_4^+$	3953.9 ±127.2	1454.8 ± 36.4	-2481.1 ± 90.8

portion of the free amino ^{15}N found in the shoot was derived from the ammonium source with an increasing contribution by the nitrate source from the 4 hour to the 8 hour harvest.

It is also evident that the ^{15}N content of the root of the $^{15}NO_3^-$ + $^{14}NH_4^+$ mixed feed plants (Table 5.4) at both the 4 and 8 hour harvests was approximately one half that of the root of the nitrate-only fed plants indicating a possible suppression of nitrate uptake by ammonium or due to the fact that the nitrate concentration of the feeding solution of the mixed feed plants was at one half of the nitrate-only fed plants.

From Table 5.6 it can be seen that the assimilation rate of $^{15}NO_3^-$ into organic compounds by sunflower plants fed 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$ increased by approximately 50% indicating a decrease in the inhibitory effect of ammonium upon the assimilation of nitrate at the 8 hour harvest. The $^{15}NH_4^+$ appears to be greatly depleted by the end of the 4 hour harvest in the 1 mM $^{14}NO_3^-$ + 1mM $^{15}NH_4^+$ fed plants as can be seen from the very high change in ^{15}N assimilation rate from the 4 hour to the 8 hour harvest.

5.3.4 Comparison of the Results of the Different Treatments

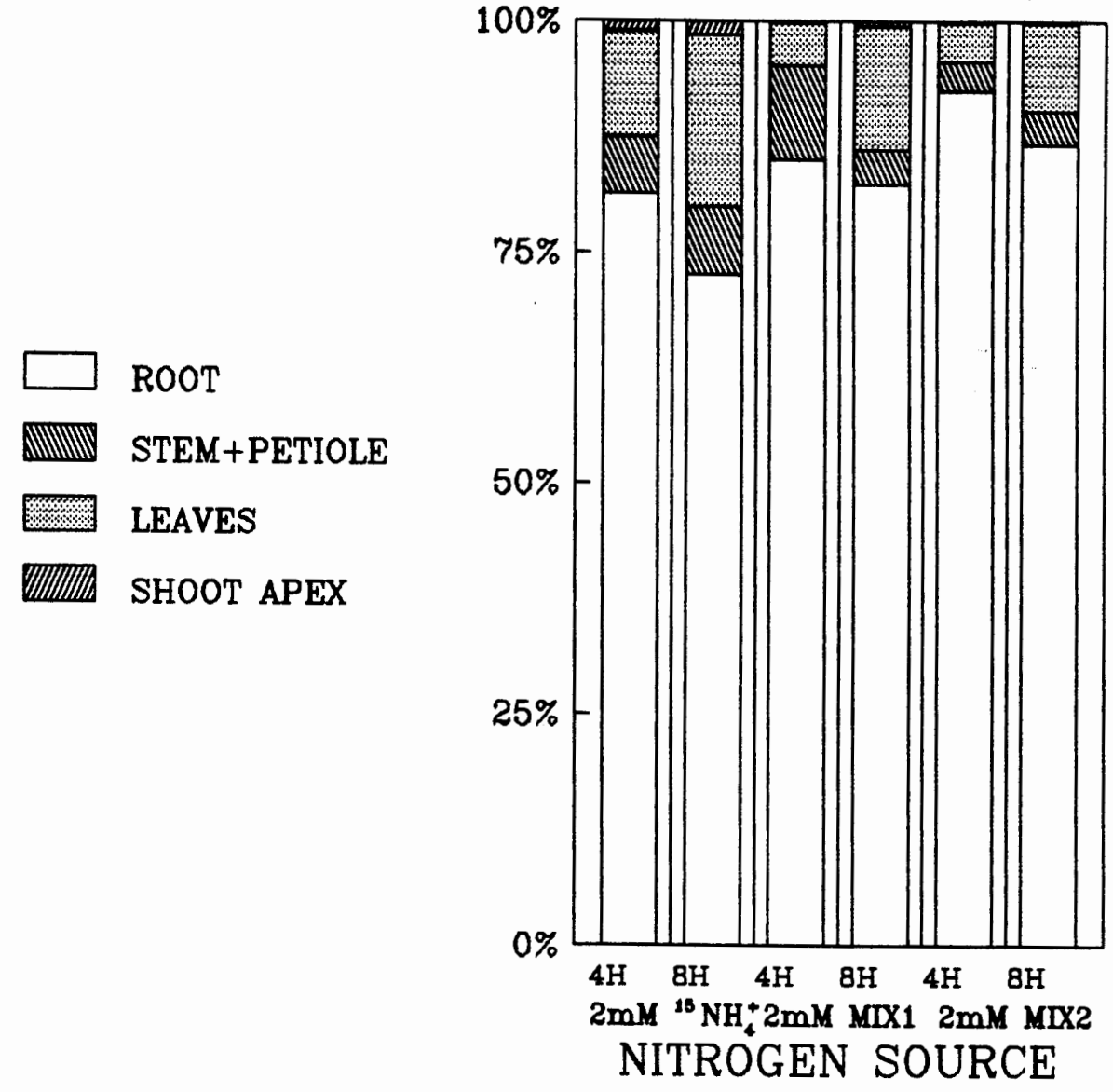
5.3.4.1 Inorganic ^{15}N

The distribution of $^{15}NH_4^+$ on a percentage basis in plants fed with $^{15}NH_4^+$ in the nutrient solution (Figure 5.2), shows that at both harvests over 70% of all of the $^{15}NH_4^+$ in the

plants was found in the root, indicating the root as the major site of ammonium assimilation in ammonium fed sunflower plants.

The decrease of ^{15}N enrichment from the 4 to the 8 hour harvest (Figure 5.3) in the $^{15}NH_4^+$-only fed plant roots, showed no change between harvests for the two mixed feed plant roots which indicated that a saturation of the $^{15}NH_4^+$ pool in the root of all three treatments had been reached by the 4 hour harvest.

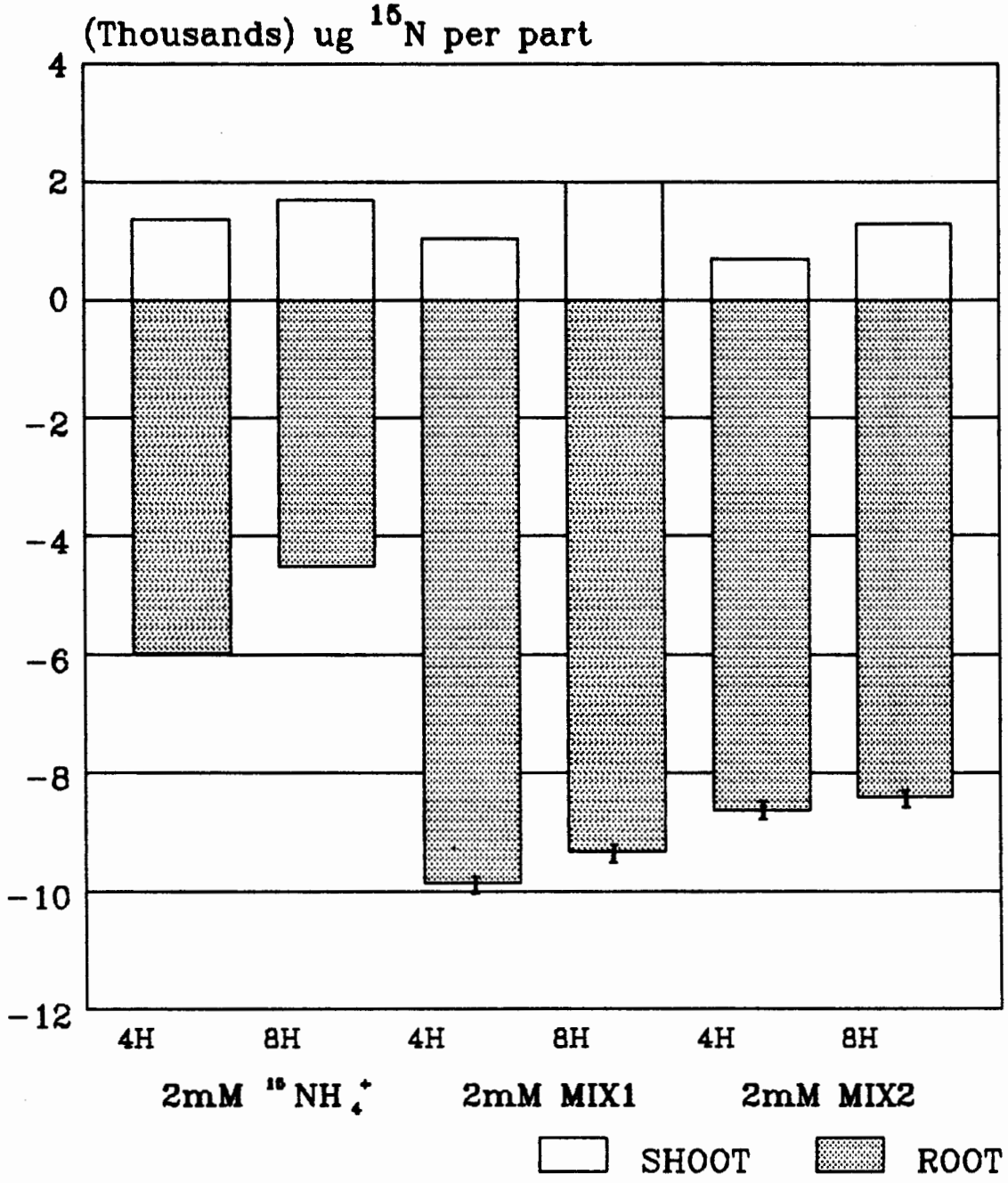
There was an overall greater ^{15}N enrichment of the nitrate (Figure 5.4) in the nitrate-only fed plants at both the 4 and 8 hour harvests than in either of the two mixed feed plants indicating that in the mixed feed plants there was a possible suppression of the nitrate uptake by the ammonium ion. Lewis, James and Hewitt, (1982a) also showed the suppression of nitrate uptake by the presence of ammonium in the nutrient solution in barley plants. This difference in $^{15}NO_3^-$ enrichment may also have been the result of the nitrate in the mixed feed plants being at one half the concentration of the nitrate of the nitrate-only fed plants.



MIX1- 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$

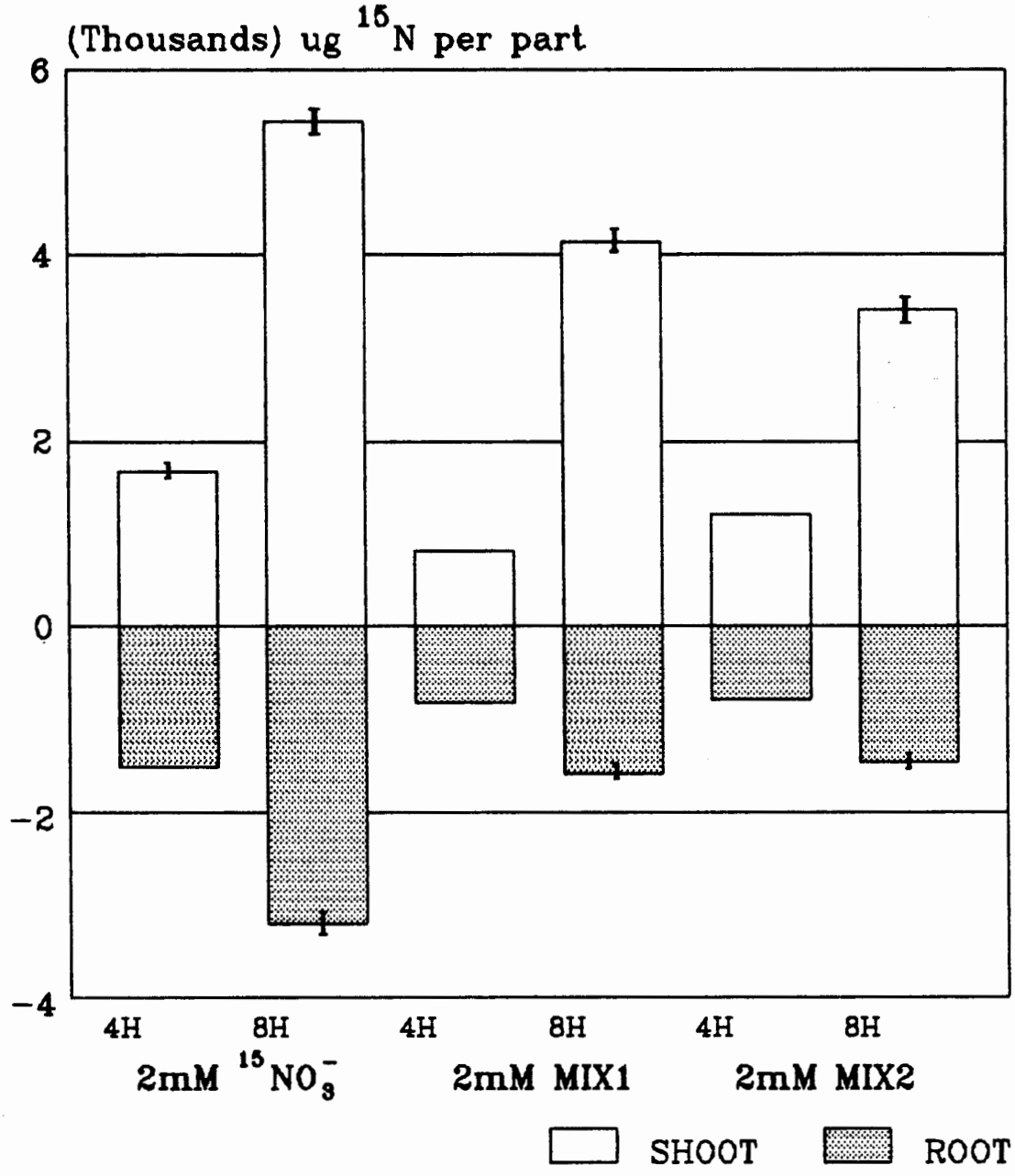
MIX2- 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$

FIGURE 5.2
Percentage distribution of $^{15}NH_4^+$ in *Helianthus annuus* plants fed 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$ and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.



MIX1- 1mM $^{15}NO_3^-$ +1mM $^{15}NH_4^+$
MIX2- 1mM $^{14}NO_3^-$ +1mM $^{15}NH_4^+$

FIGURE 5.3
$^{15}NH_4^+$ content of root and shoot of plants of Helianthus annuus fed 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$ and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.



MIX1 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX2 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$

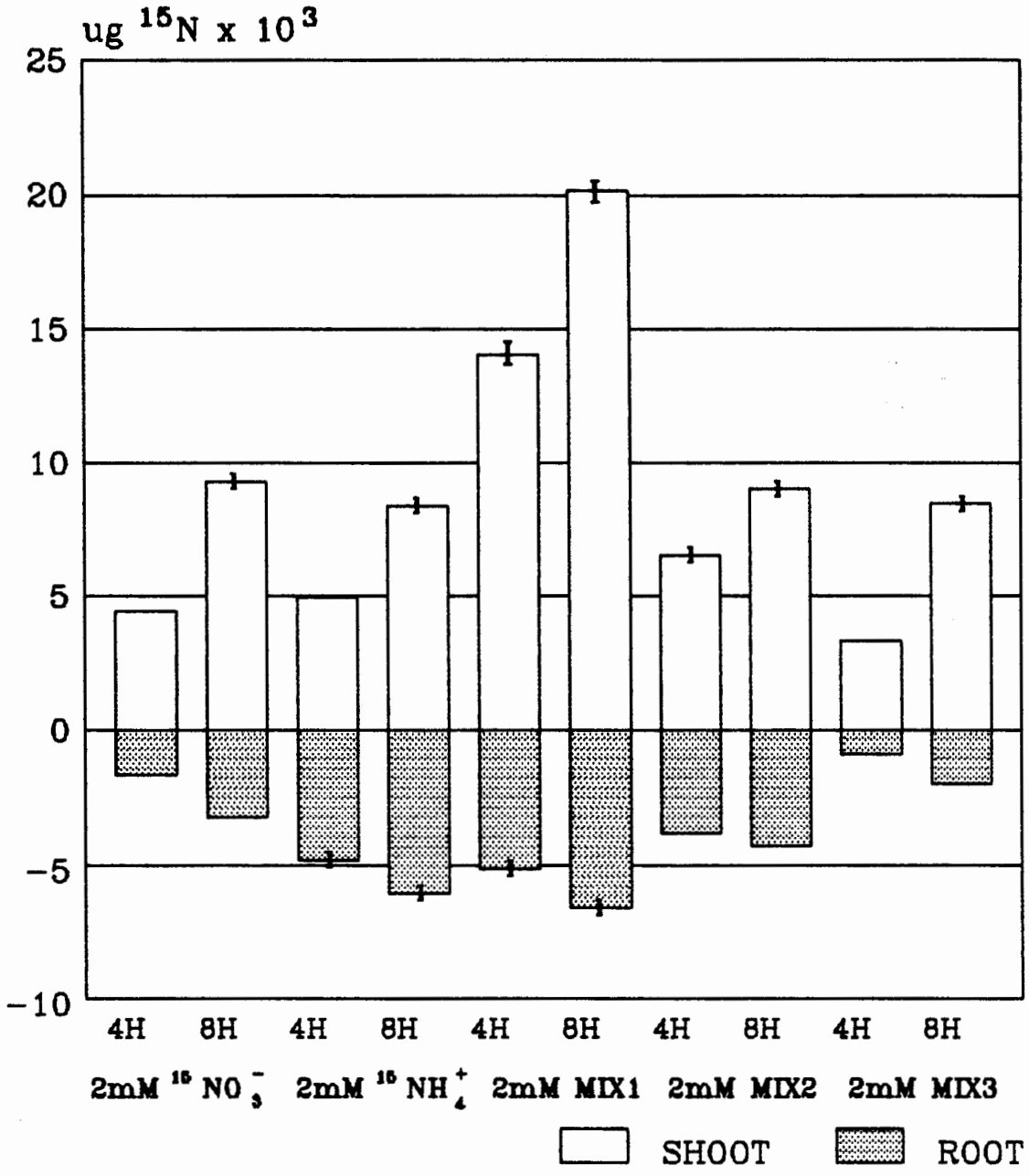
FIGURE 5.4
$^{15}NO_3^-$ content of root and shoot of plants of

The percentage distribution of $^{15}NO_3^-$ in $^{15}NO_3^-$ fed plants (Figure 5.1) shows that in all three of these experiments the percentage of ^{15}N in the root decreased from the 4 hour harvest to the 8 hour harvest, but increased in the shoot over the same interval indicating that $^{15}NO_3^-$ was accumulating in the shoot.

5.3.4.2 Free Amino ^{15}N

Figure 5.5 shows a comparison of the free amino ^{15}N content of sunflower plants fed separately with nitrate, ammonium and ammonium + nitrate. The ^{15}N content of $^{15}NO_3^-$ fed and the $^{15}NO_3^-$ + $^{14}NH_4^+$ fed roots doubled between the 4 and 8 hour harvests indicating either a continual nitrate reduction in the root or possibly that the assimilation products from nitrate which had been reduced in the leaves were returned via the phloem (Pate, 1983) to be used for root growth within 8 hours.

Figure 5.5 also shows that the free amino ^{15}N content of the shoot was the same at the 8 hour harvest for plants fed $^{15}NO_3^-$, $^{15}NH_4^+$, $^{15}NO_3^-$ + $^{14}NH_4^+$ and $^{14}NO_3^-$ + $^{15}NH_4^+$. There was an almost equal contribution from both the ammonium and the nitrate ion to the free amino fraction in the shoot of the mixed feed plants, as deduced from the fact that the ^{15}N content of the $^{15}NO_3^-$ + $^{15}NH_4^+$ mixed feed plants' shoot was double that shown for the single ^{15}N labelled plants.

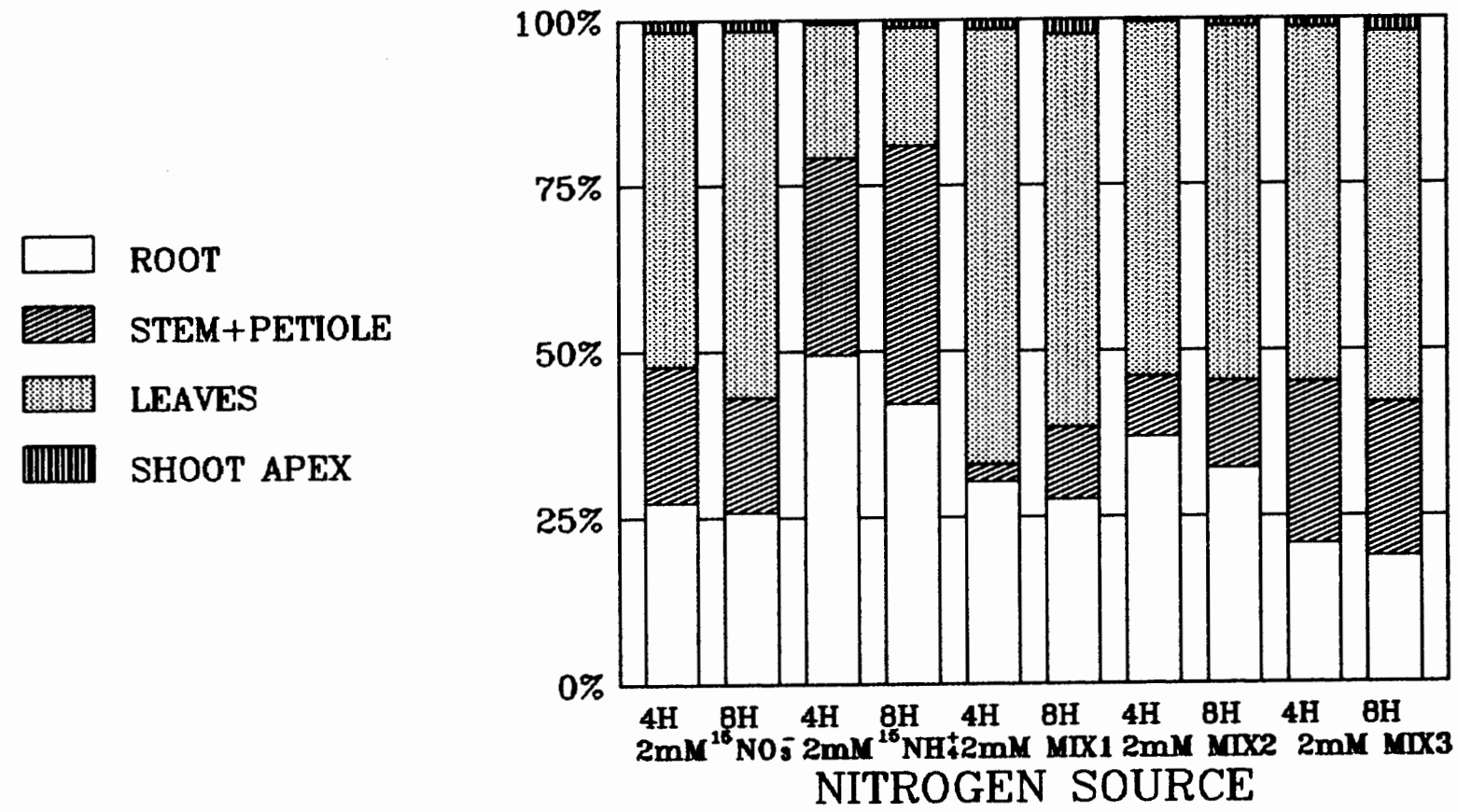


MIX1- 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX2- 1mM $^{14}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX3- 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$

FIGURE 5.5
The free amino ^{15}N of root and shoot of plants of *Helianthus annuus* fed 2 mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$, 1 mM $^{14}NH_4^+$ + 1 mM $^{15}NO_3^-$ and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.

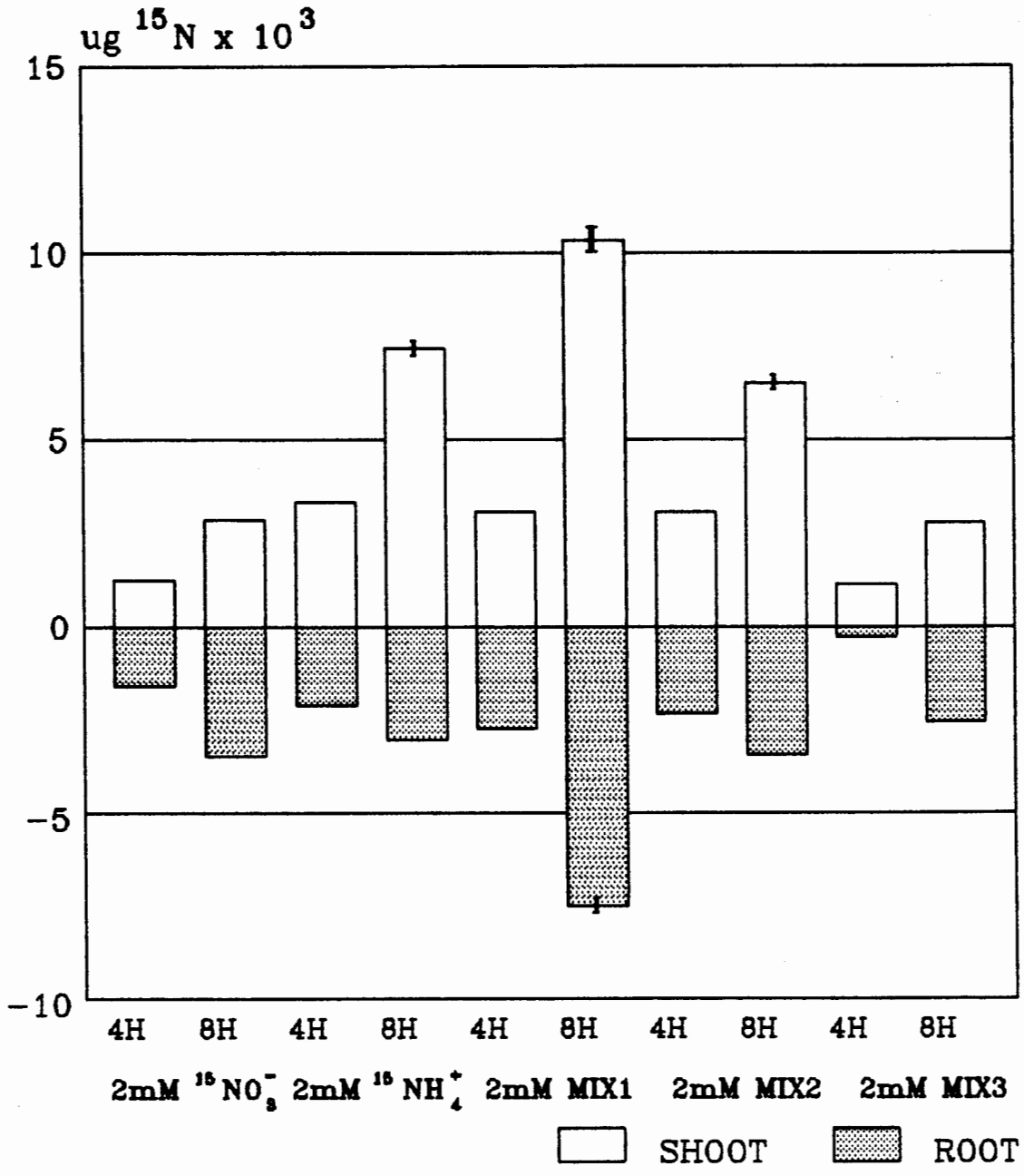
Figure 5.6 shows the percentage distribution of free amino ^{15}N between the various parts of the plants which had been fed the different nitrogen sources. The leaf was the major single location of free amino ^{15}N (more than 50% of the total in each treatment) for all plants fed with nitrate regardless of whether $^{14}NO_3^-$ or $^{15}NO_3^-$ was used, while ammonium-only fed plant leaves received only 20% of the ^{15}N at both harvests. The large free amino ^{15}N content of the $^{14}NO_3^-$ + $^{15}NH_4^+$ fed plants in comparison to the $^{15}NH_4^+$-only fed plants may have in some way been due to a translocation promoting effect of the nitrate driven potassium shuttle as shown by Lips et al, (1987).

As can be seen in Figure 5.5, the free amino ^{15}N found in the shoot apex of plants fed with $^{15}NO_3^-$ was greater than that of the ammonium-only fed plants and of the $^{14}NO_3^-$ + $^{15}NH_4^+$ mixed feed plants, indicating that a greater diversion of nitrogen to the shoot apex region originated from nitrate feeding than from ammonium feeding.



MIX1– 1mM $^{15}NO_3^-$ +1mM $^{15}NH_4^+$
MIX2– 1mM $^{14}NO_3^-$ +1mM $^{15}NH_4^+$
MIX3– 1mM $^{15}NO_3^-$ +1mM $^{14}NH_4^+$

FIGURE 5.6
Percentage distribution of free amino ^{15}N between the plant parts of *Helianthus annuus* fed 2 mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$, 1 mM $^{14}NH_4^+$ + 1 mM $^{15}NO_3^-$, and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.



MIX1- 1mM $^{15}NO_3^-$+1mM $^{15}NH_4^+$
MIX2- 1mM $^{14}NO_3^-$+1mM $^{15}NH_4^+$
MIX3- 1mM $^{15}NO_3^-$+1mM $^{14}NH_4^+$

FIGURE 5.7
Distribution of bound ^{15}N between the plant parts of *Helianthus annuus* fed 2 mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$, 1 mM $^{14}NH_4^+$ + 1 mM $^{15}NO_3^-$, and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.

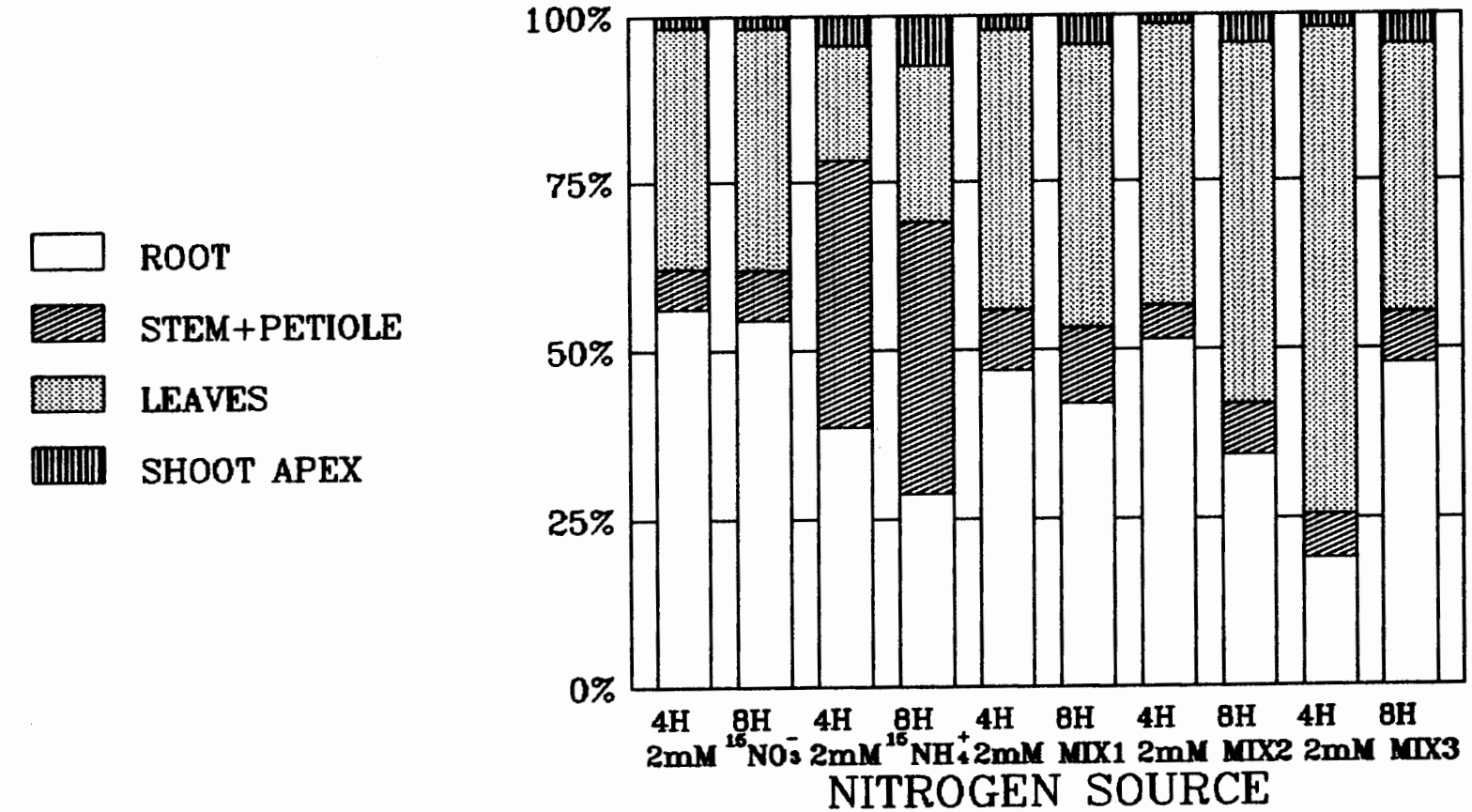
5.3.4.3 Bound ^{15}N

The incorporation of ^{15}N into the bound fraction of the shoot of the $^{15}NO_3^-$ + $^{14}NH_4^+$ fed plants (Figure 5.7), appeared to be unaffected by the presence of the ammonium ion when the 4 and 8 hour harvest results were compared to those of the nitrate-only fed plants. The bound ^{15}N content of the root of the $^{15}NO_3^-$-only fed plants was 10 times greater than that of the $^{15}NO_3^-$ + $^{14}NH_4^+$ fed plants at the 4 hour harvest reflecting the inhibitory effect of $^{15}NH_4^+$ on $^{15}NO_3^-$ uptake described in Section 5.3.4.1.

Ammonium had a greater influence upon new shoot development than nitrate. This was shown by the ^{15}N bound nitrogen of the shoot apex remaining constant between the 4 and 8 hour harvests for the nitrate-only fed plants, whereas the bound ^{15}N of all $^{15}NH_4^+$ fed plants increased (Figure 5.8).

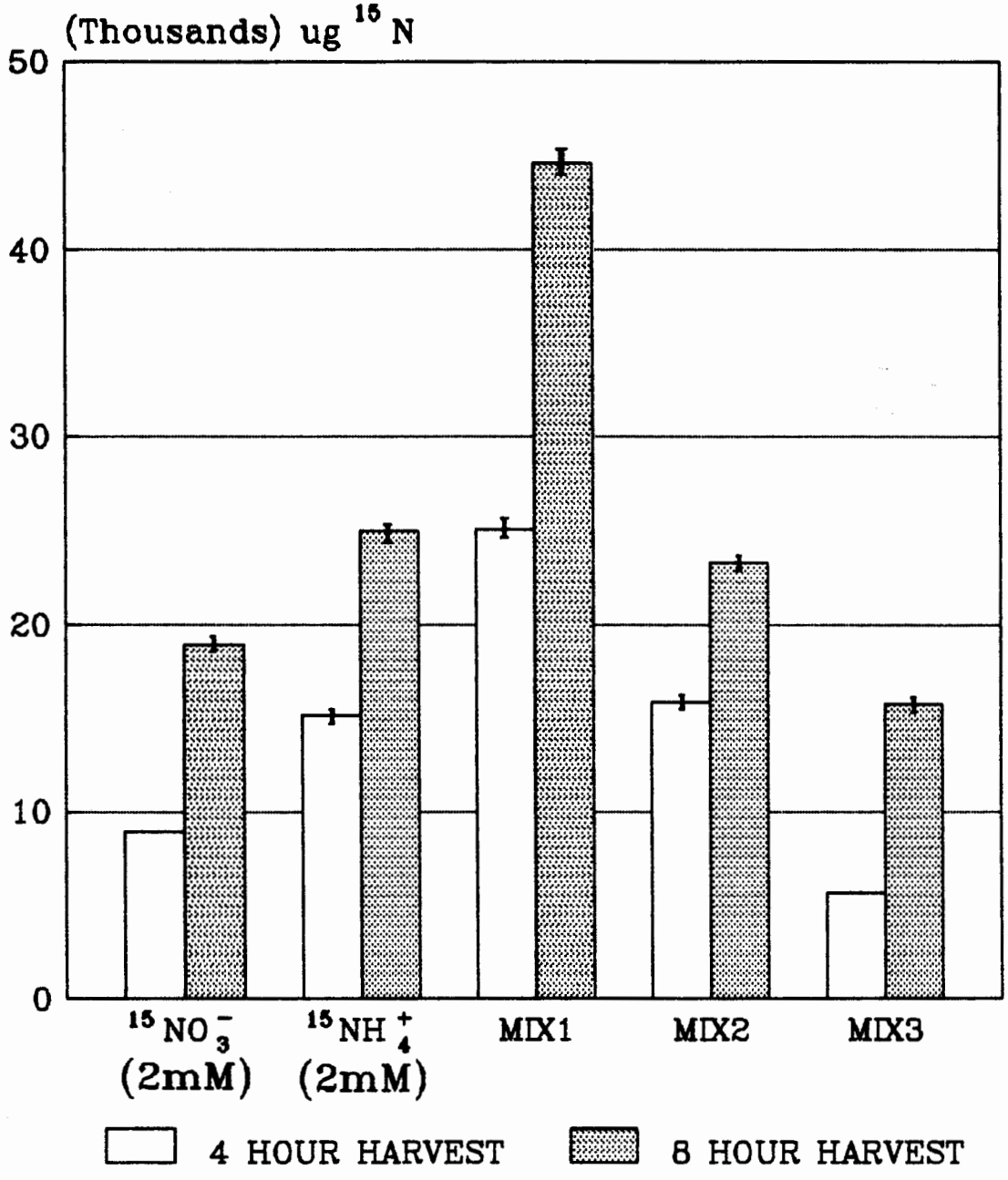
5.3.4.4 Total Organic ^{15}N

The total organic ^{15}N values for both the 4 hour harvest and 8 hour harvest of the $^{15}NO_3^-$ + $^{14}NH_4^+$ mixed feed plants were lower than those of the nitrate-only fed plants (Figure 5.9). This may be the consequence of the nitrate concentration of the mixed feed plants being one half that of the nitrate-only fed plants, or of an influence of the ammonium ion upon the uptake of nitrate. The ^{15}N content of



MIX1– 1mM $^{15}NO_3^-$ +1mM $^{15}NH_4^+$
MIX2– 1mM $^{14}NO_3^-$ +1mM $^{15}NH_4^+$
MIX3– 1mM $^{15}NO_3^-$ +1mM $^{14}NH_4^+$

FIGURE 5.8
Percentage distribution of bound ^{15}N between the plant parts of *Helianthus annuus* fed 2 mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$, 1 mM $^{14}NH_4^+$ + 1 mM $^{15}NO_3^-$, and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.



MIX1- 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX2- 1mM $^{14}NO_3^-$ + 1mM $^{15}NH_4^+$
MIX3- 1mM $^{15}NO_3^-$ + 1mM $^{14}NH_4^+$

FIGURE 5.9
Total organic ^{15}N of plants of *Helianthus annuus* fed 2 mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$, 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$, 1 mM $^{14}NH_4^+$ + 1 mM $^{15}NO_3^-$ and 1 mM $^{15}NH_4^+$ + 1 mM $^{14}NO_3^-$ for 4 h and 8 h.

the $^{15}NO_3^-$ + $^{15}NH_4^+$ mixed feed plants was greater than that of any of the other ^{15}N fed plants at both harvests. This ^{15}N content was equal to the sum of the contents of the two single source ^{15}N fed plants for both harvests, indicating that although the concentration of each of the nitrogen sources was one half of that used for the single source nitrogen fed plants, the net result was an addition of the ^{15}N contents of the two different nitrogen sources which were assimilated in different parts of the plant.

From Table 5.6 the supply of $^{15}NH_4^+$ appears to be depleted by the 8 hour harvest in the mixed feed plants, therefore only the 4 hour harvest assimilation rates will be considered. The uptake of $^{15}NH_4^+$ was 65% more rapid than that of the $^{15}NO_3^-$ in the mixed feed plants. Also, the rate of assimilation into organic compounds in the 1 mM $^{15}NO_3^-$ + 1 mM $^{15}NH_4^+$ fed plants was equal to the combined rates of the 2 mM $^{15}NO_3^-$-only fed plants plus the 2 mM $^{15}NH_4^+$ fed plants indicating that nitrogen from the combined source was more rapidly assimilated than from either source singly.

5.4 Conclusions

The apparently slower uptake of $^{15}NO_3^-$ nitrogen observed in the mixed feed plants compared with the nitrate-only fed plants may result from a repression by ammonium of nitrate uptake as was shown by Schrader *et al*, (1972) in corn, Zevenboom and Mur, (1981) in *Oscillatoria agardhii* cultures;

and Lewis et al, (1982a) in barley. This apparently slower uptake could also be the result of the concentration of nitrate in the nutrient solution being one half that of the nitrate-only fed plants.

There was a low shoot ammonium concentration in all plants supplied with ammonium (i.e. mixed as well as single source). This low ammonium concentration in the shoot has also been observed with barley (Chadwick, 1985), maize (Murphy, 1985) and rice (Muhammed and Kumazawa, 1974a).

When sunflower plants were grown with ammonium as the only nitrogen source, root masses were lower than that of nitrate-only grown plants (Chapter 3, Section 3.3.1). Warncke and Barber (1973) postulated that the reduction in root mass exhibited by ammonium grown maize plants was due to the acidity around the roots which increased as the ammonium was consumed. This hypothesis does not hold in the present experiment as the pH of the nutrient solution was kept between pH 6.8 and pH 7 through the inclusion of calcium carbonate in the nutrient feeding solution (Chapter 2, Section 2,1; Hewitt, 1966).

It would appear from the S:R ratio of 1.8 for the bound ^{15}N in the $^{15}NH_4^+$-only fed plants that the compounds transported via the phloem to the roots are either used to assimilate ammonium or are returned via the xylem to the shoot (Pate,

1983) with very little incorporation into the bound nitrogen in the root. The S:R ratio for free amino ^{15}N in ammonium-only fed plants indicated that the root of the plants retained very little of the assimilated ^{15}N. It is well known that assimilated ammonium nitrogen from the root is transported via the xylem to the growing regions of the shoot while photoassimilates (Shiroya, 1977), carbon skeletons and amino compounds (Pate, 1983) are translocated via the phloem to the root to be used in the assimilation of more ammonium.

In spite of the high concentration of $^{15}NH_4^+$ found in the roots of both the ammonium-only and mixed feed plants (Figure 5.3), very little $^{15}NH_4^+$ was found in the shoot indicating the root as the major site of ammonium nitrogen assimilation for sunflowers.

Although nitrate uptake from the ammonium plus nitrate feeding solution was apparently suppressed by the ammonium ion, the presence of nitrate appears to aid in the translocation of assimilated nitrogen to the shoot.

In spite of this suppression of nitrate uptake by ammonium, the assimilation of organic compounds by mixed feed plants was greater than that for plants fed either nitrogen source alone.

Ammonium was taken up more rapidly than nitrate as was shown by the rates of ^{15}N assimilation into organic compounds.

The results in the present experiment agree with those of Lewis, Soares and Lips (1986) who found that ^{15}N assays of plant organs and xylem sap of hydroponically grown barley plants indicated a compartmentation of N assimilation between root and shoot according to N source: nitrate in shoots, ammonium in roots, with both organs active in nitrate + ammonium assimilation.

The rapid uptake of ammonium, combined with the increasing uptake of nitrate, gave the plants both large shoots from ammonium feeding and large roots from nitrate feeding. From the agricultural viewpoint, mixed nitrogen source feeding thus results in the production of plants with superior growth features than either nitrogen source singly.

CHAPTER 6

CHASE FEEDING OF ^{15}N WITH ^{14}N IN MATURE SUNFLOWER PLANTS

6.1 Introduction

The purpose of this experiment was to determine what effect newly supplied nitrogen to the root had upon the fruit at the stage when the fruit was approximately 90% filled.

Ammonium + nitrate has been demonstrated to be a superior nitrogen source for plant growth over either nitrate or ammonium as sole nitrogen source for a variety of plants: sunflower (Chapter 3, Section 3.3.1, 3.3.5 and 3.4); melon, cucumber, watermelon, okra, egg plant, tomato, sweet pepper, pea (Ikeda and Osawa, 1979); barley (Lewis and Chadwick, 1983).

In his chase feed experiments using ^{15}N, Yoneyama (1983) found that there were no significant differences in the distribution of nitrogen absorbed at different times of growth among the protein fractions of the grain of wheat plants. His chase experiments were, however, carried out over a three week interval between application of the N^{15} and harvesting and were only performed with nitrate nitrogen. These experiments did not reflect the short term distribution of newly incorporated nitrogen or the effect of ammonium or ammonium + nitrate as nutrient source.

6.2 Experimental Methods

Helianthus annuus plants were allowed to develop to the point where the seed was approximately 90% filled (which took about 10 weeks). The 10 week growth stage was chosen for experimentation since this is the point at which sunflower plants show maximum dry matter content in all parts, and, with the exception of the fruit (Hocking and Steer, 1983), begin to lose dry matter. ^{15}N as ammonium, nitrate, or ammonium + nitrate was fed hydroponically as described in Chapter 2, Section 2.2.3. After 8 hours the ^{15}N nutrient solution was replaced with ^{14}N nutrient solution and the plant allowed to complete a normal 24 hour cycle from the time that the ^{15}N chemicals were introduced. At the end of the 24 hour period the plants were harvested as described in Chapter 2, Section 2.3 and extraction of free amino compounds, nitrate and/or ammonium was carried out as described in Chapter 2, Section 2.3. Samples of the free amino compounds, nitrate and/or ammonium plus the bound nitrogen of root, stem plus petiole, leaves, base of the capitulum and fruit were prepared as described in Chapter 2, Section 2.4 and ^{15}N analysis carried out as described in Chapter 2, Section 2.5. The leaves were divided into four sections: small expanding leaves just below the capitulum, upper half of the fully expanded leaves, lower half of the fully expanded leaves and the bottom older leaves (including senescent leaves).

The nitrate only hydroponic feeding solutions were tested for ammonium content with Nessler's reagent as described in Chapter 2, Section 2.8.3 and both the fresh and old solutions at each nutrient change were found to be free of ammonium.

Nitrate determinations were performed with Szechrome NAS on the new and old solutions of the ammonium-only fed plants at each change of the hydroponic solutions as described in Chapter 2, Section 2.8.2. There was no nitrate found at any time during the 10 week time course.

6.3 Results and Discussion

6.3.1 2 mM ^{15}N-Nitrate Feeding

As can be seen from Table 6.1 there was very little free ^{15}N-nitrate remaining in any part of the plant. The free amino ^{15}N content of the shoot was 8 times that of the root with the fruit containing 33% of the shoot ^{15}N. The shoot also contained 30% more bound ^{15}N than the root with one quarter of the shoot bound ^{15}N being found in the fruit.

Table 6.1 shows that very little of the newly assimilated ^{15}N had been distributed to the bottom leaves with the bound

TABLE 6.1

mg N plant^{-1} and ^{15}N enrichments (A%E) of the bound-N, free amino compound-N and nitrate-N fractions of fruit, base of capitulum, stem + petiole, leaves (4 divisions) and root of 10 week old sunflower plants fed 2 mM $^{15}NO_3^-$ for 8 h and chased with 2 mM $^{14}NO_3^-$ for 16 h. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

Plant Part	Bound-N			Free Amino Compound-N			Nitrate-N		
	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}
Fruit	128.6 ± 4.7	0.63 ±0.02	810.9 ±55.6	84.1 ±1.9	1.50 ±0.03	1261.5 ± 54.3	0.1 ±0.0	0.04 ±0.00	0.1 ±0.0
Base of Capitulum	80.3 ±0.2	0.18 ±0.00	144.5 ± 0.4	70.2 ±2.1	1.24 ±0.03	870.5 ±47.7	0.5 ±0.0	0.06 ±0.00	0.3 ±0.0
Stem ± Petiole	92.9 ±1.7	0.49 ±0.01	455.2 ±17.8	42.8 ±1.1	1.87 ±0.04	800.4 ±38.1	5.4 ±0.1	0.18 ±0.00	9.7 ±0.0
Top Leaves	48.5 ±0.8	0.48 ±0.01	232.8 ± 8.8	24.8 ±0.3	2.31 ±0.02	572.9 ±11.9	2.1 ±0.1	0.22 ±0.00	4.6 ±0.2
Upper Leaves	208.1 ± 6.2	0.42 ±0.00	874.0 ±26.1	20.9 ±0.2	1.13 ±0.01	236.2 ± 4.3	2.8 ±0.0	0.12 ±0.00	3.4 ±0.0
Lower Leaves	197.6 ± 6.3	0.27 ±0.01	533.5 ±37.4	12.4 ±0.1	0.43 ±0.01	53.3 ±1.7	1.2 ±0.0	0.08 ±0.00	1.0 ±0.0
Bottom Leaves	61.5 ±3.1	0.18 ±0.01	110.7 ±12.0	4.2 ±0.0	0.20 ±0.00	8.4 ±0.0	0.2 ±0.0	0.05 ±0.00	0.1 ±0.0
Root	318.7 ±14.5	0.69 ±0.03	2199.0 ±200.0	76.2 ±1.5	0.61 ±0.01	464.8 ±16.9	7.6 ±0.2	0.13 ±0.00	9.9 ±0.1
Shoot ug 15N			3161.6 ±158.1			3803.2 ±158.0			19.2 ±0.3

nitrogen for these leaves exhibiting the least ^{15}N incorporation for the entire plant. The presence of both free amino ^{15}N and bound ^{15}N found mainly in the upper and top leaves, but not in the older and scenesing leaves on the lower part of the shoot, indicated that reduction and assimilation of nitrate only took place in the upper part of the shoot.

Blacklow (1982) found that senescing flag leaves of winter wheat fed ^{15}N nitrate were able to assimilate $^{15}NO_3^-$ and translocate the assimilate to the grain within three days. In the present chase feed experiment no nitrate accumulated in the fruit of the sunflower, as was also shown by Blacklow (1982) for wheat, indicating that $^{15}NO_3^-$ was reduced elsewhere in the plant. Hocking and Steer (1983) found that sunflower seeds acquire 33% of their nitrogen from redistribution from above ground parts and over 50% of the nitrogen in the stem and leaves was from redistribution at the time of 75% fruit filling. They found that during early senescence, at 75% fruit filling, the enzymes of nitrogen metabolism were still functioning . They also found that redistribution of nitrogen occurred throughout the plant despite an adequate daily supply of nitrogen, and redistribution from vegetative parts provided nearly all the nitrogen accumulated by seeds during the last 12 days of their growth.

In this experiment more than 20% of the total newly assimilated $^{15}NO_3^-$ of the nitrate-only fed plants was found in the fruit (Figure 6.1), indicating agreement with the findings of Blacklow (1982) and Hocking and Steer (1983).

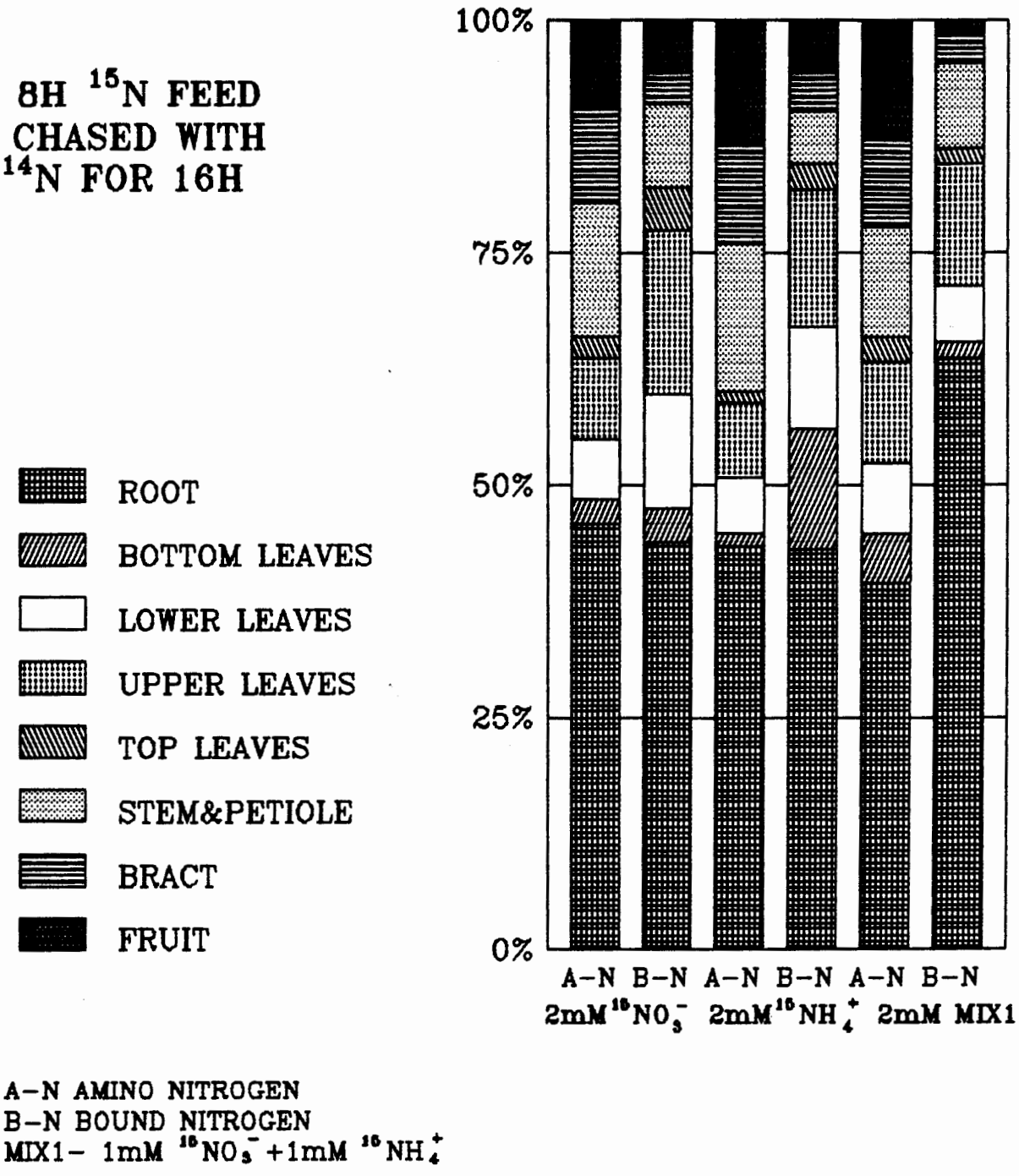
6.3.2 2 mM ^{15}N-Ammonium Feeding

As can be seen from Table 6.2, very little free ^{15}N-ammonium was found in any part of the plant indicating that the ^{15}N-ammonium taken up by the plant had been assimilated into nitrogenous compounds in the root, as already shown for the young sunflower seedlings (Chapter 5, Section 5.3.2). The feeding solution at the end of the 24 hour period was tested and found to be lacking in any ^{15}N, indicating that there was no flow of nitrogen from the root back to the nutrient solution. The shoot contained 24 times more free amino ^{15}N than the root indicating that very little of the newly incorporated ammonium was utilized in the root. This may partially account for a fresh mass shoot to root ratio greater than one whereas the nitrate and ammonium + nitrate shoot to root ratios were less than one (Chapter 3, Section 3.3, Table 3.5). The lower and bottom scenesing leaves contained less than 1% of the newly assimilated ^{15}N indicating that the lower and bottom leaves were no longer receiving newly incorporated nitrogen. The fruit contained 23% of the free amino ^{15}N of the shoot (Figure 6.1) indicating that the fruit was receiving newly incorporated ^{15}N as well as nitrogen from redistribution. These 10 week

TABLE 6.2

mg N plant^{-1} and ^{15}N enrichments (A%E) of the bound-N, free amino compound-N and ammonium-N fractions of fruit, base of capitulum, stem + petiole, leaves (4 divisions) and root of 10 week old sunflower plants fed 2 mM $^{15}NH_4^+$ for 8 h and chased with 2 mM $^{14}NH_4^+$ for 16 h. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

Plant Part	Bound-N			Free Amino Compound-N			Ammonium-N		
	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}
Fruit	88.5 ±2.7	0.68 ±0.02	601.8 ±33.1	76.7 ±2.2	1.28 ±0.04	981.8 ±59.7	0.2 ±0.0	0.02 ±0.00	0.1 ±0.0
Base of Capitulum	87.0 ±2.2	0.29 ±0.01	252.3 ±15.3	57.7 ±1.5	1.34 ±0.04	773.9 ±43.1	0.4 ±0.0	0.06 ±0.00	0.2 ±0.0
Stem ± Petiole	116.7 ± 2.7	0.66 ±0.02	770.2 ±41.7	34.4 ±0.8	2.64 ±0.06	908.2 ±42.2	1.4 ±0.0	0.10 ±0.00	1.4 ±0.0
Top Leaves	41.8 ±0.8	1.02 ±0.02	426.4 ±16.6	33.8 ±0.6	3.49 ±0.04	1179.6 ±34.7	1.8 ±0.1	0.11 ±0.00	2.0 ±0.1
Upper Leaves	275.3 ± 5.5	0.93 ±0.01	2560.3 ± 79.2	20.5 ±0.3	2.00 ±0.02	410.0 ±10.2	1.7 ±0.0	0.12 ±0.00	2.0 ±0.0
Lower Leaves	160.2 ± 2.9	0.75 ±0.00	1201.5 ± 21.8	14.4 ±0.2	0.40 ±0.01	57.6 ±2.3	1.1 ±0.0	0.08 ±0.00	0.9 ±0.0
Bottom Leaves	24.2 ±0.7	0.27 ±0.00	65.3 ±4.4	8.1 ±0.2	0.25 ±0.01	20.3 ±1.3	0.8 ±0.0	0.04 ±0.00	0.3 ±0.0
Root	285.0 ± 8.1	0.92 ±0.02	2622.0 ±133.1	22.3 ±0.7	0.82 ±0.02	182.9 ±10.3	3.9 ±0.2	0.10 ±0.00	3.9 ±0.2
Shoot ug 15N			5877.8 ±207.7			4331.4 ±193.5			10.8 ±0.3



A-N AMINO NITROGEN
B-N BOUND NITROGEN
MIX1- 1mM $^{15}NO_3^-$ +1mM $^{15}NH_4^+$

FIGURE 6.1
Chase feed of ^{15}N with ^{14}N for nitrate, ammonium and mix feed plants.

old plants showed the same incorporation pattern into the bound fraction as the 4 week old plants (Chapter 5, Section 5.3.2, Table 5.2) where the total bound ^{15}N was greater in the ammonium fed plants (Table 6.2) than in the nitrate fed plants (Table 6.1). The capitulum contained 15% of the bound ^{15}N of the shoot indicating storage of newly assimilated nitrogen in the fruit. The presence of bound ^{15}N in the top, upper and even the lower leaves indicated that the leaves were still producing protein even though the bottom leaves were senescing as the fruit was filling. The roots contained only 40% of the total ^{15}N incorporated into the plant indicating that the shoot was still the major recipient of newly assimilated nitrogen.

6.3.3 1 mM ^{15}N-Ammonium + 1 mM ^{15}N-Nitrate Feeding

Table 6.3 shows that very little free ^{15}N-ammonium or ^{15}N-nitrate remained in the plant after the 16 hour chase with ^{14}N nutrient solutions. These results agree with those shown for nitrate-only (Table 6.1) and ammonium-only (Table 6.2) fed plants.

The bottom senescent leaves exhibited virtually no ^{15}N incorporation while the other three leaf divisions each had approximately 1% ^{15}N enrichment of these bound fractions indicating that the scenescening leaves were no longer storing newly incorporated nitrogen. The fruit exhibited a 1% ^{15}N enrichment of the bound nitrogen fraction which

amounted to 14% of the total ^{15}N bound nitrogen for the shoot (Figure 6.1). The root contained almost one half of the total bound ^{15}N for the plant. The total bound ^{15}N of these plants was more than double that for the ammonium fed plants (Table 6.2) and more than treble that for the nitrate fed plants (Table 6.1).

The shoot to root ratio of free amino ^{15}N (16:1) indicated that the newly assimilated nitrogen was being transported to the shoot for possibly further leaf expansion or fruit filling. The fruit of the mixed feed plants contained the largest free amino ^{15}N pool (2077 µg) of any individual plant part. When this pool was compared to that of the nitrate fed plants (1261 µg) and the ammonium fed plants (982 µg) it would appear that the root of the mixed feed plants was contributing twice the amount of nitrogen for fruit filling than either of the single nitrogen sources fed via the plant roots. One third of the free amino ^{15}N of the shoot was found in the fruit, indicating a strong contribution of newly assimilated nitrogen to fruit filling. These results agree with those of Kato (1980) who demonstrated that the fruit of citrus trees contained ^{15}N in the form of free amino compounds seven hours after feeding either nitrate or ammonium via the roots.

TABLE 6.3

mg N plant^{-1} and ^{15}N enrichments (A%E) of the bound-N, free amino compound-N, nitrate-N and ammonium-N fractions of fruit, base of capitulum, stem + petiole, leaves (4 divisions) and root of 10 week old sunflower plants fed 1 mM $^{15}NO_3^-$ + 1 mM $^{15}NH_4^+$ for 8 h and chased with 1 mM $^{14}NO_3^-$ + 1 mM $^{14}NH_4^+$ for 16 h. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

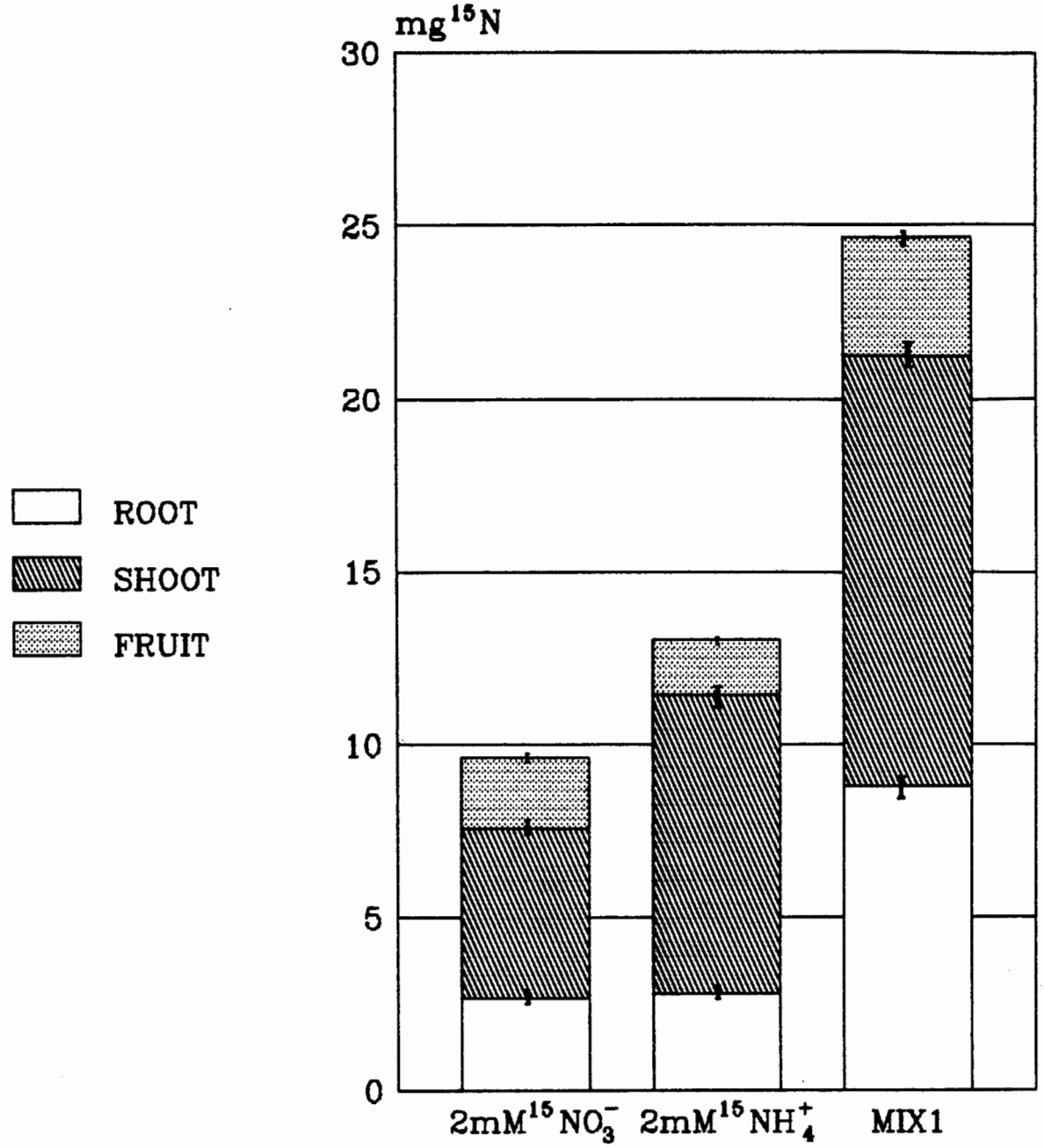
Plant Part	Bound-N			Free Amino Compound-N			Nitrate-N			Ammonium-N		
	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}	mg N Plant^{-1}	^{15}N Enrichment (A%E)	ug ^{15}N Plant^{-1}
Fruit	130.5 ± 3.9	1.01 ±0.03	1318.1 ± 79.7	92.3 ±2.8	2.25 ±0.07	2076.8 ±129.5	0.2 ±0.0	0.03 ±0.00	0.1 ±0.0	0.3 ±0.0	0.24 ±0.05	0.7 ±0.0
Base of Capitulum	82.0 ±2.1	0.96 ±0.02	787.2 ±17.4	76.1 ±2.1	1.98 ±0.06	1506.8 ± 88.5	0.4 ±0.0	0.05 ±0.00	0.2 ±0.0	0.5 ±0.0	0.08 ±0.00	0.4 ±0.0
Stem ± Petiole	85.0 ±2.1	1.68 ±0.04	1428.0 ± 70.1	25.9 ±0.7	4.26 ±0.11	1103.3 ± 59.1	5.5 ±0.1	0.19 ±0.01	10.6 ±0.6	1.7 ±0.0	0.12 ±0.00	2.0 ±0.0
Top Leaves	45.4 ±0.8	1.20 ±0.03	544.8 ±23.5	32.3 ±0.4	4.81 ±0.05	1553.6 ± 32.4	3.0 ±0.0	0.23 ±0.01	6.9 ±0.3	2.5 ±0.1	0.14 ±0.00	3.5 ±0.1
Upper Leaves	272.7 ± 3.4	1.05 ±0.01	2863.4 ± 63.3	13.7 ±0.3	3.09 ±0.03	423.3 ±10.4	3.8 ±0.1	0.17 ±0.00	6.5 ±0.1	2.2 ±0.1	0.13 ±0.00	2.9 ±0.1
Lower Leaves	238.3 ± 7.1	0.90 ±0.02	2144.7 ±113.0	11.7 ±0.2	0.55 ±0.00	64.4 ±0.7	1.7 ±0.1	0.09 ±0.00	1.5 ±0.1	1.8 ±0.0	0.09 ±0.00	1.6 ±0.0
Bottom Leaves	113.1 ± 1.4	0.05 ±0.00	56.5 ±1.0	4.6 ±0.0	0.28 ±0.01	12.9 ±0.4	0.1 ±0.0	0.04 ±0.00	0.1 ±0.0	0.9 ±0.0	0.08 ±0.00	0.7 ±0.0
Root	332.8 ± 5.2	2.51 ±0.05	8353.3 ±269.5	65.6 ±1.5	0.62 ±0.02	406.7 ±22.7	9.0 ±0.0	0.10 ±0.00	9.0 ±0.0	7.6 ±0.0	0.15 ±0.01	11.4 ±0.7
Shoot ug 15N			9142.7 ±368.0			6741.1 ±291.0			25.8 ±1.1			11.8 ±0.9

6.4 General Discussion

Despite the senescing of the lower leaves, the sunflower plants were able to assimilate and translocate nitrogen to the fruit within 24 hours (Tables 6.1, 6.2 and 6.3). This supports the findings of Hara and Sonoda (1979) in cabbage plants and Przemeck and Kucke (1986) in cereal plants that nutrient feeding up to the time of harvesting would have the effect of a higher yield. In rice, the nitrogen content of the fruit regardless of the nitrogen feeding source, would appear to come mainly from the chloroplasts of older senescing leaves (Morita, 1980) with only a small contribution coming directly from the root of the plants.

From Figure 6.2 it can be seen that nitrate-only fed plants contribute more newly assimilated ^{15}N nutrient to the fruit than ammonium-only fed plants and is more beneficial for fruit filling than ammonium. The ammonium-only fed plants contribute more newly assimilated nitrogen to the rest of the shoot than do nitrate-only fed plants. Both the nitrate-only and ammonium-only fed plants had equal but lesser amounts of newly assimilated ^{15}N in the root than did the ammonium + nitrate fed plants. The ammonium + nitrate fed plants appear to combine the results of the two single source fed plants supplying newly assimilated nitrogen to fruit, shoot and root.

It would appear that a combination of ammonium and nitrate for the nitrogen source, as opposed to either one singly, produces larger plants with a larger fruit mass and that nutrient supplied even at the stage of advanced fruit filling, is beneficial for a greater fruit fraction.



MIX1 – 1mM $^{15}NO_3^-$ + 1mM $^{15}NH_4^+$

FIGURE 6.2
Total organic ^{15}N of plants of Helianthus annuus fed 2 mM $^{15}NO_3^-$, 2 mM $^{15}NH_4^+$ and 1 mM $^{15}NH_4^+$ + 1 mM $^{15}NO_3^-$ for 8 h followed by a chase feed with ^{14}N for 16 h.

CHAPTER 7

^{15}N-NITRATE VACUUM INFILTRATION OF LEAVES OF FRUIT BEARING SUNFLOWER PLANTS

7.1 Introduction

This experiment was designed to determine the distribution of soluble nitrogen from a mature leaf which was vacuum infiltrated with nitrate to the various plant parts and in particular to the fruit. ^{15}N vacuum infiltration of selected leaves was used to ensure that any ^{15}N found in the plant only originated from those leaves. Ito and Kumazawa (1976) using vacuum infiltration of mature leaves of three week old sunflower plants with ^{15}N have shown that the label was distributed to all parts of the plant, but the majority of the isotope was directed to plant parts below the infiltrated leaf.

As was shown by xylem sap analysis in Chapter 4, Section 4.3 when ammonium or ammonium nitrate was used as the nitrogen source very little free ammonium was transported from the root to the shoot, the principal amino compound transported being glutamine. When nitrate was fed via the roots either as nitrate alone or as ammonium nitrate, the prime nitrogenous component of the xylem sap was nitrate, with glutamine as the principal amino nitrogen component (Kaiser and Lewis, 1980; Chapter 4, Section 4.3). Nitrate has been

shown to be primarily assimilated in the leaves of Helianthus annuus (Kaiser and Lewis, 1984), glutamine being the main amino compound formed (Ito and Kumazawa, 1976; Kaiser, 1978). Thus in this experiment only ^{15}N nitrate was vacuum infiltrated into the leaves of Helianthus annuus.

It has been demonstrated that 33% of the nitrogen content of sunflower seed (Hocking and Steer, 1983) is from redistribution of above ground parts while cowpea fruit gained 60% of its nitrogen from mobilization of nitrogen fixed before flowering (Peoples et al, 1983). Perez et al, (1983) have shown this redistribution to be dependent upon the level of nitrogen in the feeding solution. Values for the redistribution of nitrogen to the ears of wheat range from 100% for 0.8 milligram equivalent N/litre to nil with 12.8 milligram equivalent N/litre (Perez et al, 1983). Similar results were observed in studies on the productivity of grain sorghum by Mirhadi and Kobayashi (1980).

7.2 Experimental Methods

Vacuum infiltration of sunflower leaves using 20 mM $^{15}NO_3^-$ was carried out as described in Chapter 2, Section 2.2.2 using two opposing mature leaves of the same age near the top of the plant. Two separate experiments were carried out; (1) harvesting of feed leaves and fruit 4 hours and 8 hours after infiltration, and, (2) harvesting separately fruit, base of capitulum, leaves above feed leaves, petioles

above feed leaves, stem above feed leaves, stem and petioles of feed leaves, feed leaves, leaves below feed leaves, petioles below feed leaves, stem below feed leaves and root 24 hours after ^{15}N infiltration. It was felt that a clearer picture would be presented for the role of the mature leaf in supplying reduced N to the rest of the plant, especially the fruit, if an entire plant harvest was performed. The plant material was harvested as described in Chapter 2, Section 2.3 and the extraction of nitrogenous compounds was carried out as described in Chapter 2, Section 2.3. Samples were prepared for ^{15}N determinations as described in Chapter 2, Section 2.4 and ^{15}N analysis carried out as described in Chapter 2, Section 2.5. At the end of the 4 hour, 8 hour and 24 hour harvests, 10 ml aliquots of the remaining nutrient solution fed via the root were prepared for ^{15}N determinations as described for free amino nitrogen (Chapter 2, Section 2.4.2) and ^{15}N analysis carried out as described in Chapter 2, Section 2.5.

7.3 Results and Discussion

The results for the 4 and 8 hour harvest can be seen in Table 7.1. The ^{15}N content of only the feed leaves and fruit were determined in these short duration experiments. The ^{15}N-nitrate content remaining in the feed leaves at the 4 hour harvest was 105 µg but dropped to 20 µg at the 8 hour harvest indicating either the presence of active nitrate

TABLE 7.1

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of the nitrate, amino-N and bound-N fractions of feed leaves and fruit of 10 week old sunflower plants fed 2 mM ^{14}N-nitrate via the root and 20 mM ^{15}N-nitrate with vacuum infiltration via the feed leaves. Harvesting was done at 4 h and 8 h. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

	Harvest	Nitrate-N			Amino-N			Bound-N		
		ug N/ Plant	^{15}N Enrich-ment (A%E)	ug ^{15}N Plant	ug N/ Plant	^{15}N Enrich-ment (A%E)	ug ^{15}N Plant	ug N/ Plant	^{15}N Enrich-ment (A%E)	ug ^{15}N Plant
Feed Leaves	4 h	660.4 ±10.3	15.89 ±0.61	104.9 ± 5.1	3535.6 ± 27.3	27.98 ±0.91	989.3 ±40.0	40693.2 ± 136.3	1.20 ±0.00	488.3 ± 1.7
	8 h	647.9 ± 8.7	3.10 ±0.09	20.1 ±0.8	3479.4 ± 14.9	20.01 ±0.88	696.2 ±33.8	39851.0 ± 121.9	1.90 ±0.10	757.2 ±42.3
Fruit	4 h	113.7 ± 2.1	0.10 ±0.00	0.1 ±0.0	66204.8 ± 53.7	1.34 ±0.08	887.1 ±53.8	94791.6 ± 217.6	0.43 ±0.01	407.6 ±10.4
	8 h	103.0 ± 1.1	0.18 ±0.00	0.2 ±0.0	66347.7 ± 71.1	2.10 ±0.09	1393.3 ± 61.3	92912.7 ± 198.1	0.87 ±0.03	808.3 ±29.7

reducing enzymes in the leaf or transfer of nitrate out of the leaf to other parts of the plant. The fruit received very little of the ^{15}N-nitrate at either the 4 hour or the 8 hour harvest. There was no ^{15}N detected in the nutrient solution at the 4 hour and 8 hour harvests indicating that there had been no excretion of nitrogenous compounds from the root to the outer medium.

The decrease of free amino ^{15}N in the leaf from 1000 µg ^{15}N at the 4 hour harvest to 700 µg ^{15}N at the 8 hour harvest indicated that the reduced nitrate was transferred to the bound fraction of the leaf or transported out of the leaf to other parts of the plant. The increase in free amino ^{15}N of the fruit, from the 4 hour harvest to the 8 hour harvest, indicated that at least part of the newly reduced nitrate from the leaf was transferred to the fruit.

The incorporation of ^{15}N into the bound fraction of leaf and fruit indicated that at the 10 week stage of growth, these organs were still capable of producing protein from newly reduced nitrogen. A doubling of the bound ^{15}N in the fruit from the 4 hour harvest to the 8 hour harvest indicated that ^{15}N was transferred from the infiltrated mature leaves which were not yet at the senescing stage, to the fruit.

There was no ^{15}N detected in the nutrient solution fed to the root at the 24 hour harvest further indicating that

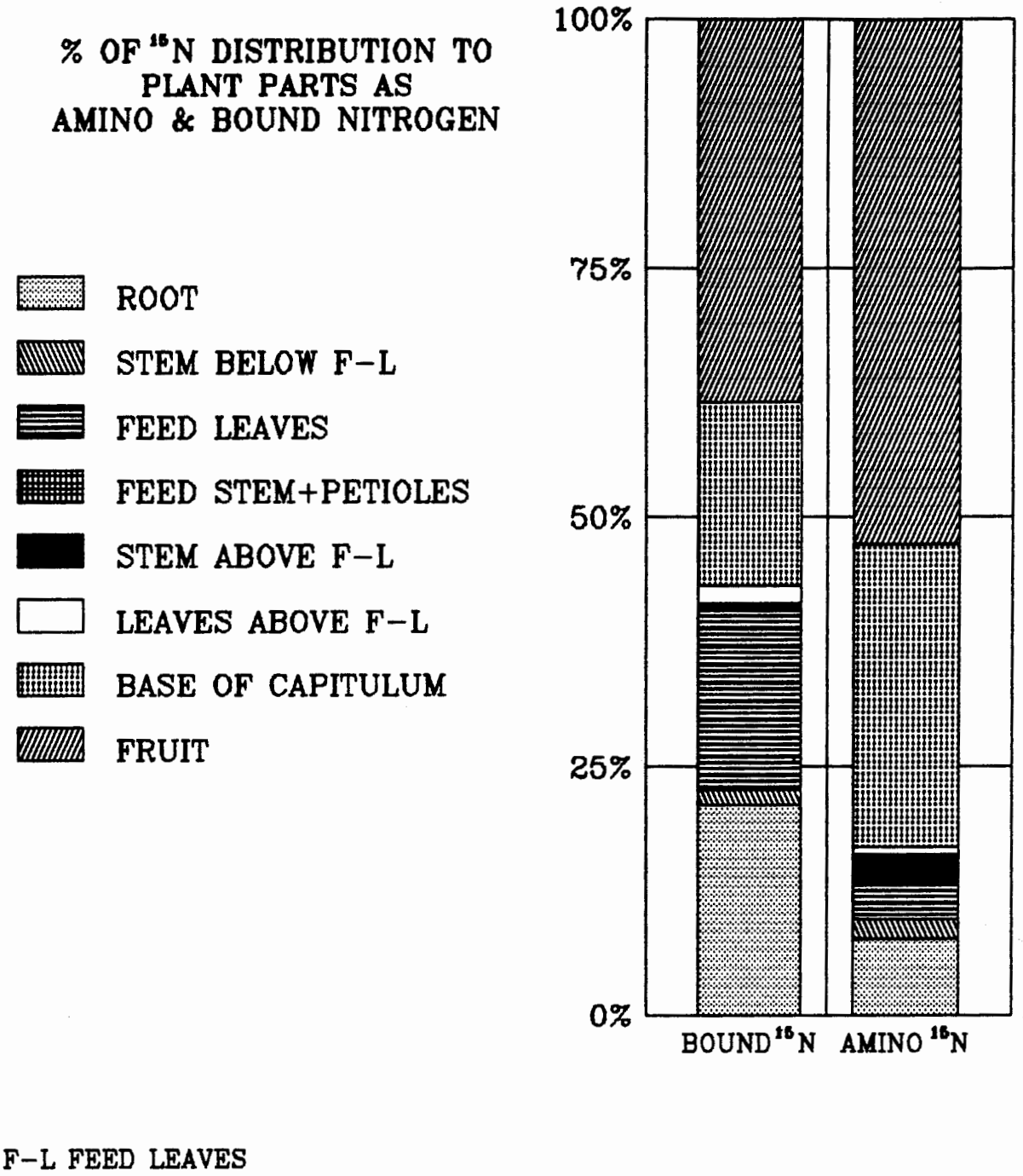
there was no excretion of nitrogenous compounds from the root to the outer medium. Table 7.2 shows the results from this entire plant harvest. As can be seen, there was very little ^{15}N-nitrate left anywhere in the plant after 24 hours indicating a virtually complete conversion of nitrate to other nitrogenous compounds. It has been demonstrated with ^{15}N-nitrate fed to senescing flag leaves of wheat that the nitrate was reduced in the flag leaf, 30-40% of the assimilate translocated to the grain and that no nitrate accumulated in the leaves or grain (Blacklow, 1982). In the present experiment the virtual complete lack of ^{15}N-nitrate anywhere in the sunflower plant and the fact that ^{15}N labelled nitrogenous compounds were located in the shoot and the root, would indicate that at this stage of fruit filling and older leaf senescence, the enzymes for nitrate reduction were still active in the leaf.

The free amino ^{15}N fraction of the different plant parts (Table 7.2) showed a downward movement of nitrogen only to the root via the stem, and not to the older leaves below the feed leaves indicating that newly incorporated nitrogen from mature leaves was not being diverted to these older, possibly senescent, leaves. The upward movement of free amino ^{15}N was to all plant parts above the feed leaves with 96% reaching the capitulum, 61% being found in the fruit of the capitulum (Figure 7.1). This indicated that the capitulum was the most actively growing plant part and that

TABLE 7.2

Nitrogen concentration plant^{-1} and ^{15}N enrichments (A%E) of the nitrate-N, soluble-N and bound-N fractions of plant parts of 10 week old sunflower plants fed 2 mM ^{14}N-nitrate via the root and 20 mM ^{15}N-nitrate with vacuum infiltration via feed leaves. Harvesting was done after a 24 h photosynthesis period. Figures have been averaged from duplicate determinations of duplicate plants and are expressed as average ± range.

	Bound N			Soluble N			Nitrate N		
	ug N/ Plant	^{15}N Enrich-ment ^{15}N (A%E)	ug ^{15}N Plant	ug N/ Plant	^{15}N Enrich-ment ^{15}N (A%E)	ug ^{15}N Plant	ug N/ Plant	^{15}N Enrich-ment ^{15}N (A%E)	ug ^{15}N Plant
Fruit	114820.4 ± 113.7	1.21 ±0.01	1389.3 ± 0.0	78958.8 ± 314.5	1.90 ±0.00	1500.2 ± 0.0	106.1 ± 1.6	0.00 ±0.00	0.0 ± 0.0
Base of Capitulum	83415.8 ± 75.1	0.80 ±0.00	667.3 ± 0.0	71288.4 ± 571.0	1.21 ±0.00	862.6 ± 0.0	518.5 ± 10.5	0.00 ±0.00	0.0 ± 0.0
Leaves above	92867.4 ± 278.6	0.07 ±0.00	65.0 ± 0.0	14275.3 ± 93.4	0.14 ±0.00	20.0 ± 0.0	2017.2 ± 29.7	0.00 ±0.00	0.0 ± 0.0
Petioles above	1942.5 ± 13.6	0.01 ±0.00	0.2 ± 0.0	793.7 ± 6.0	0.06 ±0.00	0.5 ± 0.0	161.2 ± 1.2	0.00 ±0.00	0.0 ± 0.0
Stem above	15807.6 ± 94.8	0.11 ±0.00	17.4 ± 0.0	12518.3 ± 106.7	0.63 ±0.01	78.9 ± 0.0	1131.2 ± 10.8	0.03 ±0.00	0.3 ± 0.0
Stems and Petioles fed	7320.2 ± 65.9	0.07 ±0.00	5.1 ± 0.0	572.9 ± 4.7	0.41 ±0.00	2.3 ± 0.0	462.6 ± 4.6	0.02 ±0.00	0.1 ± 0.0
Leaves fed	87551.9 ± 236.4	0.74 ±0.02	647.9 ± 0.0	3368.4 ± 28.7	3.13 ±0.04	105.4 ± 0.0	685.1 ± 8.4	0.11 ±0.01	0.8 ± 0.0
Leaves below	303127.6 ± 482.0	0.00 ±0.00	0.0 ± 0.0	43378.9 ± 389.1	0.00 ±0.00	0.0 ± 0.0	2992.2 ± 65.5	0.01 ±0.00	0.3 ± 0.0
Petioles below	11964.2 ± 94.4	0.00 ±0.00	0.0 ± 0.0	1641.0 ± 12.4	0.00 ±0.00	0.0 ± 0.0	346.8 ± 2.6	0.00 ±0.00	0.0 ± 0.0
Stem below	52143.6 ± 309.7	0.11 ±0.00	57.4 ± 0.0	28325.4 ± 241.3	0.20 ±0.00	56.7 ± 0.0	3855.1 ± 35.4	0.10 ±0.00	3.9 ± 0.0
Root	849586.3 ± 1248.9	0.09 ±0.00	764.6 ± 0.0	70005.5 ± 665.8	0.31 ±0.00	217.0 ± 0.0	5108.7 ± 44.3	0.00 ±0.00	0.0 ± 0.0
TOTAL	1532995.6 ± 2776.7		2966.4 ± 0.0	321757.2 ±2404.9		2738.1 ± 0.0	16699.5 ±206.2		4.6 ± 0.0



F-L FEED LEAVES

FIGURE 7.1
Percentage distribution of organic (free amino and bound) ^{15}N in plant parts of *Helianthus annuus* fed $^{15}NO_3^-$ by vacuum infiltration.

the infiltrated mature leaves were supplying nutrient mainly to the capitulum. Only 3% of the free amino ^{15}N was left in the feed leaves after the 24 hour period while 20% was left at the end of 8 hours (Table 7.1).

The bound ^{15}N results (Table 7.2) showed, as did the free amino nitrogen results, that the only plant parts below the feed leaves with ^{15}N incorporation into bound nitrogen were the stem (57.4 µg ^{15}N) and the root (765 µg ^{15}N). The majority of the bound ^{15}N in the plant above the feed leaves was in the capitulum (Figure 7.1) with the fruit containing 65% of that nitrogen.

Peoples et al, (1983) demonstrated with cowpea fruit that each fruit drew on all available current sources of nitrogen, but nitrogen from leaves was distributed preferentially to the closest fruit, with lower fruit sequestering most of the nitrogen transported from the roots. These results agree with the results of the 4 hour and 8 hour experiments (Table 7.1) which indicated that the mature, but not yet senescent, leaves of sunflower were capable of contributing to fruit filling. The 24 hour experiment (Table 7.2) indicated that the newly reduced nitrogen from mature leaves, although mainly translocated to the capitulum, was translocated to the root and all other actively growing regions in the shoot. These results agree with Yoneyama, Arai and Totsuka (1980) who found that 24

hours after feeding $^{15}NO_2$ gas to mature sunflower leaves, some ^{15}N was transferred from the fed leaf, first to the stem and then to young growing leaves, flower and roots with negligible transfer to other mature leaves.

When Tables 7.1 and 7.2 are compared, it can be seen that the majority of the ^{15}N-nitrate assimilated into the leaves of mature sunflower plants is transported to the capitulum. The distribution of the bound and free amino ^{15}N for the whole plant at the 24 hour harvest (Figure 7.1), however, shows that 8% of the soluble ^{15}N and 21% of the bound ^{15}N at 24 hours after ^{15}N-nitrate infiltration was found in the root indicating that the root was still assimilating nitrogen. These results agree with those of Soares and Lewis, (1986) who found that mature barley plants were still accumulating and assimilating considerable quantities of nitrogen, although at a much slower rate than in younger plants.

CHAPTER 8

NITRATE REDUCTASE AND GLUTAMINE SYNTHETASE ACTIVITY IN LEAVES AND ROOTS OF NITRATE-FED Helianthus Annuus L.

8.1 Introduction

Although nitrate reductase activity (NRA) Dirr et al, (1973) has been demonstrated in both the roots and leaves of many plants, the only significant activity of this enzyme in Helianthus annuus has been found in the root (Weissman, 1972). In spite of this finding, detached leaves of H. annuus can apparently incorporate nitrate nitrogen rapidly into nitrogen metabolism when this nitrogen is fed via the xylem stream (Kaiser and Lewis, 1980).

Nitrate reductase (NR) inactivating factors in the form of phenolic compounds and/or proteolytic enzymes may be the cause of this inability to demonstrate NRA (Loomis and Battaile, 1966; Schrader et al, 1974; Sherrard et al, 1979; Stock and Lewis, 1982). Loomis and Baittaile (1966) used a technique to extract NRA in which insoluble polyvinylpyrrolidone (PVP) was used to adsorb phenolic compounds, thus obtaining active soluble enzymes from peppermint leaves. PVP has since then been used successfully to reduce the effect of phenolic compounds as an enzyme inhibitor in the determination of NRA (Dirr et al, 1973; Klepper and Hageman, 1969; Matsumoto et al, 1979)

and glutamine synthetase activity (GSA) in a number of plants (Kang and Titus, 1980; Matsumoto et al, 1980; Riov and Brown, 1976; Stewart and Rhodes, 1977; Stewart and Rhodes, 1978). It has also been demonstrated that the addition of a protein such as BSA to the extracting medium (Schrader et al, 1974) affords protection to NRA from degradation by proteolytic enzymes after extraction. Casein has also been used as a protecting agent against proteolytic enzymes in the extraction of NRA from maize (Harel et al, 1977; Reed et al, 1980; Wallace, 1978), wheat (Sherrard et al, 1979; Sherrard and Dalling, 1978), barley (Lewis et al, 1982b) and rice suspension culture (Yamaya and Ohira, 1977).

There is some doubt as to the validity of using an in vivo NR assay. Yoneyama (1981) working with seven different plants including sunflower found that in vivo assays of NR under aerobic conditions may give underestimated results due to nitrite reduction taking place. Mills et al, (1984) found that either under air or nitrogen, less nitrite was accumulated than nitrate assimilated from tomato leaf sections suggesting that nitrite accumulation in the in vivo NR assay was not an adequate parameter for the estimation of nitrate utilization. Soares, Lips and Cresswell (1985) found that the measurement of nitrite accumulation underestimated nitrate reduction in tissue kept under conditions which allowed nitrite accumulation. They found that the ratio of nitrite accumulated to nitrate reduced,

varied with time, since the two processes have different kinetics and nitrite accumulation levels off earlier than nitrate reduction. Therefore conclusions on the functioning of NR, based on *in vivo* measurements, have to be drawn very carefully (Stulen, 1986). *In vitro* NR assay measures the potential activity of the enzyme and therefore probably overestimates the actual rate in the plant (Dale, 1976; Hageman, 1979; Stuelen, 1986). In spite of the probable overestimation in using the *in vitro* assay method, it was decided to use this procedure as described by Lewis, Watson and Hewitt, (1982b) for NRA determinations.

This investigation examines the effect of both casein and PVP as protectants against proteolytic enzymes and phenolic inhibitors respectively in the determination of NRA and GSA in the leaves and roots of *Helianthus annuus*.

8.2 Experimental Methods

Enzyme assays were carried out using *Helianthus annuus* plants which were grown on a 2 mM nitrate Long Ashton hydroponic feeding solution (Chapter 2, Section 2.1) for 4 weeks. Enzyme determinations were made between 2 - 4 hours after the start of the photoperiod as described in Chapter 2, Section 2.9.

Crude extracts of NR were prepared from leaves and roots as described in Chapter 2, Section 2.9.1 and *in vitro* NRA determined as described in Chapter 2, Section 2.9.2.

Crude glutamine synthetase (GS) extracts were obtained (Chapter 2, Section 2.9.1) for *in vitro* GSA assays (Chapter 2, Section 2.9.3) from leaves and roots of sunflower plants.

8.3 Results

Table 8.1 exhibits the effect of casein and polyvinylpyrrolidone (PVP) in the extraction medium on the *in vitro* NRA of the leaves and roots of *Helianthus annuus*. The extraction medium without casein or PVP indicated a greater NRA for the root than the leaf.
In the presence of casein in the extracting medium there was a slight increase in root NRA and a 20-fold increase in leaf NRA when compared to the NRA of extracts without protectants.

In the presence of PVP in the extracting medium there was a doubling in the root NRA and a 5-fold increase in the leaf NRA compared to the NRA of extracts without protectants.

In the presence of both PVP and casein in the extraction medium there was a 7-fold increase in the NRA assayed for the root and a 66-fold increase in the NRA assayed for the leaf.

Table 8.2 exhibits the effect of the presence of casein and PVP in the extraction medium on the *in vitro* GSA of roots and leaves of *Helianthus annuus*. In the absence of these protectants in the extraction medium, low levels of GSA were found in the leaves and roots of this plant compared with those found in spinach leaves which exhibit an *in vitro* GSA of 90 μ moles synthetase activity (Miflin, 1974). In the presence of casein in the extracting medium, root GSA increased 2.6 times and leaf GSA increased 6 times. In the presence of PVP, root and leaf GSA both increased 3-fold. With both casein and PVP present, root GSA increased 4-fold and leaf GSA increased 5-fold over that exhibited by protectant-free extracts. The leaves thus possess an *in vitro* GSA which is 6 times greater than that exhibited in the roots.

8.4 Discussion

The results exhibited by the absence of protectants in the extracting medium for NR agree with the results obtained by Weissman (1972). The considerable increase observed for the leaf NRA with the presence of casein in the extracting medium indicated the possibility of

TABLE 8.1

Nitrate reductase activity in leaf and root of *Helianthus annuus* (mean value of twelve replicates ± the standard deviation).

Extracting Medium	Root NRA (μ mol $g^{-1}fw\ h^{-1}$)	Leaf NRA (μ mol $g^{-1}fw\ h^{-1}$)
m	0.52 ± 0.4	0.37 ± 0.3
m+c	0.67 ± 0.6	7.44 ± 0.9
m+PVP	1.34 ± 0.2	1.90 ± 0.6
m+c+PVP	3.40 ± 0.6	24.70 ± 0.4

m extracting medium alone

c casein

PVP polyvinylpyrrolidone

TABLE 8.2

Glutamine synthetase activity in leaf and root of *Helianthus annuus* (mean value of twelve replicates ± the standard deviation).

Extracting Medium	Root GSA (μ mol $g^{-1}fw\ h^{-1}$)	Leaf GSA (μ mol $g^{-1}fw\ h^{-1}$)
m	5.6 ± 1.2	27.0 ± 15
m+c	14.9 ± 0.4	129.8 ± 5
m+PVP	17.1 ± 1.0	90.3 ± 22
m+c+PVP	23 ± 1.0	141.0 ± 2

m extracting medium alone

c casein

PVP polyvinylpyrrolidone

the presence of proteolytic enzymes interfering with the *in vitro* NRA assay in the leaf. The increased NRA exhibited upon the addition of PVP to the extracting medium, indicated the presence of phenolic compounds in the leaf and the root as inhibitors of *in vitro* NRA. It would appear from the results obtained with both casein and PVP in the extracting medium that both proteases and phenolic compounds inhibit *in vitro* NRA in *Helianthus annuus*, and that the leaves possess a much higher level of NRA than do the roots.

The low values obtained for GSA without protectants could have resulted from the presence of phenolic compounds and/or proteases. The presence of casein in the extracting medium produced increased values for GSA in both the roots and leaves indicating the possible interference from proteolytic enzymes. The increased values for GSA of both roots and leaves with the presence of PVP in the extracting medium indicated the possible inhibition of the *in vitro* GSA assay by phenolic compounds. The increased results from the presence of both casein and PVP indicate that both proteases and phenolic compounds inhibit *in vitro* GSA.

The presence of both casein and PVP in the extracting medium would therefore appear to be necessary in determining both

in vitro NRA and GSA in *Helianthus annuus*. The high level of activities shown for NR and GS activity would indicate a reason for the rapid loss of virtually all nitrate from the leaves in the vacuum infiltration experiments over 24 hours (Chapter 7, Section 7.3, Tables 7.1 and 7.2).

CHAPTER 9

CONCLUSIONS

This study has investigated a number of aspects of the reaction of Helianthus annuus L. to ammonium and nitrate nutrition. In this chapter the conclusions which have been drawn from the work reported in Chapters 3 - 8 are summarised and briefly discussed. These conclusions are as follows:

1. Ammonium + nitrate nutrition produces sunflower plants with greater masses than either nitrate-only or ammonium-only fed plants. Ammonium as a nutrient nitrogen source, produces 4 week old sunflower plants with greater shoot mass than those fed nitrate (Chapter 3, Section 3.1). Similarly larger root masses are produced by plants fed nitrate compared with those fed ammonium. The combined effect of the two nitrogen sources (ammonium + nitrate) produces plants with a larger root mass than nitrate fed plants and a larger shoot mass than ammonium fed plants indicating that the combined source would be most beneficial in sunflower plant growth (Chapter 3, Table 3.1). Ammonium + nitrate fed plants gained more dry mass per litre water lost than did nitrate-only fed plants which in turn gained more dry mass per litre water lost than did the ammonium-only fed plants (Chapter 3, Table 3.2). The leaf area was also shown to be greater in the mixed feed plants than in either nitrate-only

or ammonium-only grown plants (Chapter 3, Table 3.1). Thus the ammonium + nitrate fed plants have the benefit of the large root mass needed for nutrient and water uptake and the large leaf area for light interception needed for the production of photosynthate. Consequently, ammonium + nitrate fed plants have better plant growth and water use efficiency in dry mass production per litre water lost, than either ammonium or nitrate grown plants. It is therefore very important that when the combined source of ammonium + nitrate is used, the nitrification of ammonium and the denitrification of nitrate be prevented through the use of inhibitors as discussed in Chapter 1.

2. The ammonium-only fed plants exhibited a statistically lower photosynthetic rate than the nitrate or ammonium + nitrate fed plants which were shown to be closely related with respect to photosynthetic activity (Chapter 3, Section 3.3.2). This could partially explain the the overall higher plant masses of the ammonium + nitrate and nitrate fed plants than ammonium-only fed plants (Conclusion 1). Dark respiration rates were higher in mixed feed and nitrate fed plants as compared to those fed ammonium.

3. There appears to be a higher energy generation in sunflower roots fed nitrate than in those fed ammonium-only. The higher values for root respiration (Chapter 3, Table 3.6, Section 3.3.4) of plants fed nitrate indicated a higher

energy expenditure than in the ammonium-only fed plants. This energy expenditure is necessary not only for the active transport of nitrate into the roots but also the reduction of part of that nitrate in the root. The roots of the nitrate fed plants were respiring CO_2 at a slightly greater rate than the mixed feed plants and nearly 3 times as rapidly as the ammonium-only fed plants per fresh gram mass, indicating a higher requirement for energy in both the nitrate and ammonium + nitrate fed plants than in the ammonium-only fed plants.

4. Ten week old sunflower plants fed ammonium + nitrate are more water use efficient and produce greater fruit mass than plants fed nitrate or ammonium singly. As was shown for 4 week old sunflower plants (Chapter 3, Section 3.3.1), 10 week old sunflower plants fed ammonium + nitrate can combine the shoot development effect of ammonium with the root development of nitrate to produce a plant which is larger than those grown only in nitrate or ammonium (Chapter 3, Section 3.3.5). The ammonium + nitrate fed plants also produce a greater mass of fruit than either nitrogen source alone. The increased fruit mass production of the mixed feed plants, combined with increased dry mass production per litre water lost for the whole plant, have important agronomic consequences.

5. The ammonium + nitrate fed plants exhibited a higher net photosynthetic (Pn) rate than either nitrate-only or ammonium-only fed plants (Chapter 3, Figure 3.6). Pn rates were higher in the mature leaves and lowest in the youngest and oldest leaves for all three feeding treatments. The youngest leaves of the ammonium + nitrate fed plants, however, showed activity comparable to the mature leaves (Chapter 3, Figure 3.6). The Pn rates for the mature fruiting plants were much lower than those of the younger plants indicating a lower requirement for the products of photosynthesis (Chapter 3, Section 3.3.6).

6. The major amino compound transported via the xylem stream (Chapter 4, Figures 4.4a, b and c), regardless of nitrogen source, was glutamine. On a percentage basis, the glutamine content was 37% in nitrate-only fed plants, 65% in ammonium + nitrate fed plants and 74% in ammonium-only fed plants.

7. Nitrate appears to be assimilated primarily in the shoot. The ammonium-only fed plants (Chapter 4, Table 4.1 and Figure 4.1) showed no nitrate present in the xylem sap, whereas the sap of the mixed feed plants contained 50% and that of nitrate-only fed plants contained 80% nitrogen as nitrate-N. Within an 8 hour period ^{15}N results (Tables 4.1 and 4.2) indicated that nearly all of the xylem nitrate was from nitrate which was newly taken up by the roots. This

demonstrates that most of the nitrate from nitrate-only and ammonium + nitrate fed plants was transported to the shoot and further indicates the shoot as the major site of nitrate reduction.

8. Ammonium is assimilated mainly in the roots of sunflower plants. Although ammonium was contained in the nutrient solution for two of the three plants analysed, little ammonium was found in the xylem sap. This lack of ammonium, and the very high concentration of free amino compounds in the xylem sap, indicates that most of the absorbed ammonium is assimilated in the roots (Chapter 4, Table 4.1).

9. Ammonium is absorbed and assimilated more rapidly than nitrate when these nutrients are fed singly. The ammonium-only fed plants assimilated nitrogen nearly twice as fast as the nitrate-only fed plants, based on the respective ^{15}N contents of the plants at the 4 hour harvest (Chapter 5, Section 5.3.1).

10. There was a possible suppression of nitrate uptake by ammonium which decreased with time in sunflower plants fed ammonium + nitrate. From the ammonium + nitrate fed plant results, it was found that the major contributor to the free amino ^{15}N content in the root was the ammonium ion at both the 4 and 8 hour harvest (Chapter 5, Section 5.3.3). A

major portion of the free amino content in the shoot was from ^{15}N ammonium, however there was an increasing contribution by the nitrate source from the 4 hour to the 8 hour harvest (Chapter 5, Section 5.3.1). Ammonium appears to initially inhibit the incorporation of nitrogen originating from nitrate into the uptake and bound fraction in the mixed feed plants.

11. Nitrate and ammonium equally contribute to the shoot free amino nitrogen content in ammonium + nitrate fed sunflower plants. The three mixed feed experiments showed that the free amino content of the shoot at the 8 hour harvest equally came from nitrate and ammonium and the content was twice that from either source alone (Chapter 5, Section 5.3.4.2). The large free amino ^{15}N content of the $^{14}NO_3^-$ + $^{15}NH_4^+$ fed plants in comparison to the $^{15}NH_4^+$-only fed plants may have in some way been due to a translocation promoting effect of nitrate (Lips et al, 1987).

12. Nitrate, either alone or as ammonium + nitrate, contributes more nitrogen to the meristem apical region of Helianthus than does ammonium, i.e. there appears to be a greater diversion of nitrogen to the shoot apex region in young sunflower plants originating from nitrate feeding than from ammonium feeding (Chapter 5, Section 5.3.4.2).

13. Nitrogen from an ammonium + nitrate source is more rapidly assimilated than from either source singly in Helianthus. This was indicated by the total ^{15}N organic content of the mixed feed plant which showed that although the concentration of each of the nitrogen sources was one half of that used for the single source nitrogen fed plants, the net result was a summation of the ^{15}N contents of the two different nitrogen sources which were assimilated in different parts of the plant (Chapter 5, Section 5.3.4.4).

14. The enzymes for nitrate assimilation are still active in the shoot and those for ammonium assimilation are still active in the roots during fruit filling. Experiments using ^{15}N chased with ^{14}N in fruiting sunflower plants indicated that the assimilation of nitrate was taking place only in the upper part of the shoot while ammonium was being assimilated in the root (Chapter 6, Sections 6.3.1 and 6.3.2). This division of assimilation sites was also found in the young plants (Chapter 5, Sections 5.3.1 and 5.3.2).

15. Fruiting plants are still able to assimilate and translocate newly supplied ^{15}N, primarily in the capitulum. The continuation of a nitrogen supply up to the fruiting stage should thus assist in increasing the productivity of the plant (Chapter 6, Section 6.3.3). This was shown by: (a) the capitulum of $^{15}NO_3^-$ fed plants contained one half of the shoot free amino ^{15}N from nitrate reduction (Chapter 6,

Section 6.3.1), (b) in $^{15}NH_4^+$ fed plants the capitulum contained 15% of the free amino ^{15}N in the shoot (Chapter 6, Section 6.3.2), and (c) one half of the shoot free amino ^{15}N in the ammonium + nitrate fed plants was found in the capitulum.

16. The fruit is capable of storage of newly assimilated nitrogen in the fruit (Chapter 6). Although the total bound ^{15}N was greater in the ammonium fed plants (Chapter 6, Section 6.3.2) than in the nitrate fed plants (Chapter 6, Section 6.3.1), there was more bound ^{15}N in the fruit of the nitrate-only than the ammonium-only fed plants. The total bound ^{15}N for the mixed feed plants was greater than either of the singly fed plants again indicating the additive effect of the two nutrients.

17. Only senescing leaves do not benefit from newly assimilated nitrogen at the fruit filling stage. The presence of bound ^{15}N in the roots and the top, upper and even lower leaves indicated that the leaves and roots were still producing protein even though the bottom leaves were senescing as the fruit was filling. The senescing leaves were no longer receiving or storing newly incorporated ^{15}N in any of the plants (Chapter 6, Sections 6.3.1, 6.3.2 and 6.3.3).

18. The enzymes of nitrate assimilation are still very active in the leaves of mature sunflower plants at the time of fruit filling and older leaf senescence. There was very little $^{15}NO_3^-$ left anywhere in the plant 24 hours after leaf infiltration indicating a virtually complete conversion of nitrate to other nitrogenous compounds. Newly reduced nitrogen from these mature leaves, although distributed mainly to the capitulum, was translocated to the root and all other actively growing regions in the shoot (Chapter 7, Section 7.3).

19. Newly reduced nitrate from mature leaves of fruiting plants is translocated primarily to and bound in the capitulum with a minor portion to the root. From the 4 to 8 hour harvest there was a 63% increase in the free amino ^{15}N from the newly reduced $^{15}NO_3^-$ transferred from the infiltrated leaf to the fruit. At the time of the 24 hour harvest, free amino ^{15}N was found only in the roots, stem and plant parts above the feed leaves but not in the older leaves below them. The capitulum receiving 96% of the free amino ^{15}N above the feed leaves indicated that the capitulum was the most actively growing plant part and that mature leaves were supplying nutrient mainly to the capitulum (Chapter 7, Section 7.3).

20. Polyvinylpyrrolidone and casein are needed in the extraction medium for determining both *in vitro* nitrate reductase and *in vitro* glutamine synthetase. The results obtained with both casein and PVP in the extracting medium showed a 7-fold activity in the root and a 67-fold increase in the leaves. These results indicate that both proteases and phenolic compounds inhibit *in vitro* NRA in *Helianthus annuus*. The increased results from the presence of both casein and PVP (a 4 times increase in the roots and a 5 times increase in the leaves) indicate that both proteases and phenolic compounds inhibit *in vitro* GSA (Chapter 8, Section 8.2).

21. The leaves of sunflower plants have a higher nitrate reductase and glutamine synthetase activity than do the roots. The leaves exhibited a 7 times greater nitrate reductase activity and a 6 times greater glutamine synthetase activity than did the roots in sunflower plants. These results indicate the plant's response to the high percentage of newly absorbed nitrate transported via the xylem to the shoot (Chapter 4, Section 4.3).

22. Ammonium + nitrate fed plants appear to be superior in many respects to plants fed either nitrate-only or ammonium-only. Ammonium + nitrate produces plants with a larger mass than either nitrogen source singly at both the vegetative and fruiting stages of growth. The fruit mass in the mixed

feed plants is 25% greater than that in the nitrate-only fed plants and more than double that in the ammonium-only fed plants. The mixed feed plants combine the large roots of the nitrate-only fed plants to ensure adequate water and nutrient supplies with the large shoot of the ammonium-only fed plants which gives maximum surface for photosynthate production. The mixed feed plants are more water efficient, producing more mass per litre of water lost than either source singly.

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