EFFECT OF DIFFERENT NITROGEN SOURCE

ON RESPIRATORY CARBON USE

IN ROOTS OF

TRITICUM AESTIVUM L. VAR. ZARAGOZA.

by: P.J.D. WINTER

HONOURS PHYSIOLOGY PROJECT - 1988

ABSTRACT

The differences in carbon allocation to uptake or assimilatory processes between ammonium- and nitrate-fed wheat plants suggests possible differences in the overall carbon use-efficiency in root metabolism. Respiratory carbon use was estimated by measuring the carbon dioxide evolution in roots. No significant differences could be detected.

OUTLINE OF CONTENTS

INTRODUCTION

The form in which nitrogen is taken up from the environment, has been shown to have differential effects on the metabolism, as their assimilation involves different processes, each with its costs and benefits to the overall energy budget of the plant (Cox & Reisenauer 1973; Hageman 1984).

A large amount of research has been devoted to the use of nitrogen fertilizers for crop plants, and with advances being made in the development of nitrification inhibitors, the use of ammonium as nitrogen source has often been weighed up against the more commonly used nitrate fertilizers (Hageman 1984). The use of ammonium fertilizers are especially important in areas which suffer considerable nitrate losses due to leaching (Lewis 1986).

Wheat has the ability to utilize both ammonium and nitrate as nitrogen source. Nitrate is taken up actively in the roots, probably by means of an ATP-facilitated anion carrier system in the plasmalemma (Hocking, Steer & Pearson 1984), requiring an input of photosynthate from the shoot to meet the respiratory demand. The assimilation of nitrate has been shown to occur almost entirely in the shoot(Lewis, Fulton and von Zelewski 1986). Although ammonium on the other hand is predominantly taken up by simple diffusion, it requires respiratory energy for the assimilation (detoxification) in the root, as well as the import of carbon skeletons as substrate for this assimilation into amino-acids

(Lewis, Fulton & von Zelewski 1986).

These differences imply a differential carbon-allocation to roots of wheat plants raised on different nitrogen sources. Barneix *et al.* (1984) found that nitrate grown wheat plants had lower respiration rates than ammonium grown plants. In this project an estimate is obtained for differences in the amount of carbon allocated to root respiration, between plants grown hydroponically on either source or a combination of both sources, using a method of Infra red gas analysis to measure the carbon dioxide evolved during respiration in the rooting system.

MATERIALS AND METHODS

Germination

Seeds of *Triticum aestivum* L. var. Zaragoza were surface sterilized by soaking in $HgCl_2$ (0,2% w/v) for two minutes, then rinsing in deionised water (15 x 300ml). Seeds were sown in sterile Petri dishes between two moistened discs of filter paper, and placed in a germination cabinet (Conviron model G30, Controlled environments Ltd., Winnipeg, Canada) at 25°C.

Growth of Plants

After 8 days, germination was complete and each seedling was transferred to a 5 l. jar (figure 1), which was covered in foil to exclude light from the root area and prevent algal growth. Seedlings were held in place using glass wool in a section of tubing fitted in a hole in the lid, which allowed for expansion as the stems grew. The following three treatments were set up in N-free Long-Ashton nutrient solution (Hewitt 1966):

i) 8 jars + 4mM KNO_3

ii) 8 jars + 4mM NH_4Cl

iii) 8 jars + 2mM KNO_3 + 2mM NH_4Cl

$CaCO_3$ (10g) was added to each pot to act as a buffer for changes in pH associated with NH_4^+ uptake (Hageman 1984;

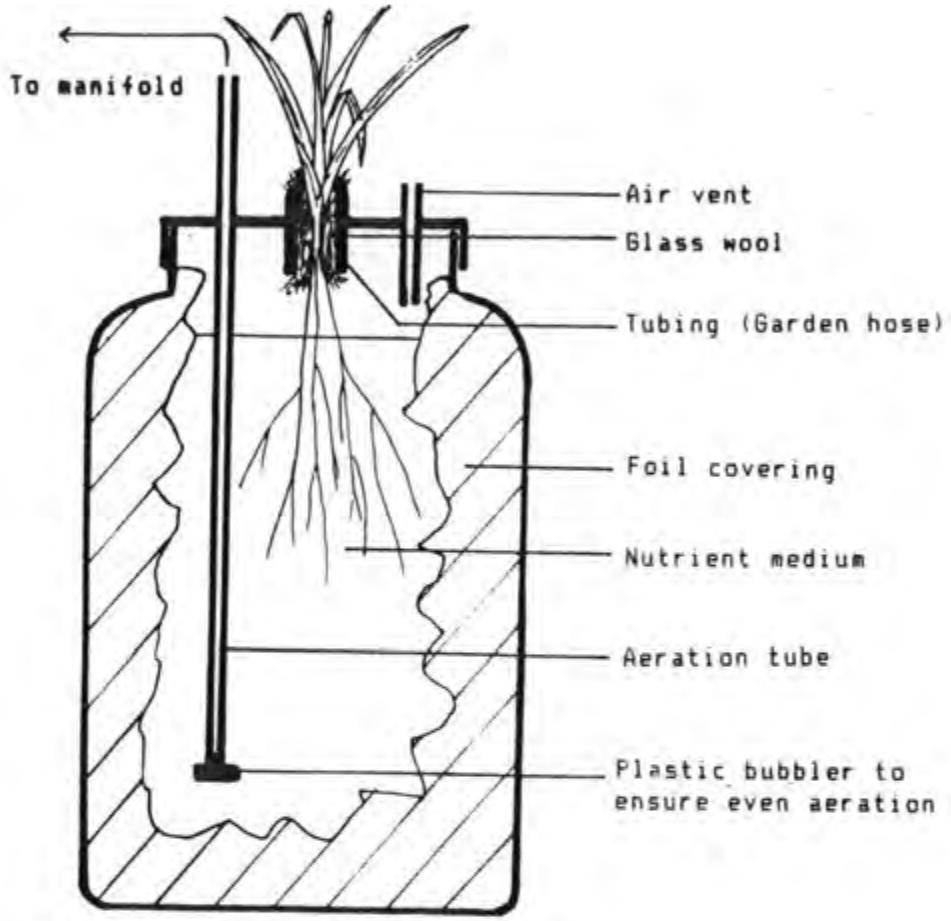

FIGURE 1 5l plastic jars in which the plants were cultivated.

Barber 1984).

Aeration was controlled using flow regulating polyethylene bubblers (Stark-Ayres, Epping) in conjunction with a manifold to maintain a steady flow of air through the solutions. This was done to prevent anaerobic conditions developing in the root medium.

The plants were grown for 5 weeks in a controlled environment growth cabinet (Conviron model E13, Controlled environments Ltd., Winnipeg, Canada) with a temperature regime of 15°C at night and 20°C during the 14hr photoperiod. Illumination was provided by Sylvania Cool White F48T12/CW/VHO flourescent tubes and 60 W incandescent lamps, at a photon flux density of 280 $\mu E.m^{-2}.s^{-1}$.

Solutions were changed every fourth day after the first week. Residual nitrate and ammonium levels were measured qualitatively using Szechrome NAS (Stock 1983) and Nessler's reagent (A.O.A.C. 1965) respectively to ensure that the nitrogen supply was adequate throughout the experiment.

The pH was maintained between 6,5 and 7,4 throughout the growing period. Changes in solution pH were monitored by measuring two random samples out of each treatment with a portable pH meter fitted with a glass electrode (PHM 80, Radiometer A/S Copenhagen, Denmark).

The plants were harvested after 36 days, separating the roots from the shoots as depicted (figure 2.). Roots were rinsed well in running de-ionized water, to wash off any remaining $CACO_3$ - deposits, then blotted dry. Fresh masses of roots and shoots were measured by difference in preweighed

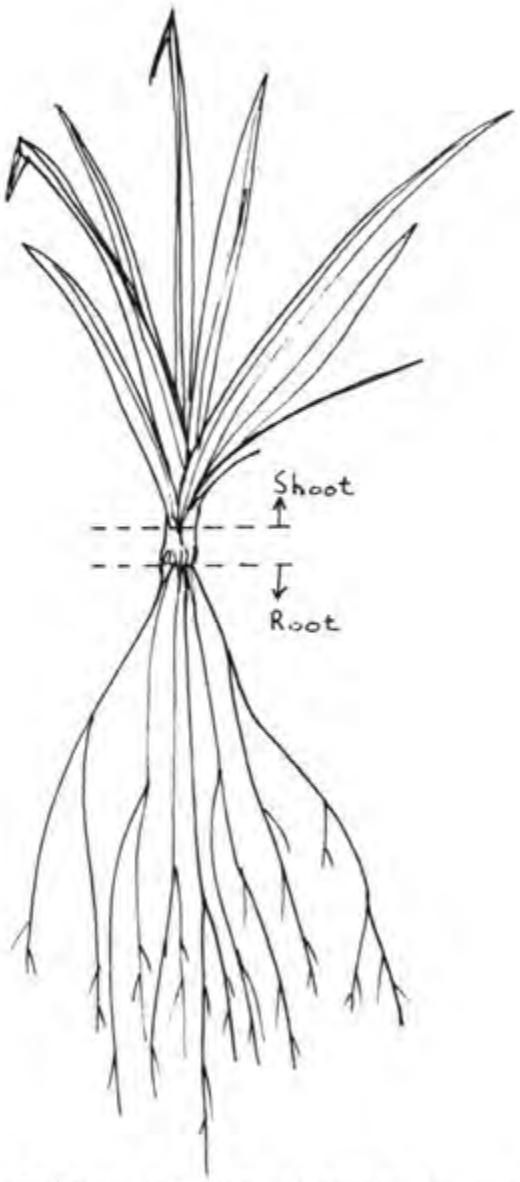

FIGURE 2 Separation of the plants into roots and shoots. For consistency of method, plants were cut just above and below the junction between stem and root, and the mid section was discarded.

paper envelopes, which were then oven-dried (80°C for 48hrs.), along with empty envelopes as controls to estimate the change in moisture content. Dry masses were measured, and corrected for moisture content.

Measurement of Photosynthetic Rates.

Photosynthesis was measured on day 35, using a portable Infra Red Gas Analyser (LCA-2, Analytical Development Company Ltd., Hoddesdon, England). Two measurements were taken, each on the third leaf from the top of different tillers. The photon flux density was standardized at c. 250 $\mu mol.m^{-2}s_{-1}$. Air temperature varied between 24,1° and 25,6°C. Relative humidity was 37%-56% at the time of measurement.

Measurement of Root Respiration.

CO_2 - evolution in the roots was measured after 34 d using an Infra Red Gas Analyser (225-MK3, Analytical Development Company Ltd., Hoddesdon, England.), set up as depicted in figure 3. The control consisted of a jar containing N-free nutrient solution to which $CACO_3$ was added. Air flow was regulated with an aquarium pump at rates of 200 or 300 $ml.min^{-1}$.

Flow rates into and out of the plant containers were balanced before the measurements were done, so that pressure remained constant in the system. This was done to avoid gas exchange between containers and atmosphere, as it was not

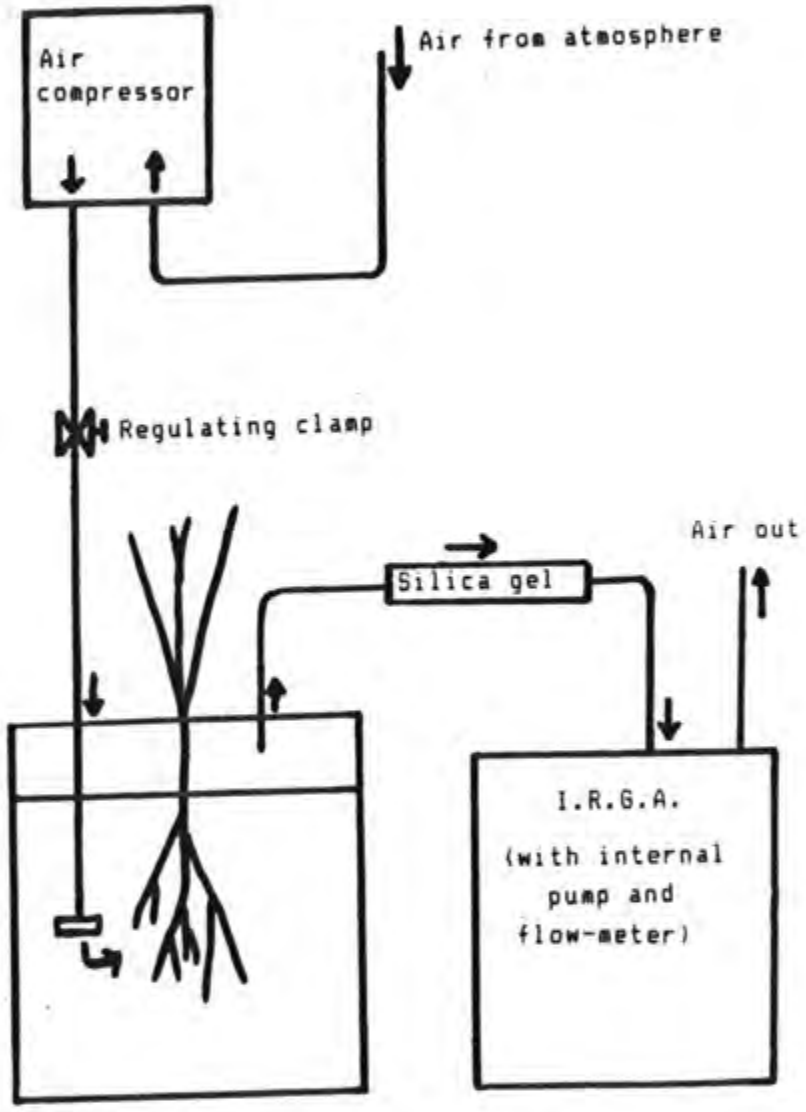

FIGURE 3 Diagram illustrating the open system used to measure CO_2-evolution in the containers.

possible to ensure total airtightness of the containers. All measurements were done in the absolute mode, after calibration against a reference mixture of 370 ppm CO_2.

Statistical analysis.

The results were analysed using one-way analysis of variance, in conjuntion with a multiple range test. The confidence limits were chosen at 95%.

RESULTS

Plant Masses

Shoot masses did not differ significantly (α=0,05) between the nitrate and ammonium treatments, but the combined nitrate and ammonium treatment shoot mass was significantly higher than both of these (fig.4.).

The only significant difference in root mass was between the ammonium and combined source treatments (fig. 4.). The lower root mass of NH_4^+ -grown plants was more consistent throughout the sample than that of NO_3^- -grown plants, where large within-sample variation was observed (fig. 5).

Shoot to root ratios (fig. 6) differed significantly (α=0,0006) between the two N-sources, with the combined source value close to that of NH_4^+, which was the highest (1 : 4,4 ; SE=0,19), compared to the NO_3^- value of 1 : 2,4 (SE=0,28).

Photosynthesis

Photosynthetic rates (fig.7) were similar on a per unit leaf area basis for all three treatments (fig.7). There was only a slight tendency for the ammonium-fed plants to show higher rates.

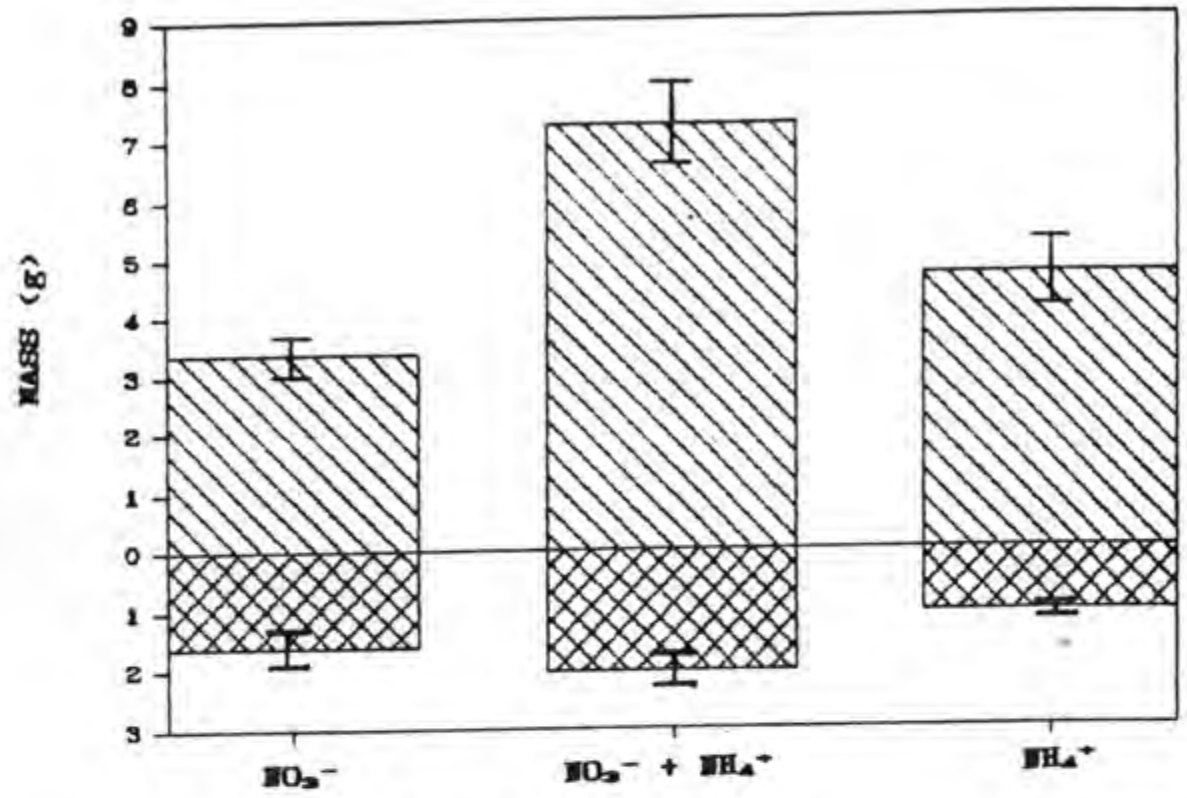

FIGURE 4 Shoot and root dry mass for the three treatments. (mean and s.e.) ROOT MASS SHOOT MASS

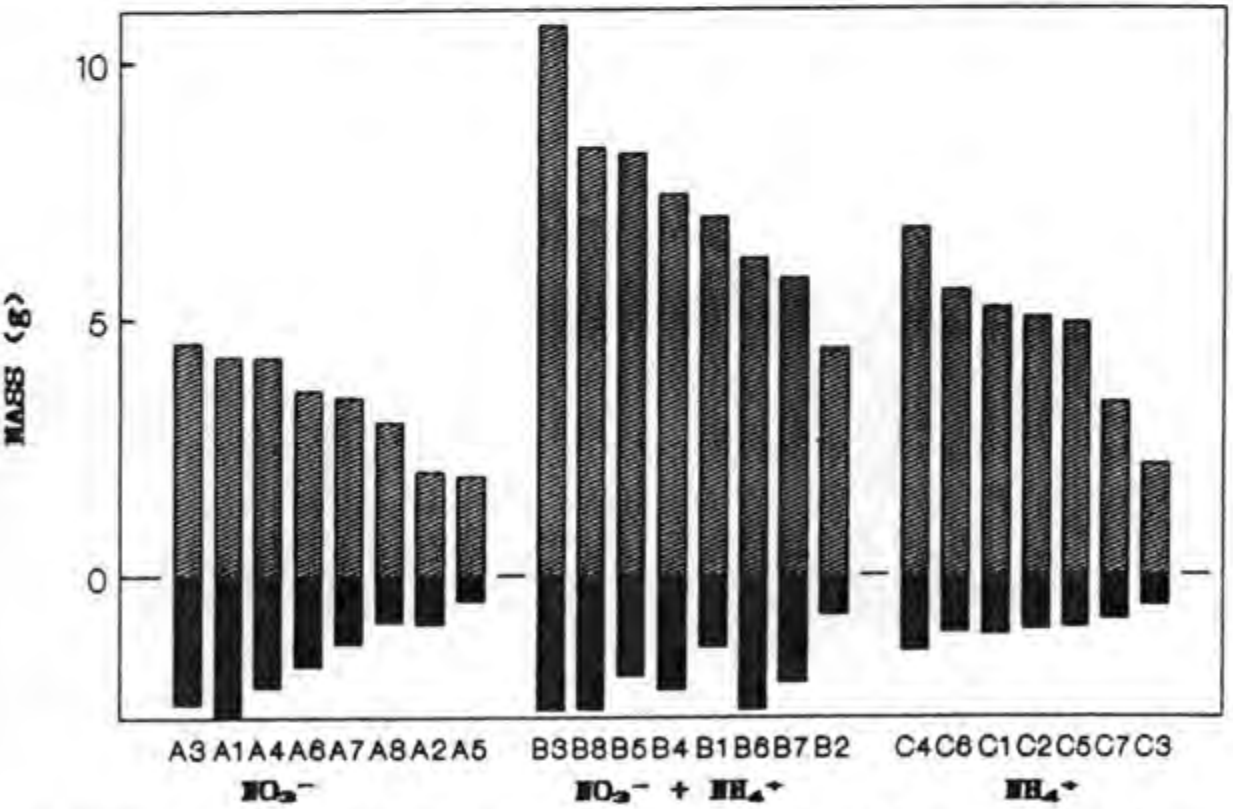

FIGURE 5 Variation of dry mass of shoots and roots within the three treatments. ROOT MASS SHOOT MASS

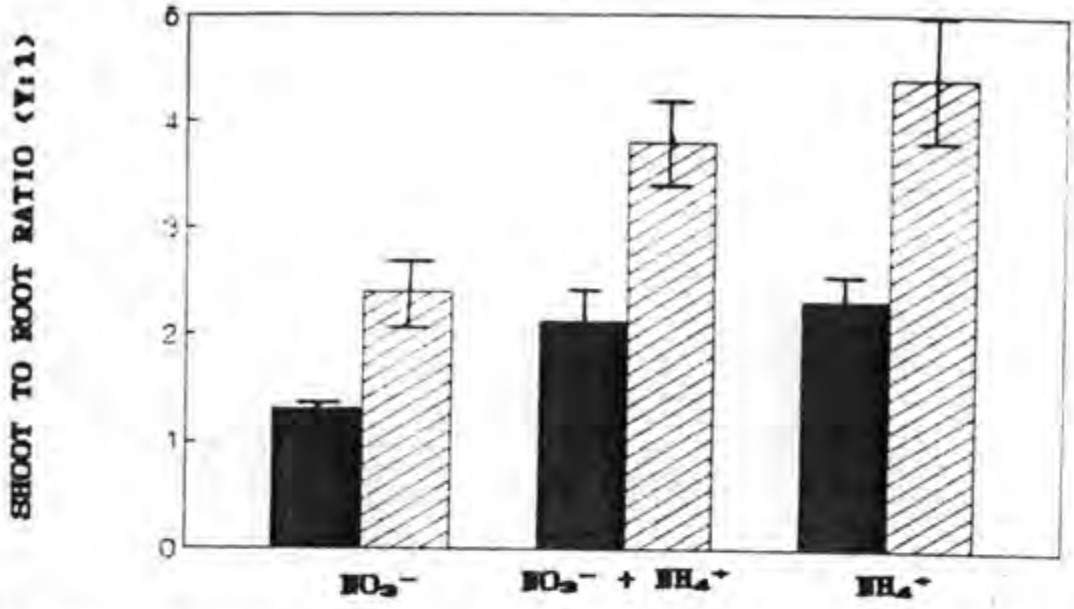

FIGURE 6 Shoot to root ratios for the three treatments, determined on a dry and fresh mass basis. The ordinate scale represents shoot mass relative to a root mass of 1. (mean and s.e.) fresh dry

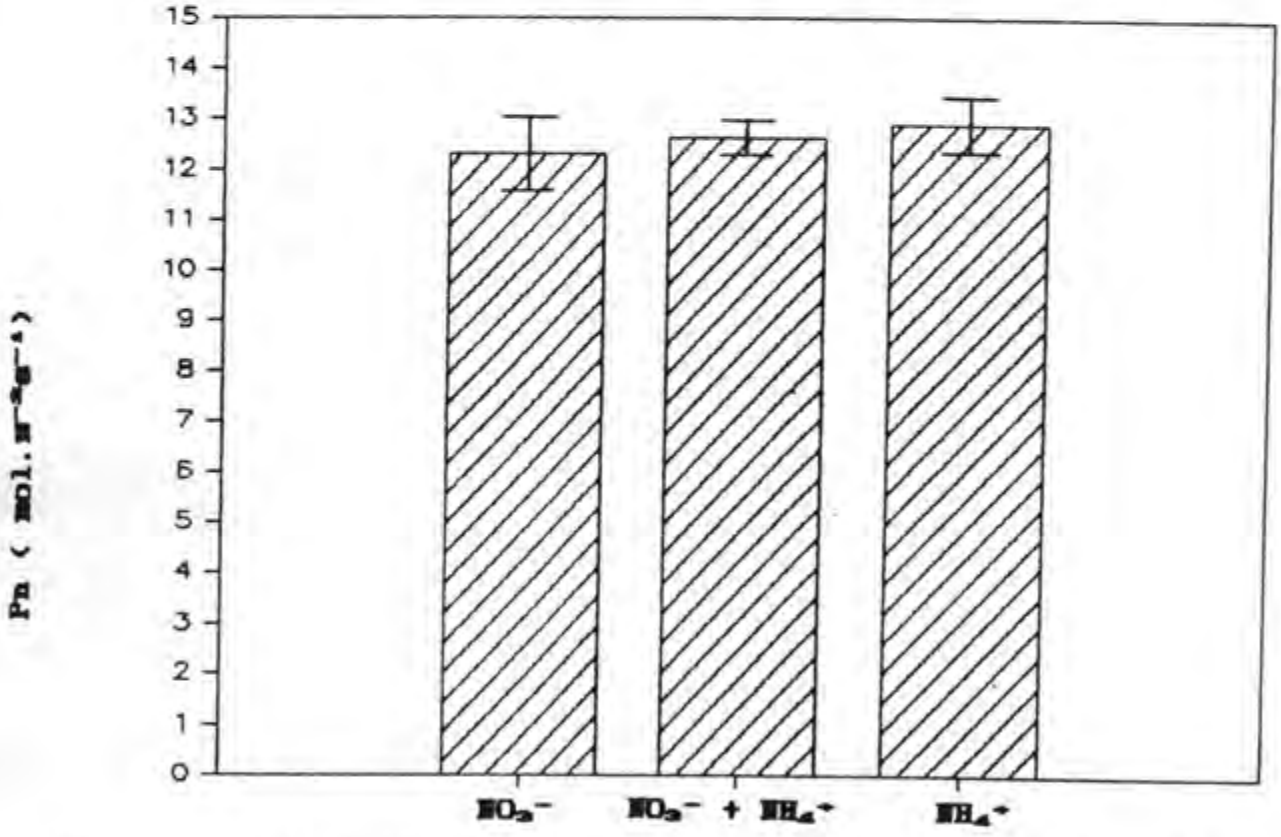

FIGURE 7 Photosynthetic rates per unit leaf area for the three treatments. (mean and s.e.)

Respiration Rates

Total root respiration did not differ between treatments (fig. 8). Variation within treatments obscured the slight variation between treatments (fig. 9). NO_3^- showed a tendency for the highest respiration rates in most replicates, and NH_4^+ for the lowest rates, with combined N-source values intermediate.

Likewise, for respiration rates expressed on a dry mass basis (fig.10), no differences were detected under the conditions of the experiment [1]. If any, there was a trend toward higher total respiration rates in the NH_4^+-treatment. The sample mean for the combined source treatment was lower than for the others, although the outliers in the latter could account for this trend (fig. 11).

1. Variation due to lack of control over experimental conditions are elaborated on in the discussion.

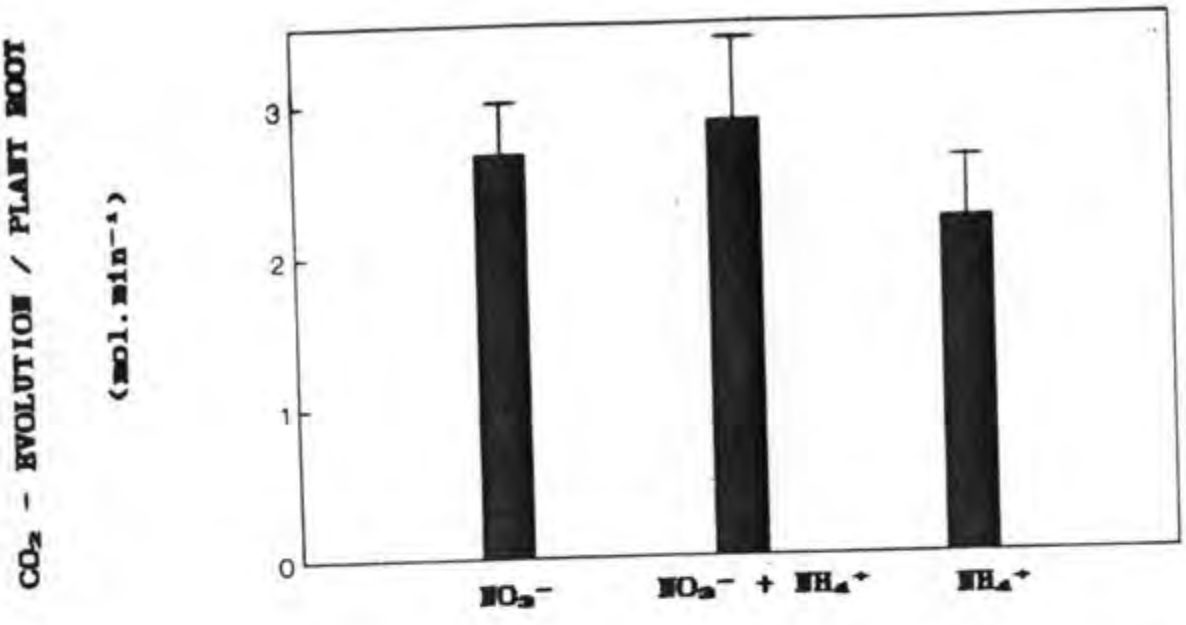

FIGURE 8 Total root respiration rates expressed as carbon dioxide evolution per plant root for the three treatments. (mean and s.e.)

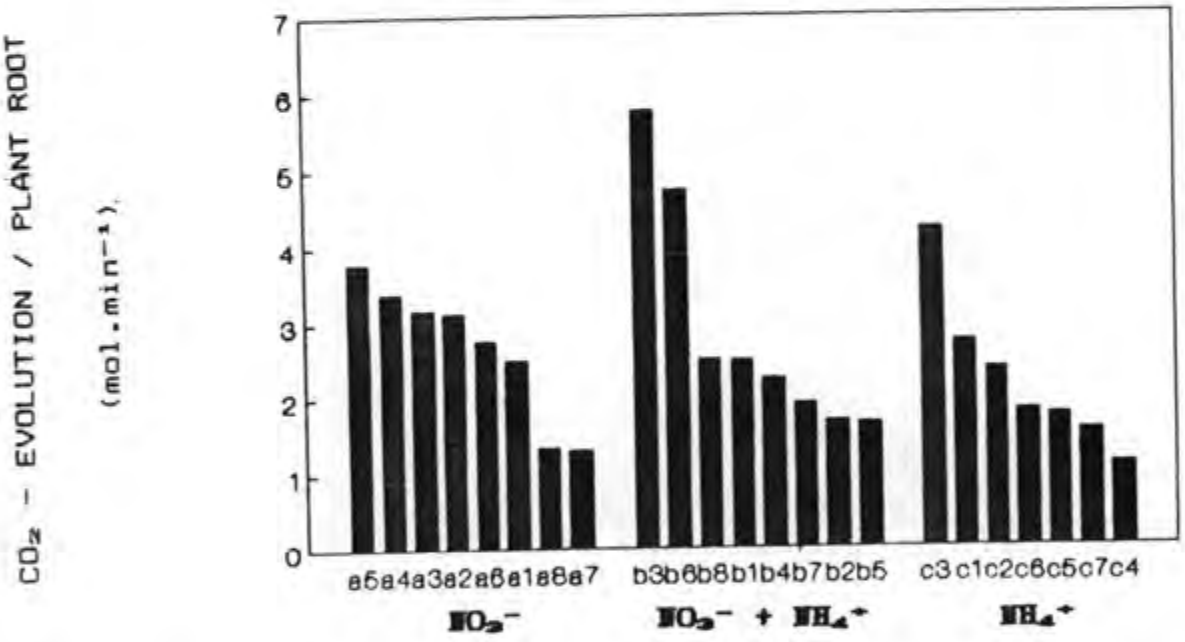

FIGURE 9 Variation of total root respiration rates within treatments.

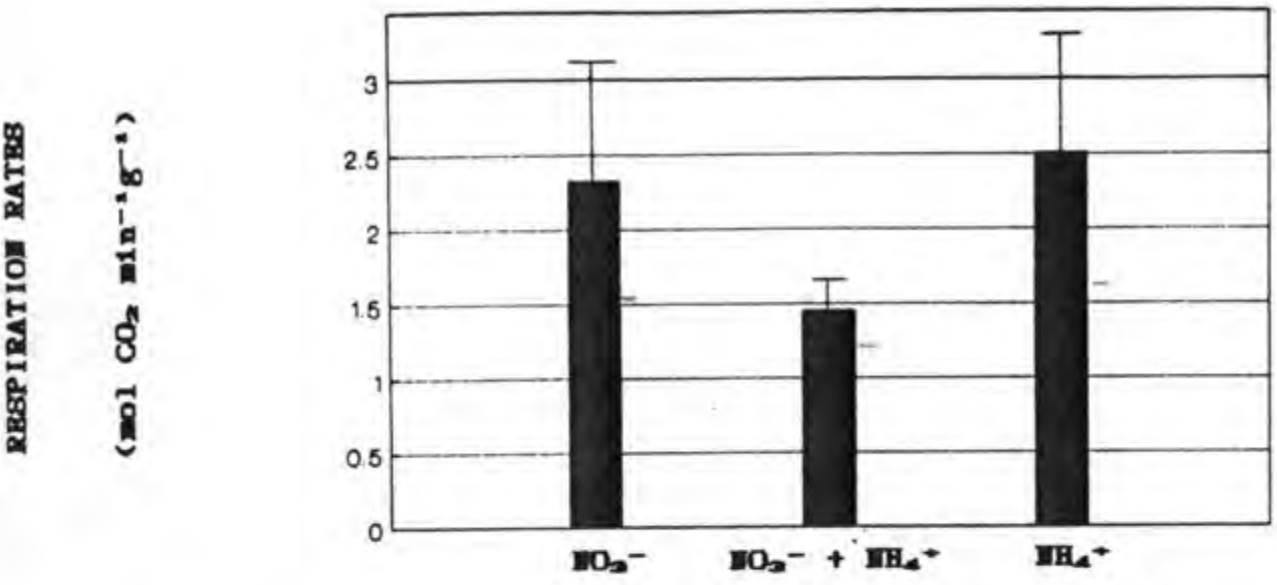

FIGURE 10 Root respiration rates per unit root mass (dry) for the three treatments. (mean and s.e.)

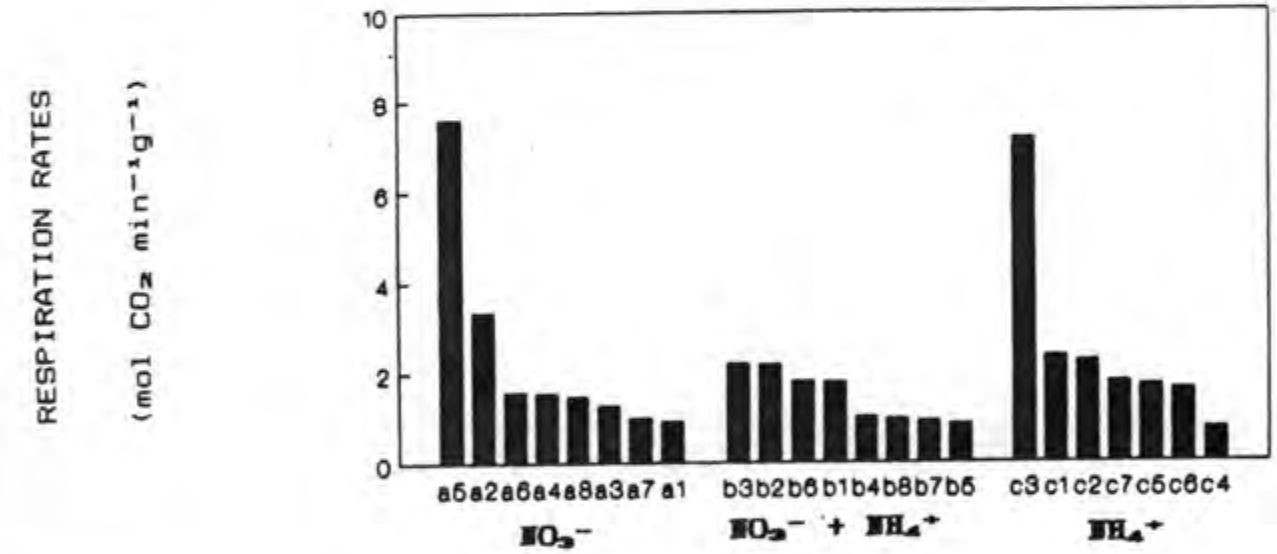

FIGURE 11 Variation of root respiration rates within treatments.

DISCUSSION

Plant masses

Results correspond within reasonable limits to what was found in previous experiments on *Triticum aestivum* (Lewis, Fulton & von Zelewski 1986), *Hordeum vulgare* (Lewis, Soares & Lips 1986), and *Pisum sativum* (de Visser & Lambers 1983) regarding root masses and root to shoot ratios. The high ratio in ammonium fed plants due to root stunting is typical. An exception to these results is that the ammonium treatment shows higher shoot mass than the nitrate treatment, resulting in higher total mass, whereas previously the ammonium-fed plants were smaller (Lewis, Fulton & von Zelewski 1986).

Photosynthesis

Under the conditions of this experiment then, wheat plants responded better to ammonium feeding in terms of vegetative yield than to nitrate feeding. This could be expected from the higher uptake rate of nitrogen as ammonium than nitrate (Lewis & Chadwick 1983; Lewis, Soares and Lips 1986), and the corresponding slightly higher (although not significantly so) photosynthetic rates, probably due to an increase in protein and thus RuBisco levels in the leaves (Cox & Reisenauer 1973; Golvano & De Felipe 1986). The relatively high pH could have led to the nitrate grown plants being adversely affected, as high pH restricts NO_3^-

availability and favours ammonium uptake (Novoa & Loomis 1984).

The high vegetative yield of the combined source treatment has been suggested to be due to an increase in carbon allocation to the structural framework (Lewis, Soares & Lips 1986). This can be afforded because of reciprocal suppression of the negative effects of each source *per se* at normal N-feeding levels, and the combined benefits of a rapid absorption rate and site division for assimilation between root and shoot (Lewis *et al.*,1986).

Respiration

Based on the evidence that energy requirements for the active uptake of nitrate, and for root growth and maintenance are higher than those for ammonium assimilation (de Visser & Lambers 1983; de Visser 1984), respiration rates were expected to be higher in nitrate fed plants, as was found for *Pisum sativum* (de Visser & Lambers 1983; de Visser 1984). Results fail to show this for *Triticum aestivum*, however, the tendency rather being toward lower rates in the nitrate treatment. This corresponds to root respiration rates measured in *Plantago* (Blaquiere, Hofstra & Stulen 1987) and in wheat (Barneix *et al.* 1984). These experiments are however, strictly not comparable, as the experimental procedures were different to that used here.

The expectation that respiratory carbon availability in the root is limiting to respiration does not find any support

in these results, as ammonium-grown plants show respiration rates equal to those of nitrate-grown plants despite photosynthate being additionally allocated as substrates for amino-transfer reactions in the detoxification process.

From the similarity observed between the treatments, we can only speculate on the following possible interpretations:

1) Respiration rates (carbon energy expenditure) in plants raised on different nitrogen sources are the same and respiration only differs in the way that this energy is allocated to different processes. In other words, the energy required for the active uptake of nitrate, as well as for the growth and maintenance of the root system, roughly equals the cost of ammonium assimilation into amino acids and their recycling through the root.

2) Respiration rates are sensitive to slight changes in experimental conditions, resulting in large variation within the treatments.

3) Respiration rates may be different, but poor design of the experiment or control of conditions during growth or measurement, could have lead to readings which are not representative of the actual situation (e.g. the order in which the measurements were taken).

Causes of variation in measurements

The large amount of variation in respiration rates within treatments can be attributed to several factors. These may be divided into physiological and methodological factors:

A) Physiological causes of variation

1) A severe limitation to this type of investigation where a generalizaton is sought to explain a phenomenon, is the necessary restriction to small sample sizes, bringing the natural genotypic variation into play. This effect is aggravated in that single plants were used which may not be genotypes representative of the population.

2) Pathogenic effects on plant metabolism cannot be excluded, despite precautions taken to ensure sterile conditions.

B) Methodological causes of variation.

1) Because the air source used during the measurements was not the same as that in the controlled environmental cabinet, there was a time lapse for the saturation of nutrient media with the measuring air. This disruption of the equilibrium condition within the system, could have lead to false readings during the equilibration period.

2) The presence of carbon in the $CaCO_3$ which was added to the nutrient media, could further complicate this equilibrium.

3) The time of measurement could have biased the readings, as treatments were not randomized, so that readings of the nitrate treatment were obtained in the morning, whereas those of the ammonium treatment were obtained in the afternoon.

4) The plastic bubblers used to control aeration of the nutrient solutions were found to be ineffective, particularly due to clogging up with $CaCO_3$ precipitate, so that they had to be replaced regularly. Some of the plants may therefore have been temporarily exposed to anaerobic conditions, which could affect their metabolism. Equal aeration rates could not be maintained, so that there could have been different responses between plants to the standard measurement condition.

5) The plants received no aeration for a period of up to twelve hours, due to a mechanical failure in the air compressor, on the day before the measurements were taken. This complication severely hampered the control of the experimental conditions.

6) Difficulty was experienced in sealing the jars to avoid analytical air escaping from the system or outside air

from entering it.

7) The presence of respiring or photosynthesizing microflora in the measuring vessels could alter the readings obtained. Slight traces of algae were detected at the root bases, growing in the light filtering through the glass wool.

8) The I.R.G.A. could not measure accurately the [CO_2] higher than 500 ppm. Instrumental error therefore makes a large contribution toward the detected variation.

Suggestions for Improving the Method

Other than by adopting a totally different approach to the experiment, such as by growing the plants in soil as was done by Warembourg *et al.* (1982) and trapping the carbon dioxide in NaOH solution, the following improvements to the existing method can be made:

1) A larger sample size is advisable, but may be impractical, as it is restricted by the time required for the measurement of each replicate.

2) Replicates consisting of several genotypes should be used. This can be done by growing several plants in a pot, but the total respiration rate will be too high to be recorded with the current instrumentation, unless the

CO_2 is diluted in some way.

3) Measurements should be done in the differential mode, i.e. measuring both the reference and the sample simultaneously,using a single air source. This again depends on the instrumental limitations.

4) Respiration rates with and without inhibition of nitrogen uptake and assimilation should be measured, in order to obtain an estimate of the respiration associated with other processes such as growth and maintenance. Uncouplers of oxidative respiration could also be used to establish the contribution of non-phosphorylating respiration.

ACKNOWLEDGEMENTS

I am grateful to Professor O.A.M.Lewis for suggesting the topic of this project, and for his patient supervision throughout the year.

I am also greatly indebted to the following people for rendering their services and advice: D.T.Barnes, M-T.Deignan, A.Jenssen, Dr.J.J.Kaiser, T.v.d.Leij and F.v.d.Heyden.

Literature Cited

A.O.A.C. (1965) Official Methods of Analysis. 10th ed. Hurwitz,W. (ed.) Association of Agricultural Chemists, Washington d.c.

BARBER,S.A. (1984) Nutrient balance and nitrogen use. In: Nitrogen in crop production. Hauck, R.D. (ed.) American Society of Agronomy, USA.

BARNEIX,A.J., H.BRETELER & S.C.VAN DE GEIJN 1984 Gas and ion exchange in wheat roots after N-supply. Physiol. Plant. 24:288-290

BEN ZIONI,A., VAADIA,Y. & LIPS,S.H. (1971) Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiol. Plant. 24: 288-290

BLAQUIERE,T HOFSTRA,R. & STULEN,I. (1987) Ammonium and nitrate nutrition in *Plantago lanceolata* and *Plantago major* L. ssp. *major*. Plant and Soil 104:129-141

COX,R.J. & REISENAUER,H.M. (1973) Growth and ion uptake by wheat supplied nitrogen as nitrate or ammonium or both. Plant and Soil 38:363-380

DE VISSER,R. (1984) Efficiency of respiration and energy requirements of N-assimilation in roots of *Pisum sativum*. In: Interactions between energy and nitrogen metabolism in *Pisum sativum* R.de Visser. Drukkerij van Denderen B.V. Groningen.

DE VISSER,R. & LAMBERS,H. (1983) Growth and efficiency of root respiration of *Pisum sativum* as dependent on the source of nitrogen. Physiol. Plant.58:533-543

GOLVANO,M.P. & DE FELIPE,M.R. (1986) Effect of N-nutrition on Photosynthetic apparatus of wheat during tillering. In: Fundamental, ecological and agricultural aspects of nitrogen metabolism.H.Lambers, J.J.Neeteson and I.Stulen (eds.) Marthinus Nijhoff, Dordtrecht.

GUTSCHICK,V.P. (1981) Evolved strategies of nitrogen aquisition by plants. American Naturalist 118:607-637

HAGEMAN,R.H. (1984) Ammonium nutrition versus nitrate nutrition in higher plants. Biological Reviews 53:465-510

HEWITT,E.J. (1966) Sand and water culture methods used in the study of plant nutrition. Tech. commun. N 22 (revised) Commonwealth Bureau of Horticultural and Plantation Crops.

HOCKING,P.J., B.T.STEER & C.J.PEARSON (1984) Nitrogen nutrition of non-leguminous crops: A review. Part 1. Field Crop Abstracts 37:625

LEWIS,O.A.M. (1986) Plants and nitrogen. Edward Arnold, London.

LEWIS,O.A.M. & CHADWICK,S (1983) A ^{15}N investigation into nitrogen assimilation in hydroponically-grown barley (*Hordeum vulgare L. cv. Clipper*) in response to nitrate, ammonium and mixed nitrate and ammonium nutrition. New Phytol. 95:635-646.

LEWIS,O.A.M., SOARES,M.I.M & LIPS,S.H. (1986) A photosynthetic and ^{15}N investigation of the differential growth response of barley to nitrate, ammonium and nitrate + ammonium nutrition. In: Proceedings of a symposium on nitrogen metabolism in higher plants. H.Lambers (ed.) Nijhoff/Junk Dordtrecht.

LEWIS,O.A.M., FULTON,B.A. & VON ZELEWSKI,A.A.A. (1986) The differential distribution of carbon in response to nitrate, ammonium and nitrate + ammonium nutrition in wheat.

NOVOA,R. & LOOMIS,R.S. (1981) Nitrogen in plant production. Plant and soil 58:177-204

STOCK,W.D. (1983) An evaluation of some manual colourimetric methods for the determination of inorganic nitrogen in soil extracts. Communications in soil science plant analysis 14:925-936

WAREMBOURG,F.R., MONTANGE,D. & BARDIN,R. (1982) The simultaneous use of $^{14}CO_2$ and $^{15}N_2$ labelling techniques to study the carbon and nitrogen economy of legumes grown under natural conditions. Physiol. Plant. 56:46-55