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Utilization of Urea, Ammonia, Nitrite, and Nitrate by Crop Plants  
in a Controlled Ecological Life Support System (CELSS)

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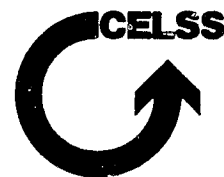
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in a Controlled Ecological Life Support System (CELSS)

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## PREFACE

This report contains 2 papers which consider the utilization of various nitrogen compounds by crop plants in controlled ecological life support systems. Discussed are the selection of crop varieties for efficient production using urea, ammonia, nitrite, and nitrate, and the assimilation of mixed nitrogen sources by cereal leaves and roots.

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# Selection of Crop Varieties for Efficient Production Using Urea, Ammonia, Nitrite, and Nitrate

## Introduction

Our work is determining how the presence of each of the expected N products from waste processing --  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and urea -- affect the assimilation of the others by wheat seedlings.

The plant can assimilate these compounds in both leaves and roots. We first determined the interactions in detached leaves and roots to ascertain where the different N sources were mainly metabolized and which were more sensitive to interactions. The N compounds had a relatively small effect on each other's metabolism in leaves; however, the effects were profound in detached roots. Nitrate had little effect on the uptake and assimilation of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  in roots. In contrast, both  $\text{NO}_2^-$  and  $\text{NH}_4^+$  inhibited  $\text{NO}_3^-$  uptake. Ammonium slightly decreased  $\text{NO}_3^-$  reduction while  $\text{NO}_2^-$  almost totally inhibited  $\text{NO}_3^-$  reduction.  $\text{NH}_4^+$  slightly decreased  $\text{NO}_2^-$  uptake and reduction. Nitrite almost totally inhibited  $\text{NH}_4^+$  uptake.

During the past year we have begun studies to determine the interactions of the N sources on each other's assimilation using intact plants. We have also increased the N sources up to three components:  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$ . This report will deal with the results obtained using intact wheat seedlings.

Materials and Methods

Wheat seeds (variety Yecora Rojo) were soaked overnight in Vita-Vax fungicide, then thoroughly rinsed and germinated on cheesecloth suspended over a 1/4 strength Hoagland's solution in darkness at 25°C and 65% relative humidity. Maintenance of a relative humidity of 65% or less was necessary to prevent fungal contamination. The seedlings were grown 4 days in darkness, then 6 days with a 16 h day ( $500 \mu\text{E}/\text{m}^{-2}\text{sec}^{-1}$ ), 8 h night cycle at a 25°C day temperature and 15°C nighttime temperature. Relative humidity was maintained at 65%.

Ten to 15 seedlings were then placed in 40 to 50 ml of 1/4 strength Hoagland's solution containing 1 mM of each N compound to be studied. Water-saturated air was bubbled through the test solutions. Samples of 0.4 ml were removed at 0.5 h intervals to determine the disappearance of each N source. Uptake was determined as the amount disappearing from solution. This method mimics the manner of nutrient removal from the substrate medium expected when crops are grown in solution culture.

Assimilation of N sources. This was determined by subtracting the concentration of each N source in the plant from the amount that was taken up.

$\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  analyses.  $\text{NO}_3^-$  was determined by HPLC.  $\text{NO}_2^-$  and  $\text{NH}_4^+$  were determined by HPLC and colorimetrically. Both methods gave consistently close and reproducible results.

Analysis of plant samples. Plants from each treatment were ground in water in mortar and pestle and centrifuged at 30,000

K x g for 15 min. Aliquots of the supernatant were analyzed for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  as described above.

The experiments were all replicated at least 3 times, and 3 replicates per treatment were used. The error bars on the graphs represent the variation occurring at each point on the curve.

## Results and Discussion

Lower concentrations of N sources were used in these initial experiments since any interactions are more rapidly seen. The lower concentrations (1 to 3 mM) are also considered to be more "physiological." During the next year, concentrations of N sources will be increased up to 10 mM, that commonly used in many nutrient solutions and which will likely be used in CELSS.

$\text{NO}_3^-$  uptake. Nitrate uptake had a lag period of about 2 h before linear uptake occurred with time and concentration (Fig. 1A, B). The wheat plants were capable of totally depleting the solution of  $\text{NO}_3^-$  (Table 1).

The lag period is apparently the time required for induction of the  $\text{NO}_3^-$  uptake system.  $\text{NO}_3^-$  uptake was linear from about 1 mM  $\text{NO}_3^-$  to total depletion of  $\text{NO}_3^-$  from the solution. Other work has shown a typical Michaelis-Menten plot for  $\text{NO}_3^-$  uptake between 0 and 1 mM  $\text{NO}_3^-$  with a steady-state rate between 0.5 and 1 mM  $\text{NO}_3^-$ . When plants are allowed to deplete the  $\text{NO}_3^-$  from the nutrient solution from a high to a low concentration, they did not show such a mechanism. Apparently their ability increased to take up  $\text{NO}_3^-$  from a solution becoming progressively more dilute.



$\text{NO}_2^-$  uptake. The concentration of  $\text{NO}_2^-$  often rapidly decreased when the plants were added to the solution (Fig. 2A, B). We do not understand this, and we are setting up experiments to determine why  $\text{NO}_2^-$  was lost. Subsequently, a long lag of about 4 h occurred before  $\text{NO}_2^-$  was taken up linearly. The plants also completely depleted the concentration of  $\text{NO}_2^-$  (Table 1). The lag in the rate of uptake could also be partially due to the necessity to induce an  $\text{NO}_2^-$  uptake system.

$\text{NH}_4^+$  uptake. Uptake of  $\text{NH}_4^+$  began immediately after the plants were placed in the uptake solution and was linear with time and concentration to total depletion of  $\text{NH}_4^+$  from the solution (Fig. 3A, B; Table 1). The plants took up  $\text{NH}_4^+$  extremely efficiently.

Uptake from a solution containing  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . Nitrate was not taken up for about 7 h when present with  $\text{NO}_2^-$  (Fig. 4A, B). After uptake began, it was linear with time and concentration to total depletion of  $\text{NO}_3^-$  from solution (Table 1).

Nitrite showed an initial early loss from solution as pointed out above. After about 2.5 h uptake was slow but quite linear with time and concentration of  $\text{NO}_2^-$  in solution until all of it was depleted (Fig. 4 AB; Table 1). Nitrate uptake did not begin until the  $\text{NO}_2^-$  concentration decreased to about 0.1 mM (Fig. 4A). After  $\text{NO}_3^-$  uptake began, its rate was almost identical to that where only  $\text{NO}_3^-$  was present (Table 2). The rate of uptake of  $\text{NO}_2^-$  was less in the presence of  $\text{NO}_3^-$  (Table 2).

Uptake from a Solution Containing  $\text{NO}_2^-$  and  $\text{NH}_4^+$ . The uptake rate of both  $\text{NH}_4^+$  and  $\text{NO}_2^-$  was decreased in the combined solution. The rate of  $\text{NH}_4^+$  uptake from a solution containing only

$\text{NH}_4^+$  was 2  $\mu\text{moles/g}$  fresh weight  $\times$  h; in the presence of  $\text{NO}_2^-$  the rate decreased to about 1  $\mu\text{mole/g}$  fresh weight  $\times$  h (Table 2). The rate of  $\text{NO}_2^-$  uptake from a solution containing only  $\text{NO}_2^-$  was 1  $\mu\text{mole/g}$  fresh weight  $\times$  h and was half that in the presence of  $\text{NH}_4^+$  (Table 2). Although the rates were slower, both  $\text{NO}_2^-$  and  $\text{NH}_4^+$  were totally depleted during the course of the experiment (Table 1).

Uptake from a Solution Containing  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . The lag period before  $\text{NO}_3^-$  uptake became linear was about 2 h longer in the presence of  $\text{NH}_4^+$  (Fig. 6 AB). The rate of  $\text{NO}_3^-$  uptake was slightly (about 20%) decreased in the presence of  $\text{NH}_4^+$  (Table 2). Nitrate uptake was slow until about 70% of the  $\text{NH}_4^+$  had disappeared from the solution, showing the preferential use of  $\text{NH}_4^+$  (Fig. 6C). Nitrate uptake then proceeded until the solution was depleted (Table 1).

Ammonium uptake began immediately and proceeded linear at a rate twice that of  $\text{NO}_3^-$  until the solution was depleted (Fig. 6CD; Table 2).

Uptake from a Solution Containing  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$ . It appeared that  $\text{NO}_3^-$  uptake was inhibited until about 60% of the  $\text{NO}_2^-$  and about 35% of the  $\text{NH}_4^+$  had disappeared from solution (Fig. 7 A-F). After 4 h  $\text{NO}_3^-$  uptake became linear and occurred until it was depleted from solution (Table 1). Its rate was about half of that when it was alone in solution (Table 2).

Nitrite uptake consistently showed quite mixed kinetics (Fig. 7 CD) with an initial rate that was almost twice that of the final (Table 2). Nitrite uptake ceased for about 3 h in the middle of

the time course (from 4 to 7.5 h). Uptake then occurred until almost all of the  $\text{NO}_2^-$  was depleted from solution. Ammonium uptake began immediately and proceeded until it was almost totally depleted (Fig. 7 EF; Table 1).

Sometimes we detected a severe inhibition of uptake of each of the three sources when all three were present in the nutrient solution which lasted for up to 10 h. This occurred more often in plants which were grown under low N conditions before initiation of the experiments. When plants were grown in higher concentrations of both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  before initiating the experiments, the plants quite efficiently utilized all three sources.

Assimilation of N sources. Nitrite and  $\text{NH}_4^+$  were very efficiently assimilated whether or not they were present singly or in the presence of the other two N sources (Table 3). In contrast,  $\text{NO}_3^-$  assimilation decreased when  $\text{NO}_2^-$  and  $\text{NH}_4^+$  were present in the same solution. The percentage of total N assimilated decreased when  $\text{NO}_3^-$  was present in the solution since it was not as rapidly assimilated and instead was stored in the tissues.

Control of pH. The pH drops rapidly in nutrient solutions containing  $\text{NH}_4^+$  as the N source (Fig. 8). In contrast, the pH increases when  $\text{NO}_3^-$  and  $\text{NO}_2^-$  are the sources. We have used two methods to maintain pH between about 6.5 to 7.0. These are: Adding a  $\text{K}^+$  charged cation exchange resin to the solution to adsorb  $\text{H}^+$  ions or adding  $\text{CaCO}_3$  to buffer either  $\text{H}^+$  or  $\text{OH}^-$  coming out of plant roots. Of the two methods, the use of  $\text{CaCO}_3$  was superior.

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The use of appropriate mixtures of mixed N sources may partially allow pH regulation. However, this depends on how the inhibitory effects on uptake are finally overcome.

#### Summary

The wheat plants readily take up  $\text{NH}_4^+$  and  $\text{NO}_2^-$  but discriminate against  $\text{NO}_3^-$  especially in the presence of  $\text{NO}_2^-$ . The N status of the plant before it receives the combined N sources seems to be important to its ability to take up and assimilate them. The plants store more  $\text{NO}_3^-$  in the presence of the combined sources apparently because  $\text{NO}_3^-$  reduction is somewhat inhibited. Overall the wheat plant shows a great ability to use the combined sources even though it appears to sequentially use  $\text{NO}_3^-$ .

Table 1. Percent removal of each N component from nutrient solutions by wheat plants during 13 h.

N Components in Nutrient Solution	% Uptake of Each Component			Total Utilization of all N Sources %
	$\text{NO}_3^-$	$\text{NO}_2^-$	$\text{NH}_4^+$	
$\text{NO}_3^-$ (Fig. 1)	100			100
$\text{NO}_2^-$ (Fig. 2)		100		100
$\text{NH}_4^+$ (Fig. 3)			100	100
$\text{NO}_3^- + \text{NO}_2^-$ (Fig. 4)	100	100		100
$\text{NO}_2^- + \text{NH}_4^+$ (Fig. 5)		100	100	100
$\text{NO}_3^- + \text{NH}_4^+$ (Fig. 6)	100		100	100
$\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$	80	95	99	89

Table 2. Uptake rates determined from linear portions of curves from figures 1 through 6.

N Components in Nutrient Solution	Uptake $\mu\text{moles/g fresh weight} \times \text{h}$		
	$\text{NO}_3^-$	$\text{NO}_2^-$	$\text{NH}_4^+$
$\text{NO}_3^-$ (Fig. 1 AB)	1.5		
$\text{NO}_2^-$ (Fig. 2 AB)		1.0	
$\text{NH}_4^+$ (Fig. 3 AB)			2.0
$\text{NO}_3^- + \text{NO}_2^-$ (Fig. 4 AB)	1.4	0.6	
$\text{NH}_4^+ + \text{NO}_2^-$ (Fig. 5 AB)		0.5	1.1
$\text{NO}_3^- + \text{NH}_4^+$ (Fig. 6 A-D)	1.2		2.0
$\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ (Fig. 7 A-F)	0.7	(1.5 initial) (0.8 final)	

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Table 3. Assimilation of N sources from nutrient solutions.

A.	Uptake			Concn			At			t <sup>12h</sup>			Assimilation		
	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>
N components in nutrient solutions 1 mM each															
NO <sub>3</sub> <sup>-</sup>	11.42			0.86			6.03						6.25		
NO <sub>2</sub> <sup>-</sup>		5.00			0.00			0.00						5.00	
NH <sub>4</sub> <sup>+</sup>			11.16			3.71									10.57
NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup>	10.50	11.29		0.04	0.00	4.01	1.59	0.25	4.00	8.95	19.99	19.98 <sup>a</sup>			
NO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup>	11.10		11.44	0.86		3.30	7.11	0.00	3.47	4.85	4.85	16.12 <sup>b</sup>			
NO <sub>2</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup>		9.00	11.14		0.00	3.71		0.00	4.75			9.00	19.10 <sup>c</sup>		
NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup>	11.10	11.40	11.66	0.86	0.00	3.30	8.00	0.00	3.46	3.96	11.40	26.86 <sup>b</sup>			

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Table 3. (Continued)

	% Assimilation			Total N Assimilation Through $\text{NH}_4^+$ (100)	
	$\text{NO}_3^-$	$\text{NO}_2^-$	$\text{NH}_4^+$	Total Uptake of all Sources	
$\text{NO}_3^-$	55	100		55	
$\text{NO}_2^-$		100		100	
$\text{NH}_4^+$			95	95	
$\text{NO}_3^- + \text{NO}_2^-$	85	99	100	92	
$\text{NO}_3^- + \text{NH}_4^+$	44	100	99	72	
$\text{NO}_2^- + \text{NH}_4^+$		100	95	95	
$\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$	36	100	99	79	

<sup>a</sup>  $\text{NO}_3^-$  assimilated +  $\text{NO}_2^-$  assimilated

<sup>b</sup>  $\text{NO}_3^-$  assimilated +  $\text{NO}_2^-$  assimilated +  $\text{NH}_4^+$  assimilated

<sup>c</sup>  $\text{NO}_2^-$  assimilated +  $\text{NH}_4^+$  assimilated

### Figure Legends

Fig. 1. The uptake of  $\text{NO}_3^-$  from a nutrient solution containing only  $\text{NO}_3^-$  (at 1 mM) as the N source.

1A: The disappearance of  $\text{NO}_3^-$  from solution. Fresh weight of plant material was 3.91 g.

1B: The uptake of  $\text{NO}_3^-$  on a g fresh weight basis.

Fig. 2. The uptake of  $\text{NO}_2^-$  from a nutrient solution containing only  $\text{NO}_2^-$  (at 1 mM) as the N source.

1A: The disappearance of  $\text{NO}_2^-$  from solution. Fresh weight of plants was 4.09 g.

1B: The uptake of  $\text{NO}_2^-$  on a g weight basis.

Fig. 3. The uptake of  $\text{NH}_4^+$  from a nutrient solution containing only  $\text{NH}_4^+$  (at 1 mM) as the N source.

1A: The disappearance of  $\text{NO}_2^-$  from solution. Fresh weight of plants was 4.01.

1B: The uptake of  $\text{NO}_2^-$  on a g fresh weight basis.

Fig. 4. The uptake of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  from a nutrient solution containing both N sources (each at 1 mM).

1A: The disappearance of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  from solution. Fresh weight of plant material was 3.81 g.

1B: The uptake of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  on a g fresh weight basis.

Fig. 5. The uptake of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  from a nutrient solution containing both N sources (each at 1 mM).

1A: The disappearance of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  from solution. Fresh weight of plants was 3.94 g.



1B: The uptake of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  on a g fresh weight basis.

Fig. 6. The uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  from a nutrient solution containing both N sources (each at 1 mM).

1A: The disappearance of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  from solution. Fresh weight of plant material was 3.02.

1B: The uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  on a g fresh weight basis.

Fig. 7. Uptake of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  from a nutrient solution containing the three N sources (each at 1 mM).

1A: The disappearance of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  from solution. Fresh weight of plant material was 3.00.

1B: The uptake of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  on a g fresh weight basis.

Fig. 8. Changes in pH of nutrient solutions as wheat plants take up  $\text{NO}_3^-$ . Nutrient solutions were  $\frac{1}{4}$  strength Hoagland's with 1 mM  $\text{KNO}_3$ .

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Fig. 1A

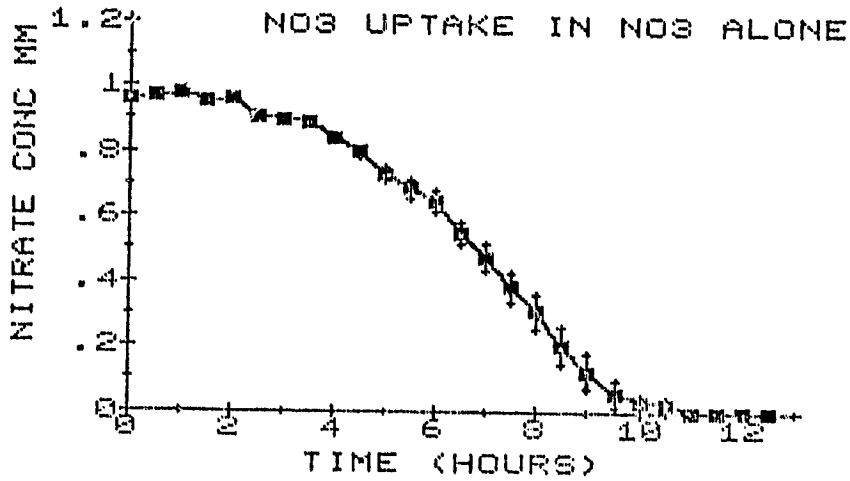
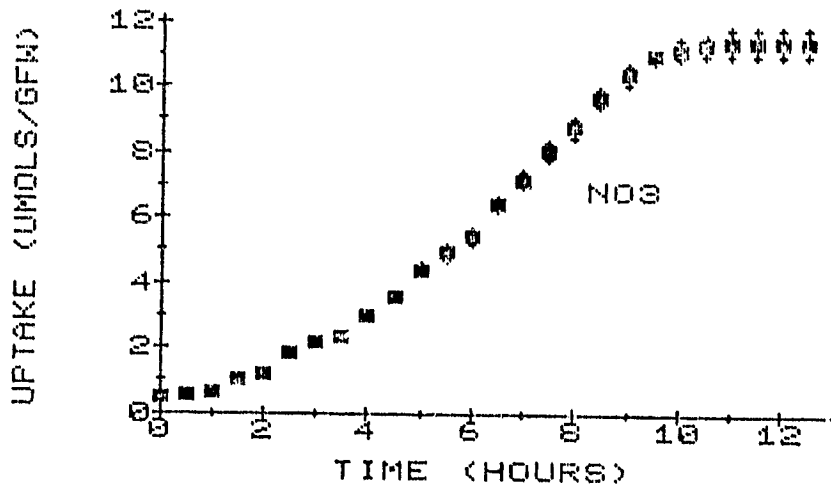


Fig. 1B



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Fig. 2A

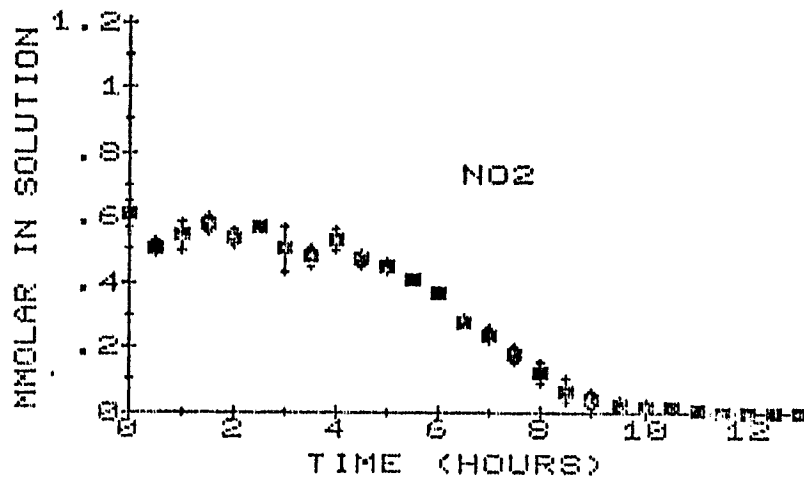
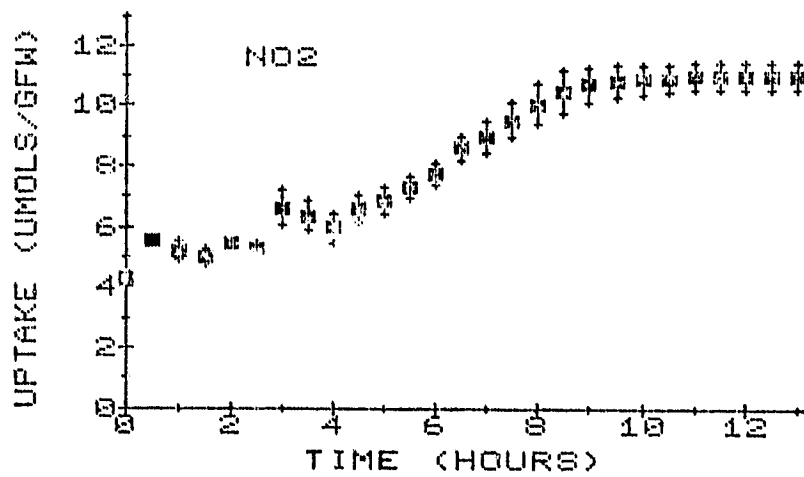


Fig. 2B



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Fig. 3A

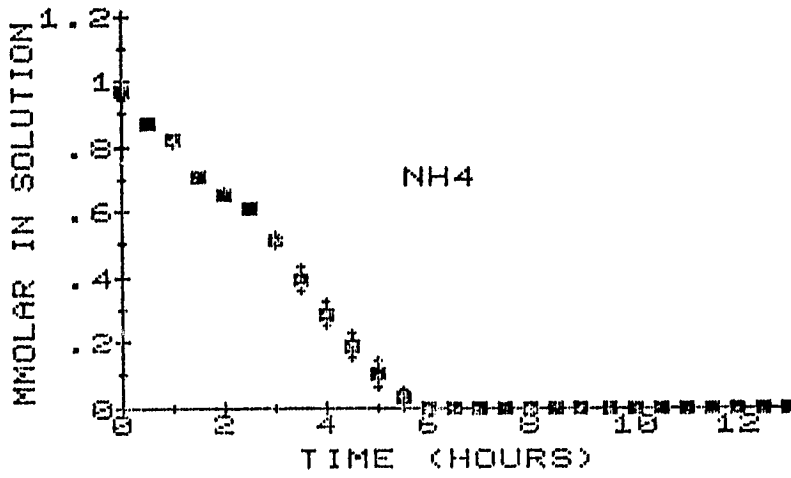
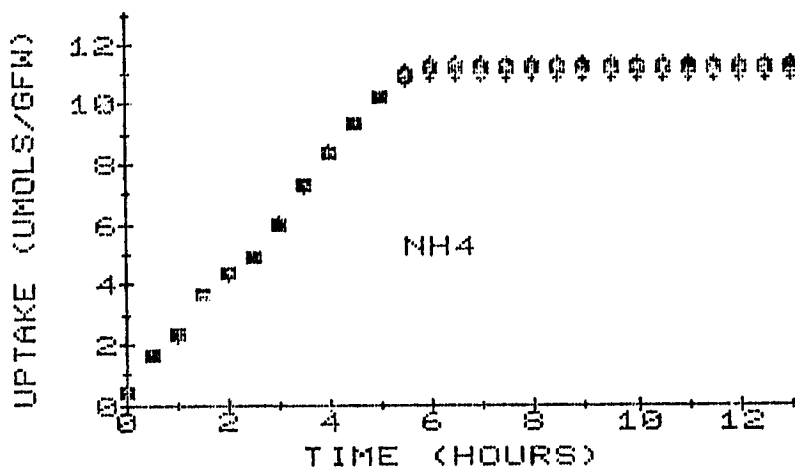


Fig. 3B



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Fig. 4A

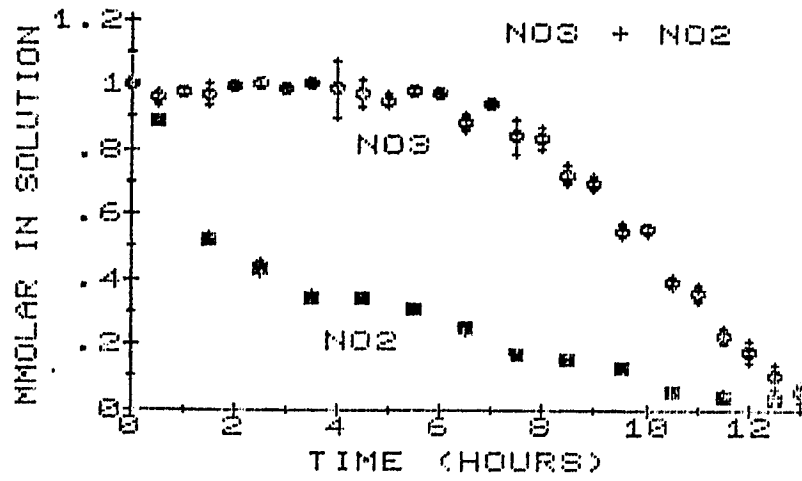
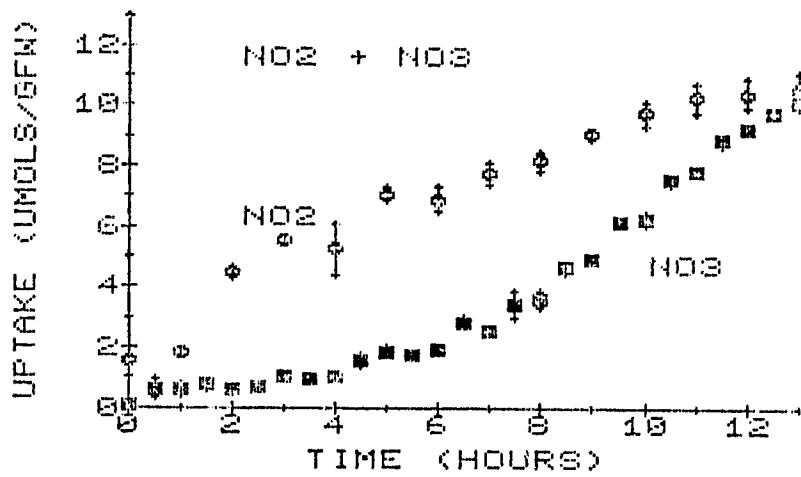


Fig. 4B



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Fig. 5A

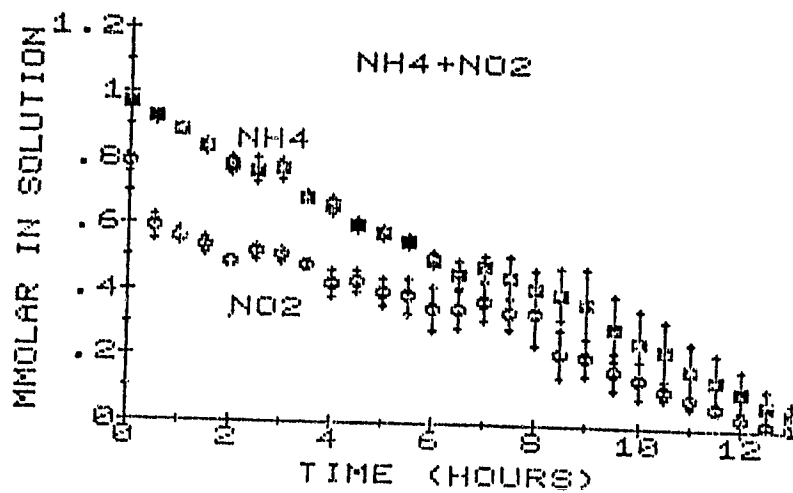
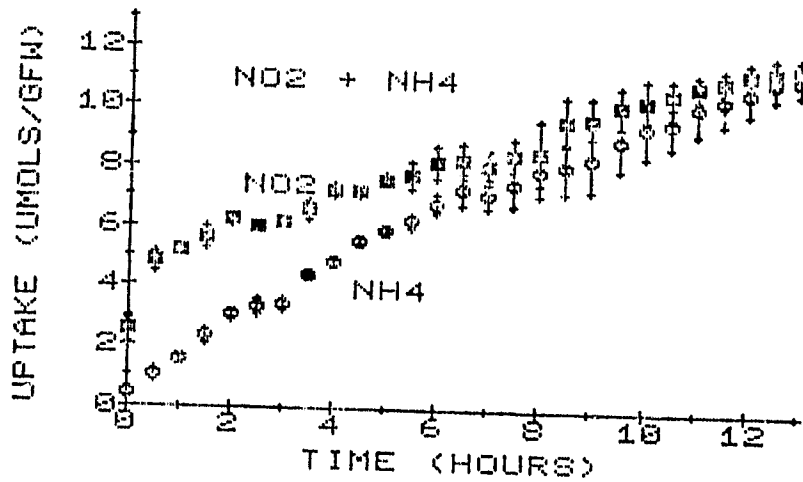


Fig. 5B



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Fig. 6A

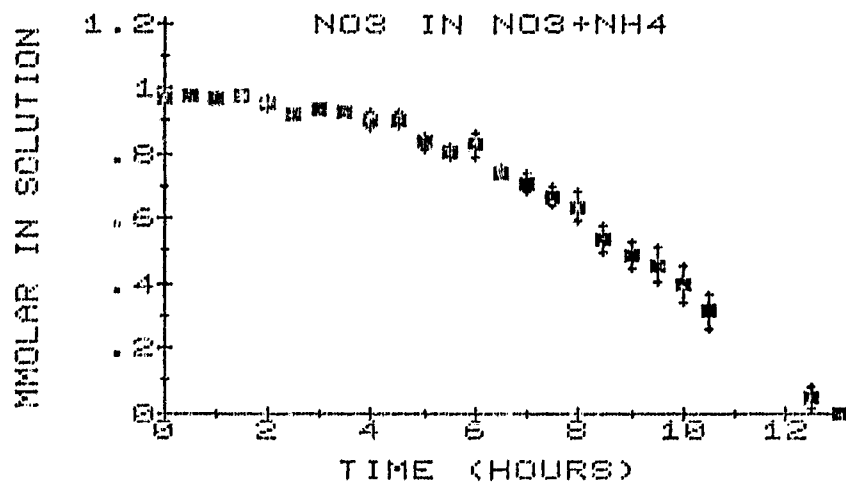
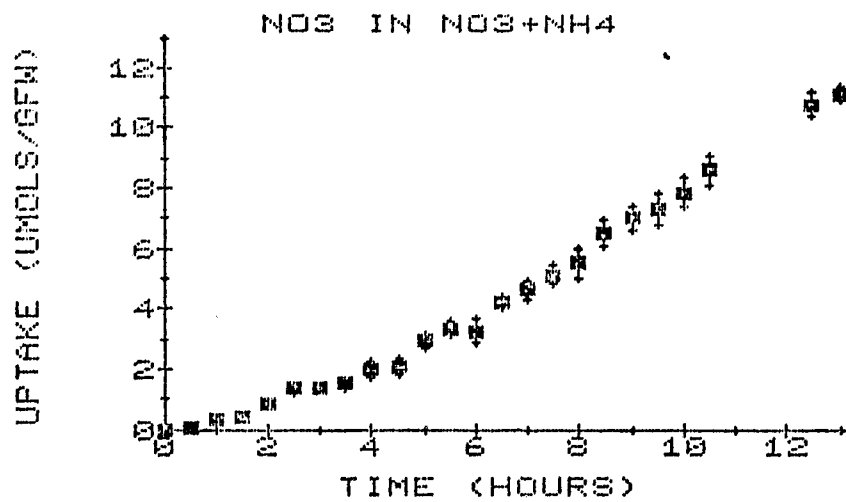


Fig. 6B



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Fig. 6C

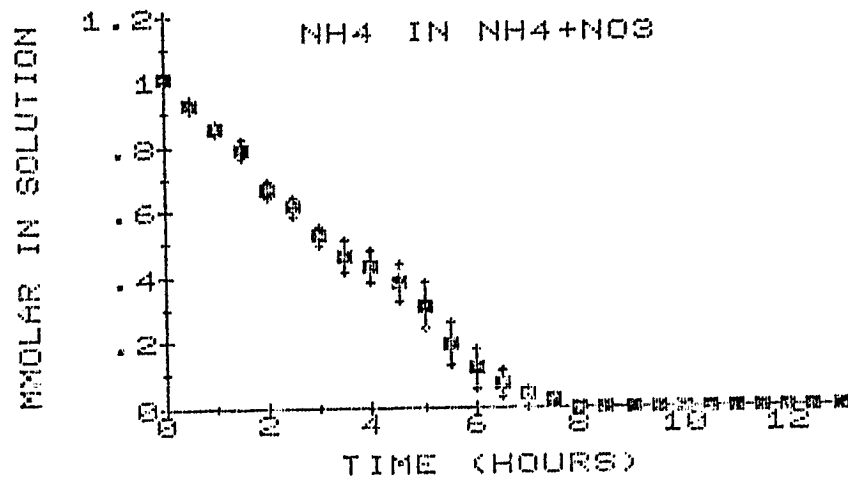
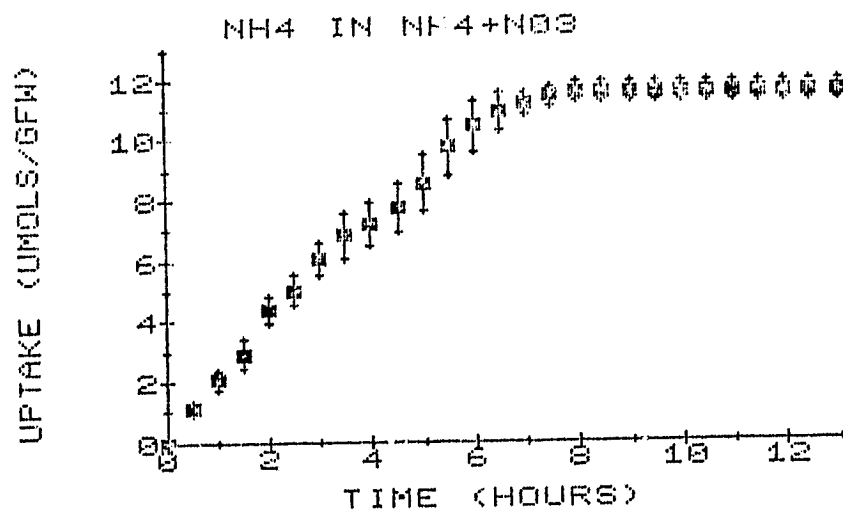


Fig. 6D





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Fig. 7A

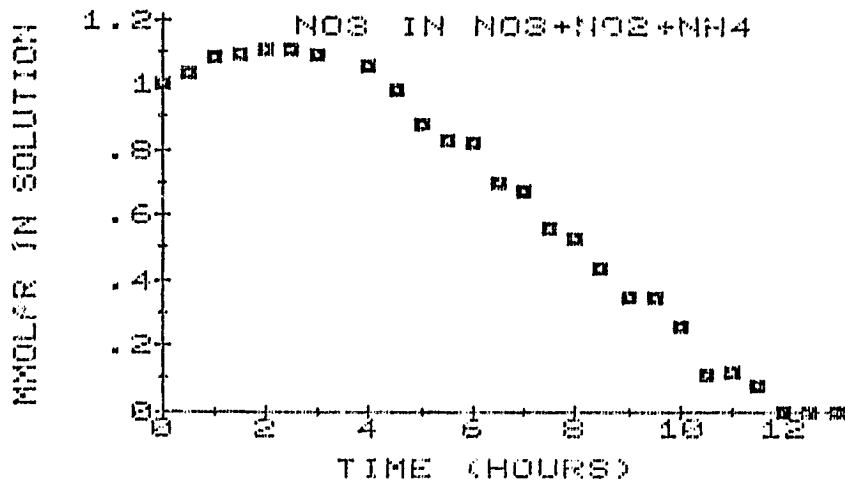


Fig. 7B

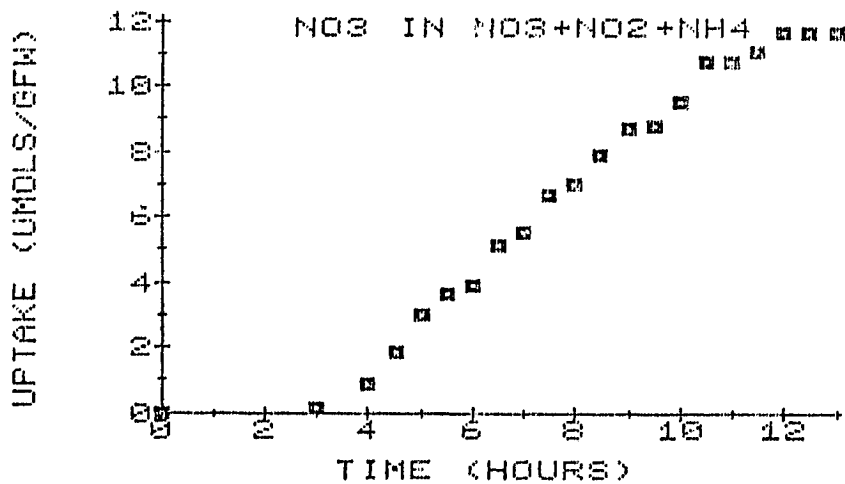


Fig. 7C

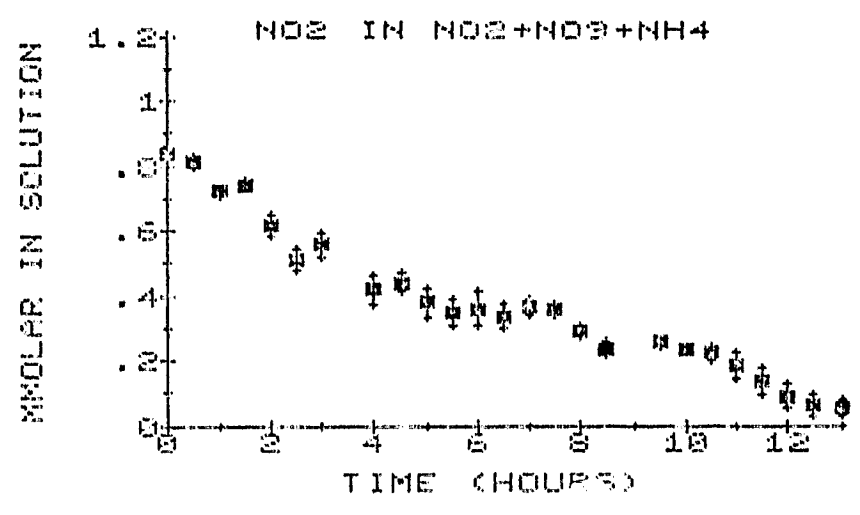
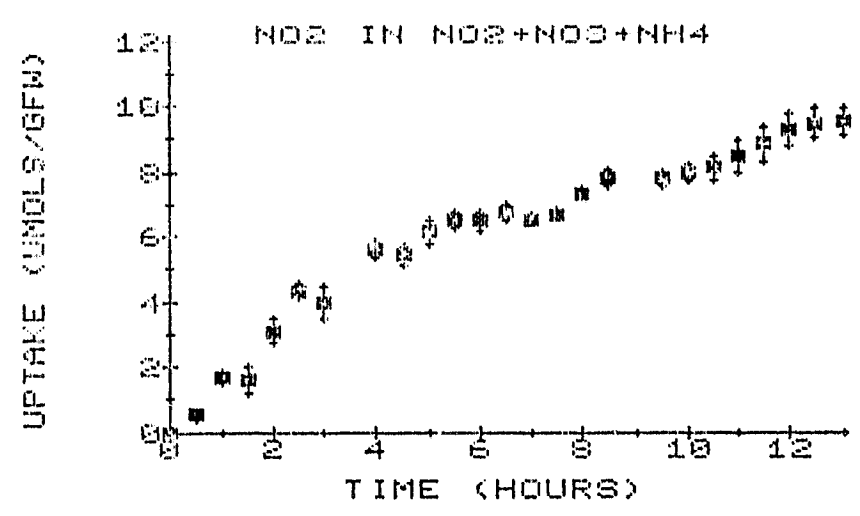


Fig. 7D



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Fig. 7E

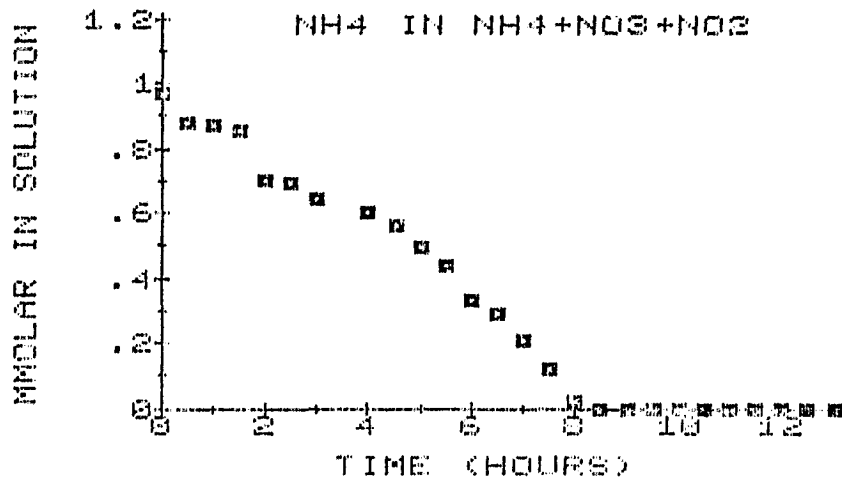
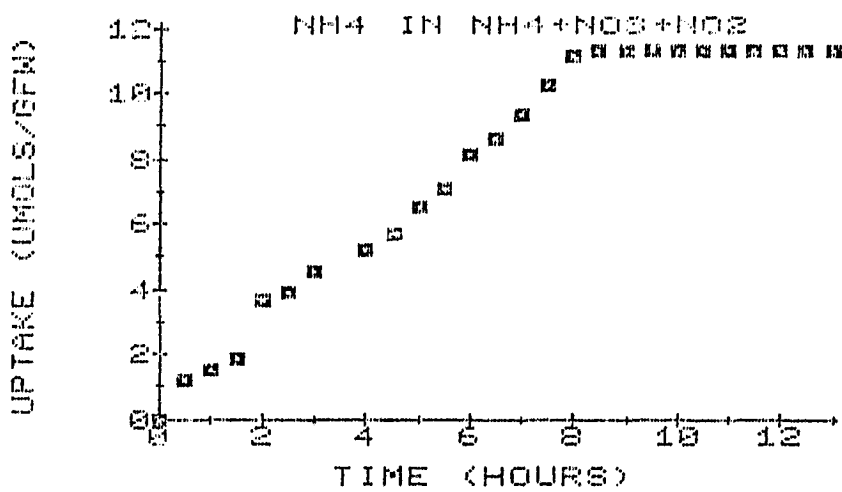
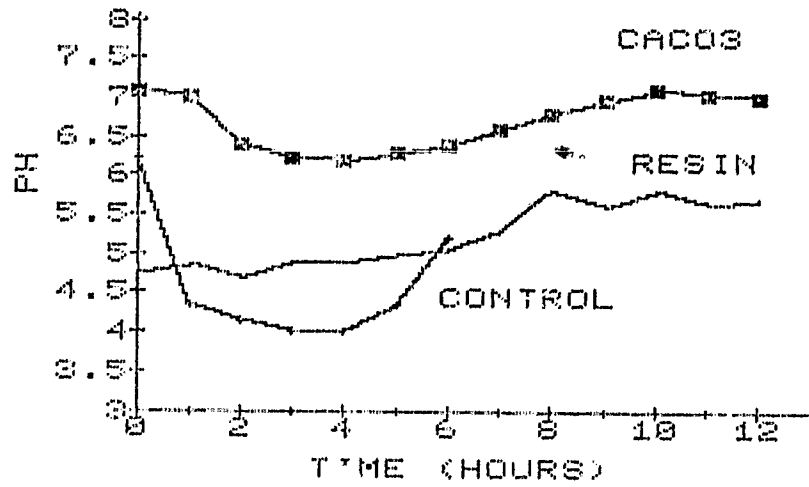


Fig. 7F



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Fig. 8



## Assimilation of Mixed-N Sources, $\text{NO}_3^-$ , $\text{NO}_2^-$ , $\text{NH}_4^+$ , and Urea by Cereal Leaves and Roots

### I. Introduction

The results discussed below represent those obtained during our current NASA Grant NSG NCC 2-99. The beginning phase was designed to determine how the presence of each of the expected N products from waste processing --  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , and urea -- would affect the assimilation of the others. Since the plant can metabolize each of these compounds in both roots and leaves, the assimilation of each in leaves, roots, and the whole plant must be determined. As described in the proposal, it is critical to determine where each is metabolized (roots or leaves), since that greatly affects the overall efficiency. The studies were done initially using detached cereal leaves. Studies with urea have just begun. The test of the proposal gives a description of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , and urea assimilation.

Initially we had great difficulty growing wheat plants hydroponically without fungal contamination. Our initial experiments showed that wheat and barley leaves responded essentially identically to the mixed N sources. Since barley had no fungal problem, we began studies with it while determining how to grow wheat hydroponically without fungal contamination. We can now grow wheat in nutrient solution without fungal contamination and are using it for all further experimentation.

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### II. Materials and Methods

Ten centimeters from each leaf tip were detached from 8-day old barley plants and placed base down in the treatment solutions. The N compounds are taken into the leaves primarily via the transpiration stream. At the end of the specified incubation periods, leaves were homogenized and assayed for N compounds. Uptake of each N component was determined by its disappearance from the external solution. The internal leaf concentration of each N component was determined from a sample of homogenate. The difference between the uptake of each specific N compound and its internal concentration represents the amount reduced or assimilated. Excised roots from wheat (Yecora Rojo) were placed in treatment solutions, and the experiments were conducted as described above for leaves. To determine the effect of one N compound on the others, the concentration of one was maintained constant while the others were varied. Nitrate and  $\text{NO}_2^-$  were determined by HPLC (Thayer and Huffaker, 1980), and  $\text{NH}_4^+$  was determined first by an ammonium-specific electrode; we currently use HPLC to assay for ammonium, since it is more sensitive and reproducible than the ammonium-specific electrode.

### III. Results

#### A. Barley Leaves

##### Effect of $\text{NO}_2^-$ on $\text{NO}_3^-$ assimilation (Tables I and II)

In light, the reduction of  $\text{NO}_3^-$  decreased about 17% as  $\text{NO}_2^-$  was increased in concentration in the external solution (Table I,

line E). Ninety-nine percent of the  $\text{NO}_2^-$  absorbed from the external solution and the  $\text{NO}_2^-$  resulting from  $\text{NO}_3^-$  reduction was inhibited (line H). Since essentially all the  $\text{NO}_2^-$  in the plant is reduced, the total N reduced from both sources (line I) increased as  $\text{NO}_2^-$  increased in the external solution. In darkness,  $\text{NO}_2^-$  had little effect on  $\text{NO}_3^-$  reduction (Table II, line E). Again, all of the  $\text{NO}_2^-$  in the plant was further reduced (line H), whereas about 40% of the  $\text{NO}_3^-$  was reduced (line G). The presence of  $\text{NO}_2^-$  increased the total N reduced in darkness (37 to 76%, line I).

Effect of  $\text{NO}_3^-$  on  $\text{NO}_2^-$  assimilation (Tables III and IV)

Increasing  $\text{NO}_3^-$  in the substrate solution had no effect on  $\text{NO}_2^-$  assimilation in light or darkness (line H). Again 98% of the  $\text{NO}_2^-$  taken up from the external solution and that formed from  $\text{NO}_3^-$  reduction was reduced. Because less  $\text{NO}_3^-$  is reduced than  $\text{NO}_2^-$ , the total N reduced decreased as  $\text{NO}_3^-$  increased in the substrate solution (line I).

Effect of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  assimilation (Tables V and VI)

As  $\text{NH}_4^+$  was increased in the external nutrient solution,  $\text{NO}_3^-$  reduction was somewhat decreased in both light and dark (line G). In light a constant amount of  $\text{NH}_4^+$  was utilized (Table V, line H), whereas in darkness,  $\text{NH}_4^+$  utilization increased as  $\text{NH}_4^+$  increased (Table VI, line H). In light the presence of external  $\text{NH}_4^+$  slightly decreased the total utilization from both sources, whereas in darkness, the presence of  $\text{NH}_4^+$  increased the total utilization of both sources (line I). Since the leaf  $\text{NO}_2^-$  concentration was negligible, essentially all  $\text{NO}_3^-$  reduced was converted

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to  $\text{NH}_4^+$ . The extent of utilization of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  was more complete in light than darkness.

Effect of  $\text{NO}_3^-$  on  $\text{NH}_4^+$  assimilation (Tables VII and VIII)

As external  $\text{NO}_3^-$  increased, more  $\text{NH}_4$  was assimilated (line H), although the total N assimilated was affected little in light (line I). In darkness,  $\text{NH}_4^+$  assimilation also increased in the presence of  $\text{NO}_3^-$  (line H); however, overall assimilation of total N decreased somewhat as  $\text{NO}_3^-$  increased (Table VIII, line I). because of low assimilation of  $\text{NO}_3^-$ . More  $\text{NH}_4^+$  was assimilated than  $\text{NO}_3^-$ .

Effect of  $\text{NH}_4^+$  on  $\text{NO}_2^-$  assimilation (Tables IX and X)

Increasing  $\text{NH}_4^+$  did not affect  $\text{NO}_2^-$  assimilation in either light or dark (line G). Each was utilized in nearly the same percentage and the total amount of N utilized from each source (line I) was affected little by increasing  $\text{NH}_4^+$ .

Effect of  $\text{NO}_2^-$  on  $\text{NH}_4^+$  assimilation (Tables XI and XII)

Almost all  $\text{NO}_2^-$  absorbed was reduced (96% or more, line G). Total utilization of  $\text{NH}_4^+$  increased in the presence of  $\text{NO}_2^-$ , from 64 to 82% in light and 59 to 81% in darkness, as  $\text{NO}_2^-$  increased in the external solution (line H). Total assimilation from both N sources (line I) increased as external  $\text{NO}_2^-$  increased.

Effect of urea on  $\text{NO}_3^-$  assimilation (Tables XIII and XIV)

Urea, supplied at a similar concentration to  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , caused a 3-fold increase in both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations, respectively, and inhibited  $\text{NO}_3^-$  reduction about 24% in light (Table XIII, lines B, H, and C, respectively). Urea was metabolized



to  $\text{NH}_4^+$  very rapidly inside the leaves (line F); the resulting  $\text{NH}_4^+$  was also rapidly assimilated (lines F and L). Increasing urea had little effect on the ratio of the N used versus the total available from all sources (line M).

In darkness urea inhibited  $\text{NO}_3^-$  reduction by 88% (lines C and D) at one-half equimolar concentration, and it totally inhibited  $\text{NO}_3^-$  reduction at an equimolar concentration (Table XIV). Urea was completely metabolized (line F); the resulting  $\text{NH}_4^+$  was also efficiently assimilated (lines K, L). The great decrease in  $\text{NO}_3^-$  reduction in the presence of urea may be due to its rapid conversion to  $\text{NH}_4^+$ , which would produce an  $\text{NH}_4^+$  concentration equal to 2 times that of  $\text{NO}_3^-$ . Because more urea and  $\text{NH}_4^+$  were assimilated than  $\text{NO}_3^-$ , the presence of urea increased the percentage of total assimilation from all N sources (line M).

Effect of  $\text{NO}_3^-$  on urea assimilation (Tables XV and XVI)

$\text{NO}_3^-$  had no effect on urea metabolism (line F) or the resulting  $\text{NH}_4^+$  assimilation in light (lines G, H, I, J) (Table XV).  $\text{NO}_3^-$  also had no effect on the percentage of total N assimilation from any N source (line K).

In darkness  $\text{NO}_3^-$ , also had no effect on either urea or  $\text{NH}_4^+$  assimilation (Table XVI). As  $\text{NO}_3^-$  concentration increased, the percentage of N metabolized from the total available amount decreased (line L) because of total inhibition of  $\text{NO}_3^-$  reduction in the presence of urea and  $\text{NH}_4^+$  (line C).

## B. Wheat Roots

### Effect of $\text{NH}_4^+$ on assimilation of $\text{NO}_3^-$ (Table XVII)

The presence of  $\text{NH}_4^+$  at equimolar concentration with  $\text{NO}_3^-$  in the external solution inhibited  $\text{NO}_3^-$  uptake 50% (line A) but only slightly decreased the  $\text{NO}_3^-$  reduced as a percentage of that taken up (line D). Hence, the major effect of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  assimilation was inhibition of  $\text{NO}_3^-$  uptake. Because more  $\text{NH}_4^+$  was assimilated than  $\text{NO}_3^-$ , the total utilization from both N sources increased as  $\text{NH}_4^+$  increased (line L).

### Effect of $\text{NO}_2^-$ on $\text{NO}_3^-$ assimilation (Table XVIII)

$\text{NO}_2^-$  greatly inhibited both  $\text{NO}_3^-$  uptake (by 50% at one-half the concentration of  $\text{NO}_3^-$  and by 75% at equimolar concentration in the nutrient solution (Line A).  $\text{NO}_3^-$  reduction was totally inhibited at the lowest concentration of  $\text{NO}_2^-$  in the external solution (line C). Because 94 to 100% of the  $\text{NO}_2^-$  was assimilated (line K), total N utilization increased as  $\text{NO}_2^-$  increased in the nutrient solution (line L).

### Effect of $\text{NO}_2^-$ on $\text{NH}_4^+$ assimilation (Table XIX)

Nitrite at one-half the concentration of  $\text{NH}_4^+$  in the external solution inhibited  $\text{NH}_4^+$  uptake 99.9% (line A); hence, essentially no  $\text{NH}_4^+$  from the external solution was utilized by the plant in the presence of a 1/2 equimolar concentration of nitrite.

### Effect $\text{NO}_3^-$ on $\text{NH}_4^+$ assimilation (Table XX)

Nitrate in the external solution slightly enhanced the utilization of  $\text{NH}_4^+$  (lines I and K) because it had no effect on  $\text{NH}_4^+$  uptake and slightly increased the percentage of  $\text{NH}_4^+$  assimilated.

Effect of  $\text{NO}_3^-$  on  $\text{NO}_2^-$  assimilation (Table XXI)

External  $\text{NO}_3^-$  had no effect on  $\text{NO}_2^-$  uptake (line A) or  $\text{NO}_2^-$  reduction (lines H, J). Since  $\text{NO}_2^-$  totally inhibited  $\text{NO}_3^-$  reduction (line E), the percentage of N used from all N sources decreased (line K) as  $\text{NO}_3^-$  increased in the external solution.

Effect of  $\text{NH}_4^+$  on  $\text{NO}_2^-$  assimilation (Table XXII)

The presence of  $\text{NH}_4^+$  in the external solution inhibited  $\text{NO}_2^-$  uptake 25% (line A) and reduction 30% (line E), at an equimolar concentration. This resulted in an overall decrease in N utilization (line M), since  $\text{NH}_4^+$  uptake (line F) was totally inhibited and  $\text{NO}_2^-$  reduction (lines D, E) was somewhat inhibited.

The effects of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  on urea metabolism and, conversely, the effect of urea on  $\text{NO}_2^-$  and  $\text{NH}_4^+$  assimilation remain to be investigated in leaves in light and darkness. Remaining to be examined as well are the interactions of urea with  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  in roots in this phase of the study. It will be interesting to determine whether  $\text{NO}_2^-$  will inhibit the absorption of urea as it does  $\text{NH}_4^+$ .

IV. Summary

In leaves, the presence of  $\text{NO}_2^-$  in the external solution increased the overall utilization of mixed N sources because of the great efficiency with which leaves reduce  $\text{NO}_2^-$ . Although  $\text{NO}_3^-$  reduction decreased as  $\text{NO}_2^-$  increased, the great utilization of  $\text{NO}_2^-$  more than made up for the decreased  $\text{NO}_3^-$  reduction in both light and darkness. As  $\text{NO}_3^-$  increased in the external solution in the presence of  $\text{NO}_2^-$ , the overall utilization of N decreased,

because a smaller portion of the  $\text{NO}_3^-$  absorbed was reduced. The assimilation of  $\text{NH}_4^+$  was increased as  $\text{NO}_3^-$  increased in the external solution in both light and darkness whereas  $\text{NH}_4^+$  tended to inhibit  $\text{NO}_3^-$  reduction.  $\text{NH}_4^+$  had no effect on  $\text{NO}_2^-$  assimilation in either light or dark;  $\text{NO}_2^-$ , on the other hand, increased the overall  $\text{NH}_4^+$  utilization in both light and dark. The leaves preferentially reduce  $\text{NO}_2^-$ , perhaps because of its toxicity.

The cereal leaves metabolized urea and the resulting  $\text{NH}_4^+$  extremely efficiently. Urea influenced  $\text{NO}_3^-$  assimilation then in essentially the same way  $\text{NH}_4^+$  did in light. In darkness, urea inhibited  $\text{NO}_3^-$  reduction almost totally. By contrast,  $\text{NH}_4^+$  inhibited  $\text{NO}_3^-$  reduction only slightly. The greater effect by urea may have been due to the systems having to assimilate twice as much  $\text{NH}_4^+$  per molecule of urea; nevertheless, the steady-state  $\text{NH}_4^+$  concentration was almost the same in the presence or absence of urea; so, the reasons for the great inhibition of  $\text{NO}_3^-$  reduction by urea in darkness remain to be worked out.

The major effect of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  assimilation in wheat roots was an inhibition of  $\text{NO}_3^-$  uptake. The amount of  $\text{NO}_3^-$  reduced compared with the amount absorbed was affected only slightly by  $\text{NH}_4^+$ . The presence of  $\text{NO}_2^-$  in external solutions containing  $\text{NO}_3^-$  or  $\text{NH}_4^+$  had greatly affected on the assimilation of  $\text{NO}_3^-$  or  $\text{NH}_4^+$ .

$\text{NO}_2^-$  substantially inhibited  $\text{NO}_3^-$  uptake and totally inhibited  $\text{NO}_3^-$  reduction at half the concentration of  $\text{NO}_3^-$ . Nitrite at half the concentration of  $\text{NH}_4^+$  totally inhibited  $\text{NH}_4^+$  uptake. Low levels of  $\text{NO}_2^-$  may have devastating effects on assimilation of

mixed-N sources that must be taken up by roots. The presence of  $\text{NO}_3^-$ , on the other hand, had little effect on the metabolism of either  $\text{NO}_2^-$  or  $\text{NH}_4^+$ .

Since  $\text{NH}_4^+$  assimilation by roots was much more efficient than  $\text{NO}_3^-$  assimilation, the efficiency of utilization from these mixed sources increased as  $\text{NH}_4^+$  increased.

GENERAL TOPICS  
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GROWTH RESPONSE  
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Table I. Effect of NO<sub>2</sub><sup>-</sup> on NO<sub>3</sub><sup>-</sup> Assimilation in Light (μmoles/g × 8 h except for % columns)

Treatment NO <sub>2</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup> (mM)	NO <sub>3</sub> <sup>-</sup> uptake A	NO <sub>2</sub> <sup>-</sup> uptake B	NO <sub>3</sub> <sup>-</sup> in leaves C	NO <sub>2</sub> <sup>-</sup> in leaves D	NO <sub>3</sub> <sup>-</sup> reduced E	Total NO <sub>2</sub> <sup>-</sup> reduced F	% NO <sub>3</sub> <sup>-</sup> reduced G	% NO <sub>2</sub> <sup>-</sup> reduced H	N reduced (%) N available I
0	5	19.5	0	6.0	0.05	13.5	69	100	69
2.5	5	23.0	16.9	9.9	0.18	29.8	57	99	75
5	5	19.4	29.8	9.3	0.53	39.4	52	99	80
	A	B	C	D	E=A-C	F=B+E-D	$\frac{C=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$

Table II. Effect of NO<sub>2</sub><sup>-</sup> on NO<sub>3</sub><sup>-</sup> Assimilation in Darkness (μmoles/g × 12 h except for % columns)

Treatment NO <sub>2</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup> (mM)	NO <sub>3</sub> <sup>-</sup> uptake A	NO <sub>2</sub> <sup>-</sup> uptake B	NO <sub>3</sub> <sup>-</sup> in leaves C	NO <sub>2</sub> <sup>-</sup> in leaves D	NO <sub>3</sub> <sup>-</sup> reduced E	Total NO <sub>2</sub> <sup>-</sup> reduced F	% NO <sub>3</sub> <sup>-</sup> reduced G	% NO <sub>2</sub> <sup>-</sup> reduced H	N reduced (%) N available I
0	5	3.8	0	2.4	0.03	1.4	37	100	37
2.5	5	4.2	6.8	2.6	0.05	8.4	38	100	76
	A	B	C	D	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$



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Table III. Effect of  $\text{NO}_3^-$  on  $\text{NO}_2^-$  Assimilation in Light ( $\mu\text{moles/g} \times 8 \text{ h}$  except for % columns)

Treatment $\text{NO}_2^- \text{NO}_3^-$ (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_2^-$ uptake	$\text{NO}_3^-$ in leaves	$\text{NO}_2^-$ in leaves	$\text{NO}_3^-$ reduced	Total $\text{NO}_2^-$ reduced	% $\text{NO}_3^-$ reduced	% $\text{NO}_2^-$ reduced	$\frac{\text{N reduced}}{\text{N available}}$ (%)
	A	B	C	D	E=A-C	F=B+E-D	$\frac{\text{G}=\text{E}(100)}{\text{A}}$	$\frac{\text{H}=\text{F}(100)}{\text{B}+\text{E}}$	$\frac{\text{I}=\text{F}(100)}{\text{A}+\text{B}}$
5 0	0	14.9	1.0	0.33	0	14.6	0	98	98
5 2.5	6.3	16.5	2.9	0.43	3.4	19.5	54	98	86
5 5.0	13.0	16.1	6.3	0.55	6.7	22.3	52	98	77

Table IV. Effect of  $\text{NO}_3^-$  on  $\text{NO}_2^-$  Assimilation in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ )

Treatment $\text{NO}_2^- \text{NO}_3^-$ (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_2^-$ uptake	$\text{NO}_3^-$ in leaves	$\text{NO}_2^-$ in leaves	$\text{NO}_3^-$ reduced	Total $\text{NO}_2^-$ reduced	% $\text{NO}_3^-$ reduced	% $\text{NO}_2^-$ reduced	$\frac{\text{N reduced}}{\text{N available}}$ (%)
	A	B	C	D	E=A-C	F=B+E-D	$\frac{\text{G}=\text{E}(100)}{\text{A}}$	$\frac{\text{H}=\text{F}(100)}{\text{B}+\text{E}}$	$\frac{\text{I}=\text{F}(100)}{\text{A}+\text{B}}$
5 0	0	5.6	0.1	0.09	0	5.5	0	98	98
5 2.5	3.2	5.9	1.6	0.09	1.6	7.4	50	98	81
5 5	7.2	6.0	2.9	0.09	4.3	10.3	60	99	77

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Table V. Effect of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  Assimilation in Light ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_3^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_3^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_3^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_3^-$ reduced	% $\text{NH}_4^+$ utilized	N reduced N available
5 0	12.3	0	1.8	1.5	10.5	9.0	85	86	86
5 2.5	13.9	6.5	3.2	1.8	10.7	15.4	77	90	75
5 5	13.6	12.2	3.9	2.6	9.6	19.2	71	88	74
A	B	C	D	D	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$

Table VI. Effect of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  Assimilation in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_3^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_3^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_3^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_3^-$ reduced	% $\text{NH}_4^+$ utilized	N reduced N available
5 0	4.4	0	1.7	1.2	2.7	1.5	61	56	34
5 2.5	4.9	2.9	2.0	1.3	2.9	4.5	59	78	58
5 5	4.5	3.2	2.2	1.7	2.3	3.8	50	69	49
A	B	C	D	D	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$

Table VII. Effect of  $\text{NO}_3^-$  on Assimilation of  $\text{NH}_4^+$  in Light ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_3^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_3^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_3^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_3^-$ reduced	% $\text{NH}_4^+$ utilized	N reduced (%) N available
0	5	8.7	0	2.7	0	6.0	0	69	69
2.5	5	11.5	1.8	2.5	3.8	12.8	69	84	75
5	5	12.2	3.9	2.6	9.7	19.3	71	88	75
A	B	C	C	C	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$

Table VIII. Effect of  $\text{NO}_3^-$  on Assimilation of  $\text{NH}_4^+$  in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_3^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_3^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_3^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_3^-$ reduced	% $\text{NH}_4^+$ utilized	N reduced (%) N available
0	5	2.5	0	0.91	0	1.6	0	64	64
2.5	5	1.8	0.9	0.74	0.4	1.5	31	66	47
5	5	2.9	1.7	0.82	0.6	2.7	26	77	52
A	B	C	C	D	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$

Table IX. Effect of  $\text{NH}_4^+$  on Assimilation of  $\text{NO}_2^-$  in Light ( $\mu\text{moles/g fr wt} \times 8 \text{ h}$ )

Treatment $\text{NO}_2^- \text{ NH}_4^+$ (mM)	$\text{NO}_2^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_2^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_2^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_2^-$ reduced	% $\text{NH}_4^+$ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
	A	B	C	D	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$
5 0	11.3	0	0.1	1.6	11.2	9.6	99	86	86
5 2.5	11.4	4.6	0.1	1.7	11.3	14.2	99	89	89
5 5	12.0	8.1	0.2	2.4	11.8	17.5	98	88	87

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Table X. Effect of  $\text{NH}_4^+$  on Assimilation of  $\text{NO}_2^-$  in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ )

Treatment $\text{NO}_2^- \text{ NH}_4^+$ (mM)	$\text{NO}_2^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_2^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_2^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_2^-$ reduced	% $\text{NH}_4^+$ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
	A	B	C	D	E=A-C	F=B+E-D	$\frac{G=E(100)}{A}$	$\frac{H=F(100)}{B+E}$	$\frac{I=F(100)}{A+B}$
5 0	2.6	0	0.01	0.6	2.6	2.0	100	77	77
5 2.5	2.1	0.6	0.01	0.8	2.1	1.9	100	70	70
5 5	2.7	2.1	0.01	0.9	2.7	3.9	100	81	81

Table XI. Effect of  $\text{NO}_2^-$  on Assimilation of  $\text{NH}_4^+$  in Light ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_2^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_2^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_2^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_2^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_2^-$ reduced	% $\text{NH}_4^+$ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
0	5	7.0	0.05	2.5	0	4.5	0	64	64
2.5	5	5.4	0.08	2.4	4.1	7.1	98	75	74
5	5	4.7	0.28	2.2	7.2	9.7	96	82	80
	A	B	C	D	E=A-C	F=B+E-D	$\frac{\text{G=E(100)}}{\text{A}}$	$\frac{\text{H=F(100)}}{\text{B+E}}$	$\frac{\text{I=F(100)}}{\text{A+B}}$

Table XII. Effect of  $\text{NO}_2^-$  on Assimilation of  $\text{NH}_4^+$  in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ )

Treatment $\text{NO}_2^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_2^-$ uptake	$\text{NH}_4^+$ uptake	$\text{NO}_2^-$ in leaves	$\text{NH}_4^+$ in leaves	$\text{NO}_2^-$ reduced	$\text{NH}_4^+$ utilized	% $\text{NO}_2^-$ reduced	% $\text{NH}_4^+$ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
0	5	2.9	0.005	1.2	0	1.7	100	59	59
2.5	5	1.7	0.007	1.0	1.7	2.4	100	71	71
5	5	2.7	0.009	0.9	2.7	3.9	100	81	81
	A	B	C	D	E=A-C	F=B+E-D	$\frac{\text{G=E(100)}}{\text{A}}$	$\frac{\text{H=F(100)}}{\text{B+E}}$	$\frac{\text{I=F(100)}}{\text{A+B}}$

Table XIII. Effect of Urea on  $\text{NO}_3^-$  Assimilation in Light by Excised Cereal Leaves  
( $\mu\text{moles/g} \times 8 \text{ h}$  except for % columns)

Treatment $\text{NO}_3^-$ urea (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ in leaves	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	Urea uptake	Urea in leaves	Urea used	$\text{NH}_4^+$ in leaves at $T_0$	$\text{NH}_4^+$ in leaves at $T_{8h}$	Total $\text{NH}_4^+$ available from $\text{NO}_3^-$ and urea	$\text{NH}_4^+$ used	% $\text{NH}_4^+$ used	N used $\frac{\text{N used}}{\text{N available}}$ (100)
	A	B	C=B-A	$\frac{D=C(100)}{A}$	E	F	G=E-F	H	I	J=C+2G+H	K=I-J	$\frac{L=K(100)}{I}$	$\frac{M=K(100)}{A+2E+H}$
5 0	8.06	0.96	7.10	88	0	0	0	1.22	1.61	8.32	6.71	80	73
5 2.5	8.35	2.35	6.0	72	5.45	0	5.45	1.22	1.93	18.12	16.19	89	79
5 5.0	8.70	3.13	5.57	64	4.51	0	9.51	1.22	3.53	25.81	22.28	86	77

Table XIV. Effect of Urea on  $\text{NO}_3^-$  Assimilation by Excised Cereal Leaves in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ )

Treatment $\text{NO}_3^-$ urea (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ in leaves	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	Urea uptake	Urea in leaves	Urea used	$\text{NH}_4^+$ in leaves at $T_0$	$\text{NH}_4^+$ in leaves at $T_{8h}$	Total $\text{NH}_4^+$ available from $\text{NO}_3^-$ and urea	$\text{NH}_4^+$ used	% $\text{NH}_4^+$ used	N used $\frac{\text{N used}}{\text{N available}}$ (100)
	A	B	C=B-A	$\frac{D=C(100)}{A}$	E	F	G=E-F	H	I	J=C+2G+H	K=I-J	$\frac{L=K(100)}{I}$	$\frac{M=K(100)}{A+2E+H}$
5 0	3.0	2.10	1.12	35.5	0	0	0	0.7	1.09	1.82	0.73	40	20
5 2.5	2.49	2.60	0.13	5	1.70	0	1.7	0.7	1.11	4.23	3.12	73.8	47
5 5.0	3.09	3.91	0.0	0	3.59	0	3.59	0.7	1.40	7.88	6.48	82.2	59

Table XV. Effect of  $\text{NO}_3^-$  on Assimilation of Urea by Excised Cereal Leaves in Light ( $\mu\text{moles/g} \times \text{h}$ ).

Treatment $\text{NO}_3^-$ urea (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ in leaves	$\text{NO}_3^-$ reduced	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	Urea uptake	Urea in leaves	Urea used	$\text{NH}_4^+$ in leaves at $T_0$	$\text{NH}_4^+$ in leaves at $T_{8h}$	Total $\text{NH}_4^+$ available from $\text{NO}_3^-$ and urea	$\text{NH}_4^+$ used	% $\text{NH}_4^+$ used	N used (100) N avail- able
	A	B	C=B-A	D=C(100) A	E	F	G=E-F	H	I	J=C+2G+H	K=J-I	L=K(100) J	M=K(100) A+2E+H	
5	0	0	0	0	8.71	0	8.71	1.22	3.01	18.64	15.63	84		
5	3.94	1.76	2.18	55	10.8	0	10.8	1.22	3.32	25.0	21.68	81		
5	8.71	3.13	5.58	64	9.51	0	9.51	1.22	3.53	25.82	22.29	86		

Table XVI. Effect of  $\text{NO}_3^-$  on Assimilation of Urea by Excised Cereal Leaves in Darkness ( $\mu\text{moles/g} \times 12 \text{ h}$ ).

Treatment $\text{NO}_3^-$ urea (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ in leaves	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	Urea uptake	Urea in leaves	Urea used	$\text{NH}_4^+$ in leaves at $T_0$	$\text{NH}_4^+$ in leaves at $T_{8h}$	Total $\text{NH}_4^+$ available from $\text{NO}_3^-$ and urea	$\text{NH}_4^+$ used	% $\text{NH}_4^+$ used	N used (100) N avail- able
	A	B	C=B-A	D=C(100) A	E	F	G=E-F	H	I	J=C+2G+H	K=I-J	L=K(100) I	M=K(100) A+2E+H
0	5	0	0	0	3.93	0	3.93	0.7	1.44	8.56	7.12	83.2	83.2
2.5	5	0.97	1.63	0	3.25	0	3.26	0.7	1.27	7.22	5.95	82.4	72.6
5	5	3.09	4.04	0	3.59	0	3.59	0.7	1.40	7.88	6.48	82.2	59.0

Table XVII. Effect of  $\text{NH}_4^+$  on Assimilation of  $\text{NO}_3^-$  by Excised (Yecora Rojo) Wheat Roots ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ in roots	$\text{NO}_3^-$ reduced	$\text{NO}_3^-$ reduced	$\text{NH}_4^+$ uptake	$\text{NH}_4^+$ in roots at $t_0$	$\text{NH}_4^+$ in roots at $t_{8h}$	Total $\text{NH}_4^+$ avail- able	$\text{NH}_4^+$ used	% $\text{NH}_4^+$ used	Total N available in roots	N used (100) N avail- able
	A	B	C=A-B	$\frac{D=C(100)}{A}$	E	F	G	H=C+E+F	I=H-G	$\frac{J=I(100)}{G}$	K=A+E+F	$\frac{L=I(100)}{K}$
1 0	23.0	16.43	6.04	26	---	1.61	1.95	7.65	5.7	75	24.61	23
1 0.5	17.28	13.63	3.65	21	18.08	1.61	3.26	23.34	20.08	86	36.97	54
1 1.0	13.78	11.20	2.58	19	32.08	1.61	8.70	36.27	27.57	76	47.47	58

Table XVIII. Effect of  $\text{NO}_2^-$  on Assimilation of  $\text{NO}_3^-$  by Yecora Rojo Wheat Roots ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NO}_2^-$ (mM)	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ in roots	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	$\text{NO}_2^-$ uptake	$\text{NO}_2^-$ in roots at $t_0$	$\text{NO}_2^-$ in roots at $t_{8h}$	Total $\text{NO}_2^-$ avail- able	Total $\text{NO}_2^-$ reduced from $\text{NO}_3^-$ available	Total N available in roots	% $\text{NO}_2^-$ reduced of total $\text{NO}_2^-$ available	N used (100) N avail- able
	A	B	C=A-B	$\frac{D=C(100)}{A}$	E	F	G	H=C+E+F	I=H-G	$\frac{J=I(100)}{G}$	K=A+E+F	$\frac{L=I(100)}{K}$
1 0	25.04	19.95	5.09	20.2	---	0.0	0.0	5.09	5.09	25.05	100	20.3
1 0.5	11.08	11.59	0	0	10.39	0.0	0.13	10.39	10.26	21.47	98.7	47.8
1 1.0	6.37	6.66	0	0	14.98	0.0	0.89	14.98	14.09	21.35	94.0	66.0



Table XIX. Effect of  $\text{NO}_2^-$  on uptake and utilization of  $\text{NH}_4^+$  by Excised Yecora Rojo Wheat Roots ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_2^-$ $\text{NH}_4^+$ (mM)	$\text{NH}_4^+$ in roots at $t_0$ at $t_{8h}$	$\text{NO}_2^-$ uptake	$\text{NO}_2^-$ in roots at $t_0$ at $t_{8h}$	$\text{NO}_2^-$ reduced	$\text{NO}_2^-$ reduced	Total $\text{NH}_4^+$ available in roots	$\text{NH}_4^+$ used	Total N available in roots	% $\text{NH}_4^+$ used	N used (100)	N avail- able		
	A	B	C	D	E	F	G=D-F	H=G(100) D	I=A+B+G	J=I-C	K=A+B+D	L=J(100) I	M=J(100) K
0	1.0	41.24	1.95	8.95	---	0.0	0.0	---	43.21	34.26	43.21	79	79
0.5	1.0	0.046	1.97	2.95	10.12	0.0	0.99	90	11.16	8.21	12.15	74	68
1.0	1.0	0	1.97	3.03	8.54	0.0	1.28	85	9.34	6.31	10.62	68	59

Table XX. Effect of  $\text{NO}_3^-$  on Uptake and Utilization of  $\text{NH}_4^+$  by Excised Yecora Rojo Wheat Roots ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NH}_4^+$ (mM)	$\text{NH}_4^+$ in roots at $t_0$ at $t_{8h}$	$\text{NH}_4^+$ uptake	$\text{NO}_3^-$ in roots at $t_0$ at $t_{8h}$	$\text{NO}_3^-$ uptake	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	Total $\text{NH}_4^+$ available in roots	$\text{NH}_4^+$ used	Total N available in roots	% $\text{NH}_4^+$ used	N used (100)	N avail- able
	A	B	C	D	E	F=D-E	G=F(100) D	H=A+B+F	I=H-B	J=A+B+D	K=I(100) H	L=I(100) J
0	1.0	32.12	1.61	11.05	---	---	33.73	22.68	33.73	67	67	67
0.5	1.0	33.71	1.61	8.54	11.55	2.24	19	29.02	46.86	77	62	62
1.0	1.0	32.08	1.61	8.7	13.78	2.58	19	27.57	57.57	76	58	58

GRAND TOTAL  
OF POOR QUALITY

Table XXI. Effect of  $\text{NO}_3^-$  on Uptake and Utilization of  $\text{NO}_2^-$  by Excised Yecora Rojo Wheat Roots ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_3^-$ $\text{NO}_2^-$ (mM)	$\text{NO}_2^-$ in roots uptake at $t_0$	$\text{NO}_2^-$ in roots at $t_{8h}$	$\text{NO}_3^-$ uptake in roots	$\text{NO}_3^-$ reduced in roots	$\text{NO}_3^-$ reduced	% $\text{NO}_3^-$ reduced	Total $\text{NO}_2^-$ available in roots	Total $\text{NO}_2^-$ reduced	Total N available in roots	% $\text{NO}_2^-$ reduced of available $\text{NO}_2^-$	N used (100)	N available
	A	B	C	D	E	F	G	H=A+F	I=A+B+F-C	J=A+B+D	$\frac{K=I(100)}{H}$	$\frac{L=I(100)}{J}$
0 1.0	18.87	0.0	1.39	---	---	---	18.87	17.48	18.87	94.8	94.8	
0.5 1.0	17.92	0.0	0.44	8.82	10.13	0	17.92	17.48	26.64	97.5	65.6	
1.0 1.0	18.07	0.0	0.59	11.37	12.48	0	18.07	16.48	30.55	96.7	57.2	

Table XXII. Effect of  $\text{NH}_4^+$  on Uptake and Utilization of  $\text{NO}_2^-$  by Excised Yecora Rojo Wheat Roots ( $\mu\text{moles/g} \times 8 \text{ h}$ )

Treatment $\text{NO}_2^-$ $\text{NH}_4^+$ (mM)	$\text{NO}_2^-$ uptake	$\text{NO}_2^-$ in roots at $t_0$	$\text{NO}_2^-$ in roots at $t_{8h}$	$\text{NO}_2^-$ reduced uptake	% $\text{NO}_2^-$ reduced	$\text{NO}_2^-$ reduced	$\text{NH}_4^+$ in roots at $t_0$	$\text{NH}_4^+$ in roots at $t_{8h}$	Total $\text{NH}_4^+$ available in roots	$\text{NH}_4^+$ used	% $\text{NH}_4^+$ used of total available	Total N available in roots	N used (%)	N available
	A	B	C	D=A-C	$\frac{E=D}{A}$	F	G	H	I=D+F+G	J=I-H	$\frac{K=J(100)}{I}$	L=A+B+F+G	$\frac{M=J(100)}{L}$	
0.1 0.0	11.48	0.0	0.81	10.67	93	---	1.97	2.27	12.65	10.37	82	13.15	79	
0.1 0.5	9.61	0.0	1.56	8.05	83	0	1.97	2.32	10.02	7.7	77	11.58	66	
0.1 1.0	8.64	0.0	1.26	7.37	85	0	1.97	3.03	9.34	6.31	68	10.60	60	

ORIGINALITY OF POOR QUALITY