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

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CELSS

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 -- NO_3^-, NO_2^-, 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 NO_2^- and NH_4^+ in roots. In contrast, both NO_2^- and NH_4^+ inhibited NO_3^- uptake. Ammonium slightly decreased NO_3^- reduction while NO_2^- almost totally inhibited NO_3^- reduction. NH_4^+ slightly decreased NO_2^- uptake and reduction. Nitrite almost totally inhibited 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: NO_3^-, NO_2^-, and NH_4^+. This report will deal with the results obtained using intact wheat seedlings.

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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 $\mu E/m^{-2} sec^{-1}$), 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.

NO_3^-, NO_2^-, and NH_4^+ analyses. NO_3^- was determined by HPLC. NO_2^- and 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 NO_3^-, NO_2^-, and 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.

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 NO_3^- (Table 1).

The lag period is apparently the time required for induction of the NO_3^- uptake system. NO_3^- uptake was linear from about 1 mM NO_3^- to total depletion of NO_3^- from the solution. Other work has shown a typical Michaelis-Menten plot for NO_3^- uptake between 0 and 1 mM NO_3^- with a steady-state rate between 0.5 and 1 mM NO_3^-. When plants are allowed to deplete the 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 NO_3^- from a solution becoming progressively more dilute.

NO_2^- uptake. The concentration of 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 NO_2 was lost. Subsequently, a long lag of about 4 h occurred before NO_2^- was taken up linearly. The plants also completely depleted the concentration of NO_2^- (Table 1). The lag in the rate of uptake could also be partially due to the necessity to induce an NO_2^- uptake system.

NH_4^+ uptake. Uptake of NH_4^+ began immediately after the plants were placed in the uptake solution and was linear with time and concentration to total depletion of NH_4^+ from the solution (Fig. 3A, B; Table 1). The plants took up NH_4^+ extremely efficiently.

Uptake from a solution containing NO_3^- and NO_2^-. Nitrate was not taken up for about 7 h when present with NO_2^- (Fig. 4A, B). After uptake began, it was linear with time and concentration to total depletion of 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 NO_2^- in solution until all of it was depleted (Fig. 4 AB; Table 1). Nitrate uptake did not begin until the NO_2^- concentration decreased to about 0.1 mM (Fig. 4A). After NO_3^- uptake began, its rate was almost identical to that where only NO_3^- was present (Table 2). The rate of uptake of NO_2^- was less in the presence of NO_3^- (Table 2).

Uptake from a Solution Containing NO_2^- and NH_4^+. The uptake rate of both NH_4^+ and NO_2^- was decreased in the combined solution. The rate of NH_4^+ uptake from a solution containing only

NH_4^+ was 2 µmoles/g fresh weight x h; in the present of NO_2^- the rate decreased to about 1 µmole/g fresh weight x h (Table 2). The rate of NO_2^- uptake from a solution containing only NO_2^- was 1 µmole/g fresh weight x h and was half that in the presence of NH_4^+ (Table 2). Although the rates were slower, both NO_2^- and NH_4^+ were totally depleted during the course of the experiment (Table 1).

Uptake from a Solution Containing NO_3^- and NH_4^+. The lag period before NO_3^- uptake became linear was about 2 h longer in the presence of NH_4^+ (Fig. 6 AB). The rate of $NO3^-$ uptake was slightly (about 20%) decreased in the presence of NH_4^+ (Table 2). Nitrate uptake was slow until about 70% of the NH_4^+ had disappeared from the solution, showing the preferential use of 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 NO_3^- until the solution was depleted (Fig. 6CD; Table 2).

Uptake from a Solution Containing NO_3^-, NO_2^-, and NH_4^+. It appeared that NO_3^- uptake was inhibited until about 60% of the NO_2^- and about 35% of the NH_4^+ had disappeared from solution (Fig. 7 A-F). After 4 h 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 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 NO_3^- and NH_4^+ before initiating the experiments, the plants quite efficiently utilized all three sources.

Assimilation of N sources. Nitrite and 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, NO_3^- assimilation decreased when NO_2^- and NH_4^+ were present in the same solution. The percentage of total N assimilated decreased when 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 NH_4^+ as the N source (Fig. 8). In contrast, the pH increases when NO_3^- and NO_2^- are the sources. We have used two methods to maintain pH between about 6.5 to 7.0. These are: Adding a K^+ charged cation exchange resin to the solution to adsorb H^+ ions or adding $CaCO_3$ to buffer either H^+ or OH^- coming out of plant roots. Of the two methods, the use of $CaCO_3$ 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 NH_4^+ and NO_2^- but discriminate against NO_3^- especially in the presence of 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 NO_3^- in the presence of the combined sources apparently because 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 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 NO_3^-	NO_2^-	NH_4^+	Total Utilization of all N Sources %
NO_3^- (Fig. 1)	100			100
NO_2^- (Fig. 2)		100		100
NH_4^+ (Fig. 3)			100	100
NO_3^- + NO_2^- (Fig. 4)	100	100		100
NO_2^- + NH_4^+ (Fig. 5)		100	100	100
NO_3^- + NH_4^+ (Fig. 6)	100		100	100
NO_3^- + NO_2^- + 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 µmoles/g fresh weight x h NO_3^-	NO_2^-	NH_4^+
NO_3^- (Fig. 1 AB)	1.5		
NO_2^- (Fig. 2 AB)		1.0	
NH_4^+ (Fig. 3 AB)			2.0
NO_3^- + NO_2^- (Fig. 4 AB)	1.4	0.6	
NH_4^+ + NO_2^- (Fig. 5 AB)		0.5	1.1
NO_3^- + NH_4^+ (Fig. 6 A-D)	1.2		2.0
NO_3^- + NO_2^- + 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.

	Uptake			Concn At To			Concn At t_{12h}			Assimilation		
A.	µmoles/g			µmoles/g			µmoles/g			µmoles/g		
N components in nutrient solutions 1 mM each	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-	NH_4^+
NO_3^-	11.42			0.86			6.03			6.25		
NO_2^-		5.00			0.00			0.00			5.00	
NH_4^+			11.16			3.71			4.30			10.57
$NO_3^- + NO_2^-$	10.50	11.29		0.04	0.00	4.01	1.59	0.25	4.00	8.95	19.99	19.98[a]
$NO_3^- + NH_4^+$	11.10		11.44	0.86		3.30	7.11	0.00	3.47	4.85	4.85	16.12[b]
$NO_2^- + NH_4^+$		9.00	11.14		0.00	3.71		0.00	4.75		9.00	19.10[c]
$NO_3^- + NO_2^- + NH_4^+$	11.10	11.40	11.66	0.86	0.00	3.30	8.00	0.00	3.46	3.96	11.40	26.86[b]

Table 3. (Continued)

B.

	% Assimilation NO_3^-	% Assimilation NO_2^-	% Assimilation NH_4^+	$\frac{\text{Total N Assimilation Through } NH_4^+}{\text{Total Uptake of all Sources}}$ (100)
NO_3^-	55	100		55
NO_2^-		100		100
NH_4^+			95	95
$NO_3^- + NO_2^-$	85	99	100	92
$NO_3^- + NH_4^+$	44	100	99	72
$NO_2^- + NH_4^+$		100	95	95
$NO_3^- + NO_2^- + NH_4^+$	36	100	99	79

[a] NO_3^- assimilated + NO_2^- assimilated

[b] NO_3^- assimilated + NO_2^- assimilated + NH_4^+ assimilated

[c] NO_2^- assimilated + NH_4^+ assimilated

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Figure Legends

Fig. 1. The uptake of NO_3^- from a nutrient solution containing only NO_3^- (at 1 mM) as the N source.

1A: The disappearance of NO_3^- from solution Fresh weight of plant material was 3.91 g.
1B: The uptake of NO_3^- on a g fresh weight basis.

Fig. 2. The uptake of NO_2^- from a nutrient solution containing only NO_2^- (at 1 mM) as the N source.

1A: The disappearance of NO_2^- from solution. Fresh weight of plants was 4.09 g.
1B: The uptake of NO_2^- on a g weight basis.

Fig. 3. The uptake of NH_4^+ from a nutrient solution containing only NH_4^+ (at 1 mM) as the N source.

1A: The disappearance of NO_2^- from solution. Fresh weight of plants was 4.01.
1B: The uptake of NO_2^- on a g fresh weight basis.

Fig. 4. The uptake of NO_3^- and NO_2^- from a nutrient solution containing both N sources (each at 1 mM).

1A: The disappearance of NO_3^- and NO_2^- from solution. Fresh weight of plant material was 3.81 g.
1B: The uptake of NO_3^- and NO_2^- on a g fresh weight basis.

Fig. 5. The uptake of NO_2^- and NH_4^+ from a nutrient solution containing both N sources (each at 1 mM).

1A: The disappearance of NO_2^- and NH_4^+ from solution. Fresh weight of plants was 3.94 g.

1B: The uptake of NO_2^- and NH_4^+ on a g fresh weight basis.

Fig. 6. The uptake of NO_3^- and NH_4^+ from a nutrient solution containing both N sources (each at 1 mM).

1A: The disappearance of NO_3^- and NH_4^+ from solution. Fresh weight of plant material was 3.02.
1B: The uptake of NO_3^- and NH_4^+ on a g fresh weight basis.

Fig. 7. Uptake of NO_3^-, NO_2^-, and NH_4^+ from a nutrient solution containing the three N sources (each at 1 mM).

1A: The disappearance of NO_3^-, NO_2^- and NH_4^+ from solution. Fresh weight of plant material was 3.00.
1B: The uptake of NO_3^-, NO_2^-, and NH_4^+ on a g fresh weight basis.

Fig. 8. Changes in pH of nutrient solutions as wheat plants take up NO_3^-. Nutrient solutions were $\frac{1}{4}$ strength Hoagland's with 1 mm KNO_3.

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Fig. 1A

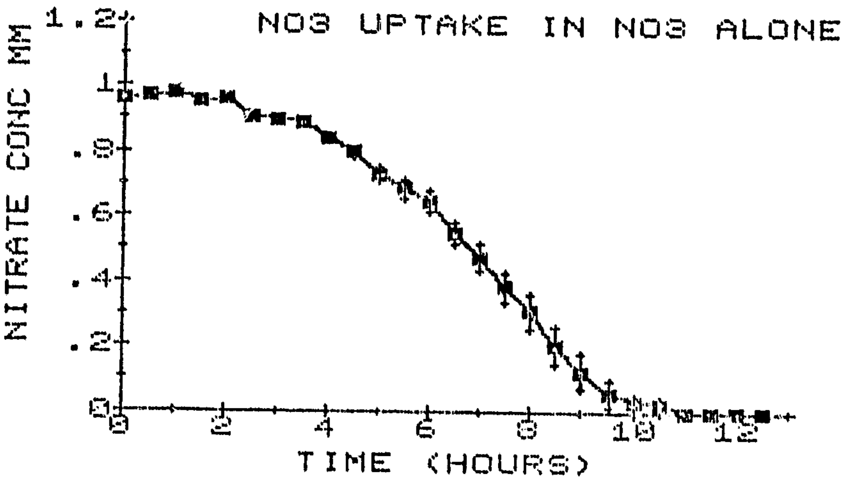

Fig. 1B

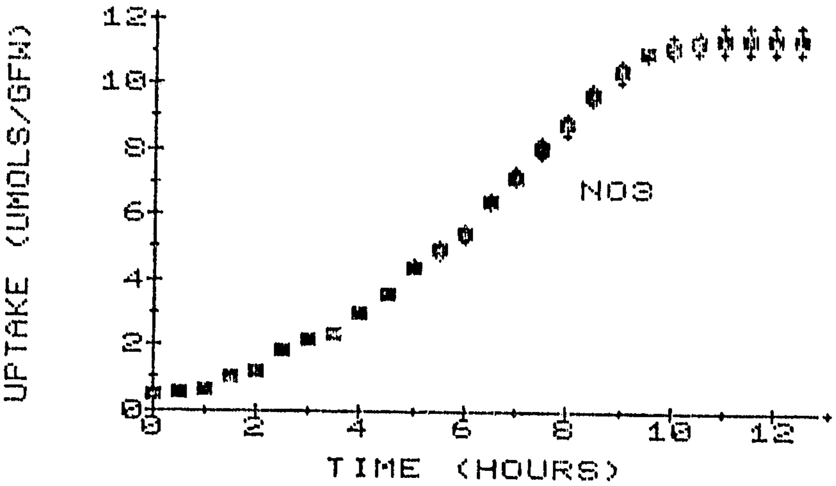

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Fig. 2A

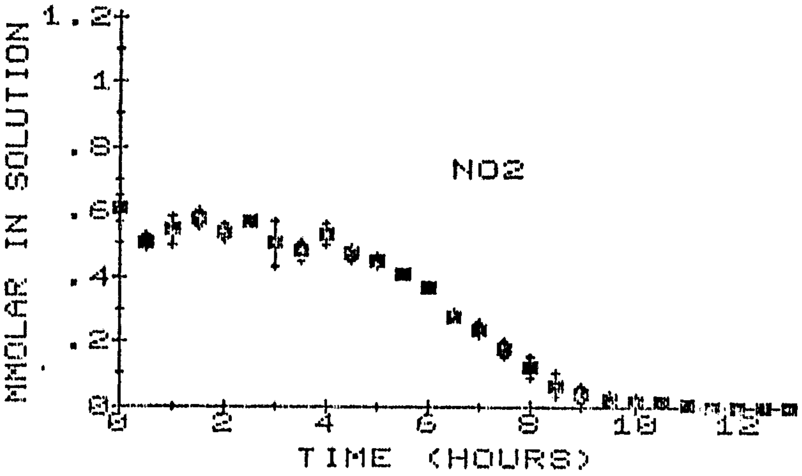

Fig. 2B

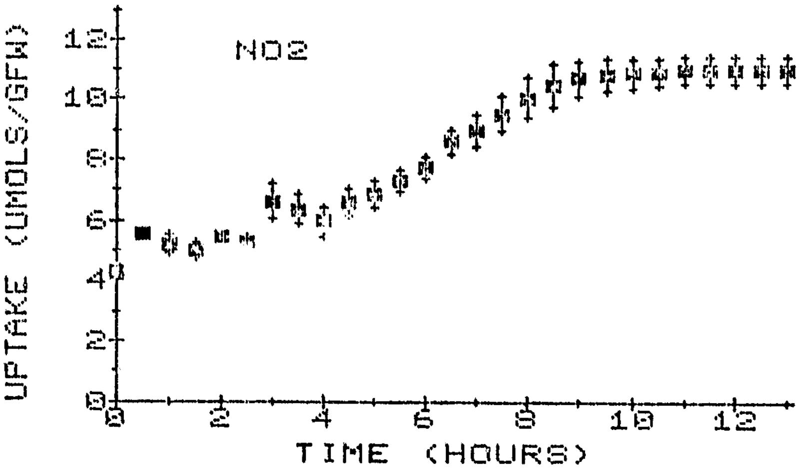

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Fig. 3A

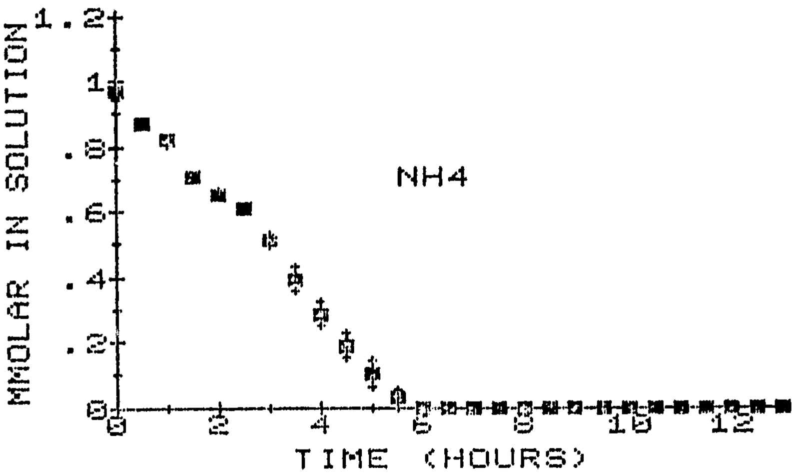

Fig. 3B

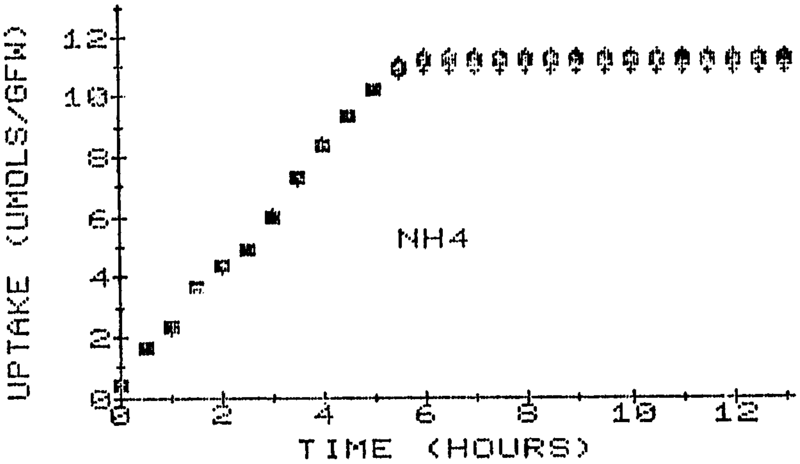

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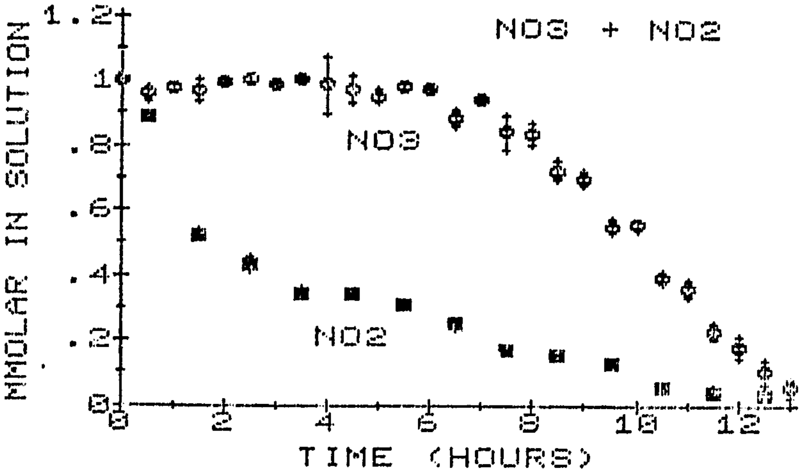
Fig. 4A



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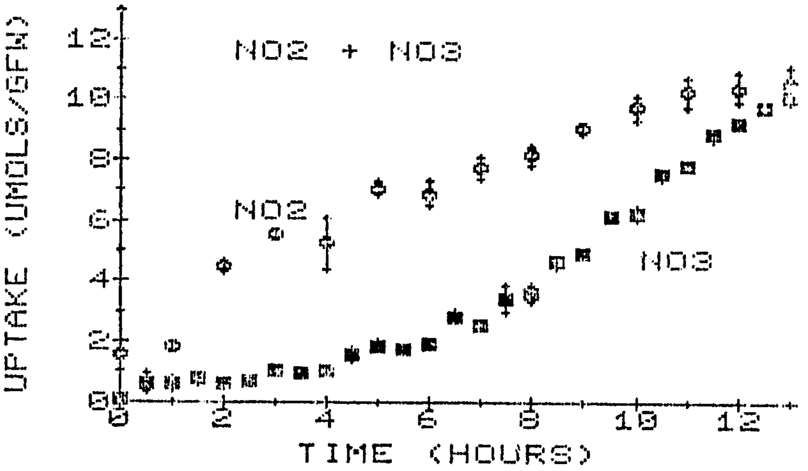

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Fig. 5A

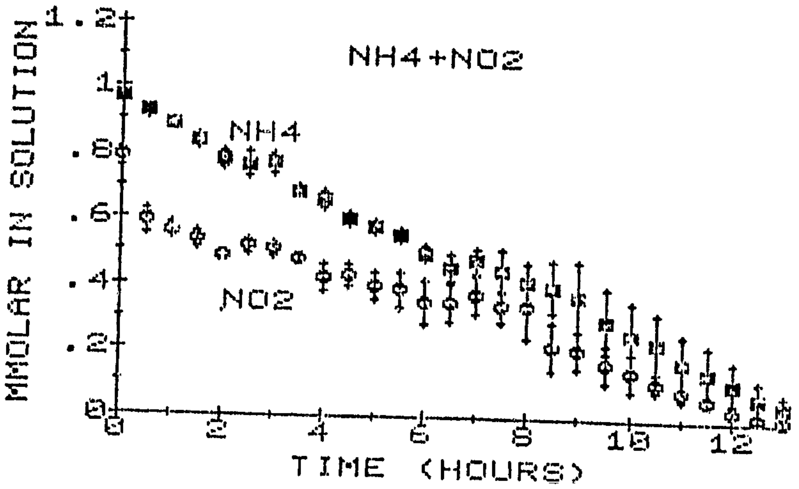

Fig. 5B

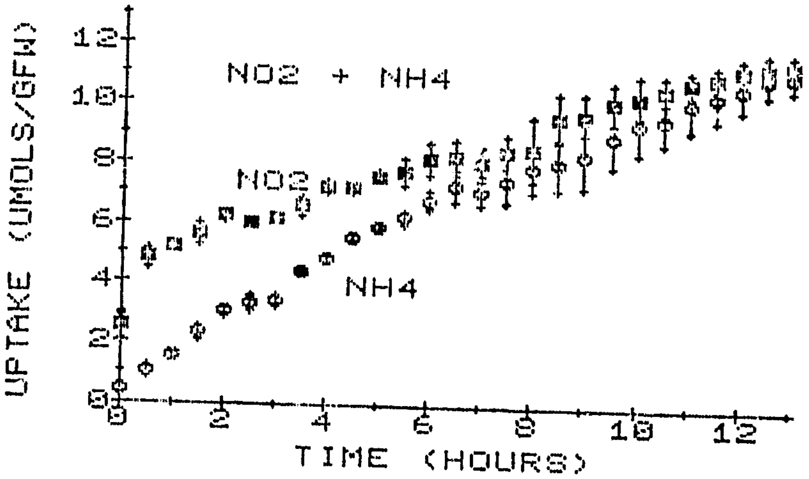

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Fig. 6A

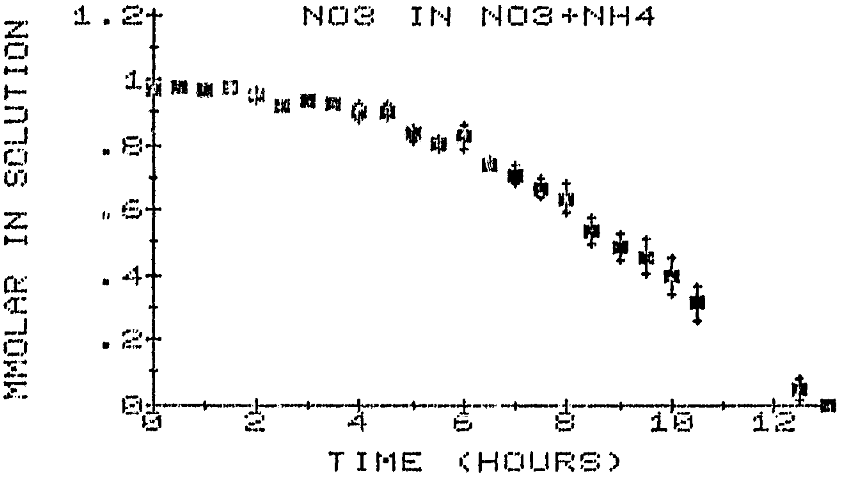

Fig. 6B

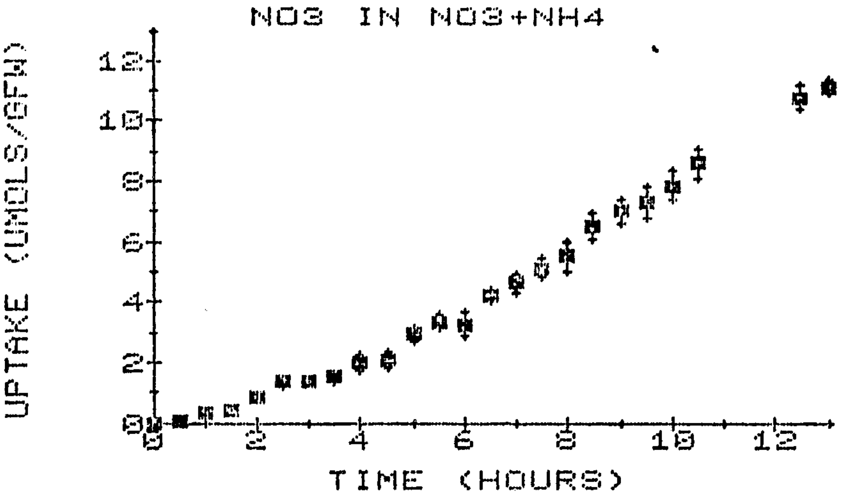

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Fig. 6C

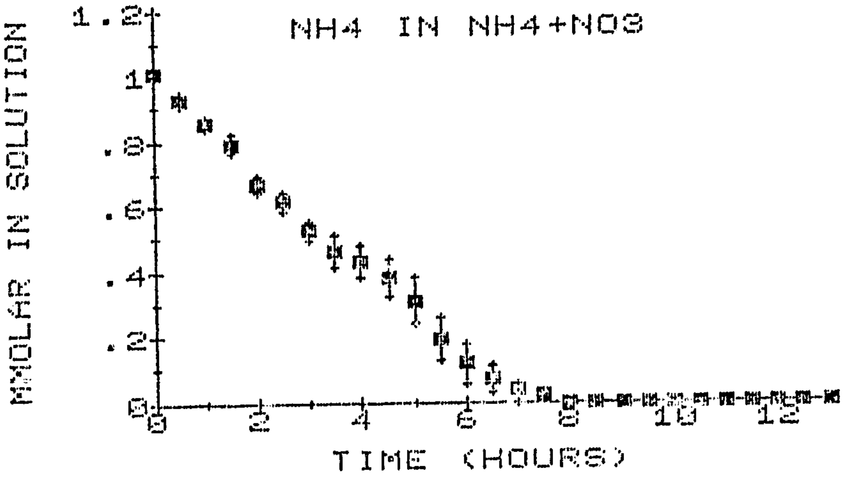

Fig. 6D

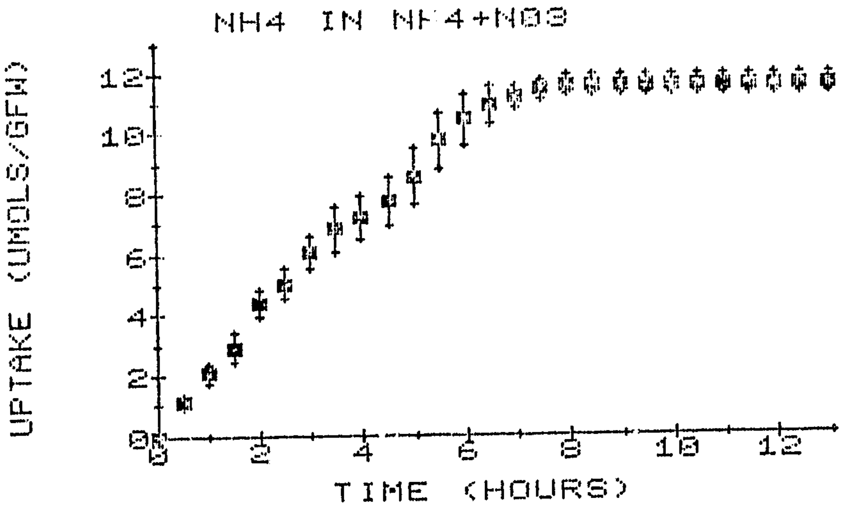

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Fig. 7A

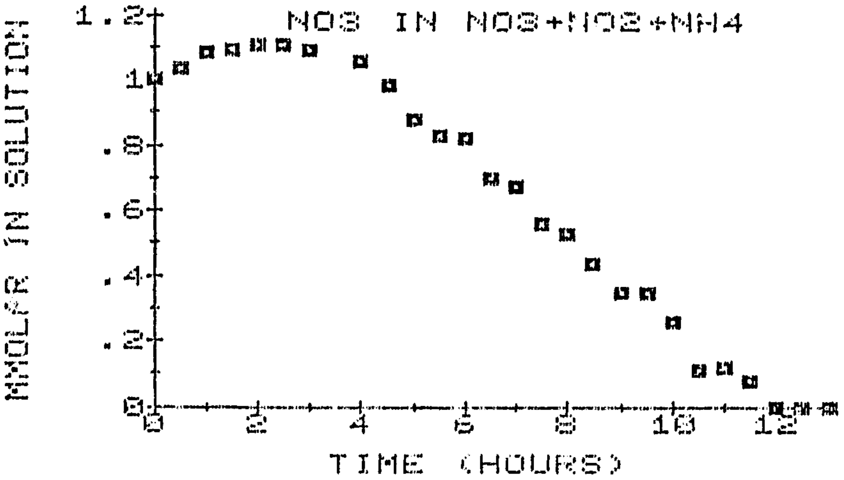

Fig. 7B

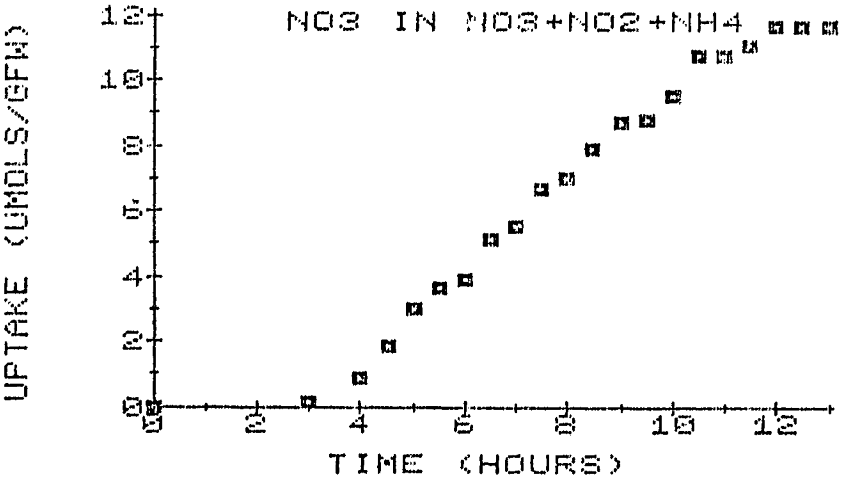

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Fig. 7C

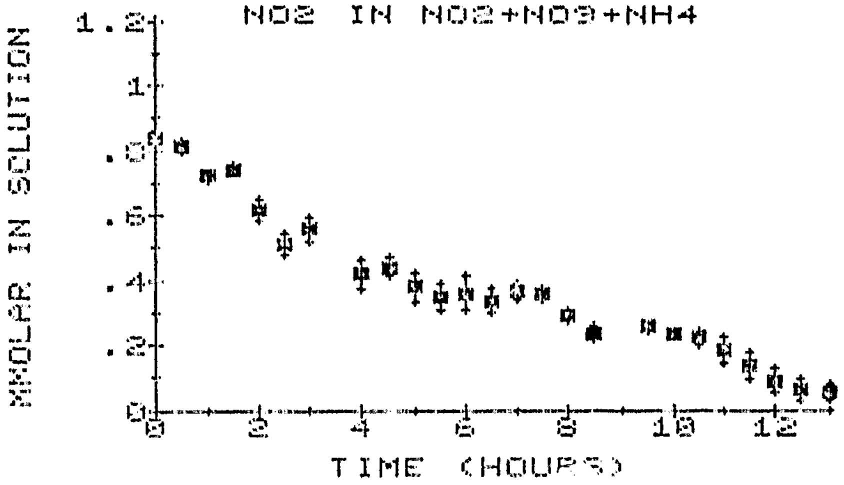

Fig. 7D

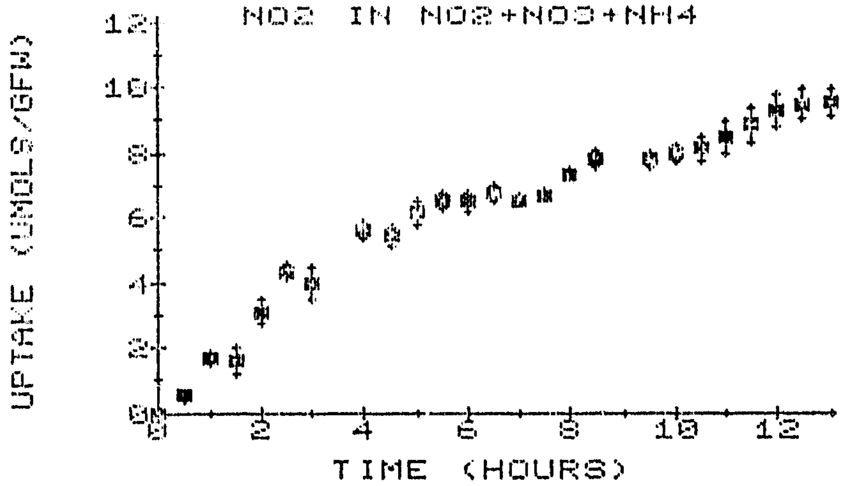

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Fig. 7E

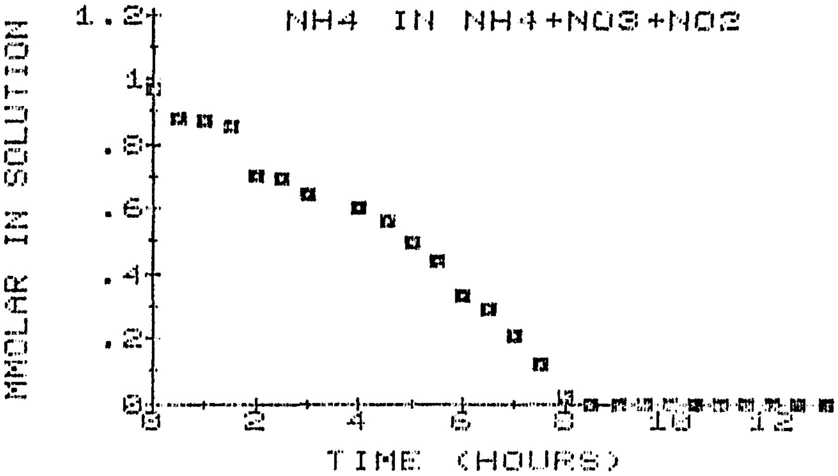

Fig. 7F

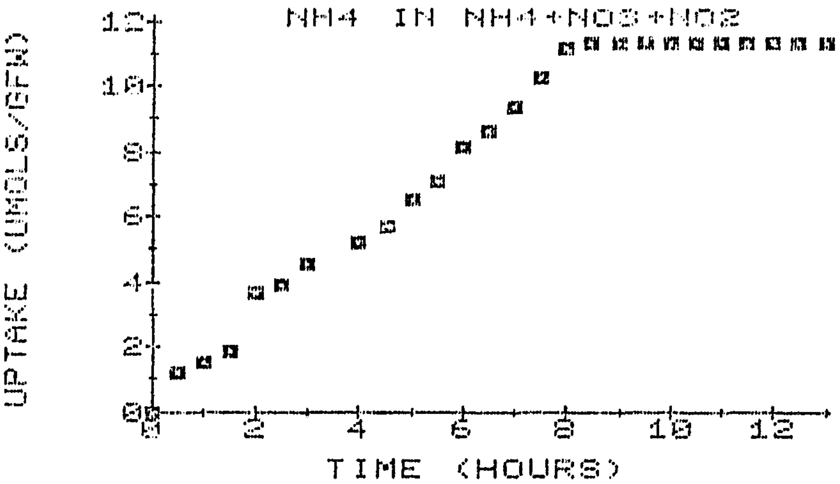

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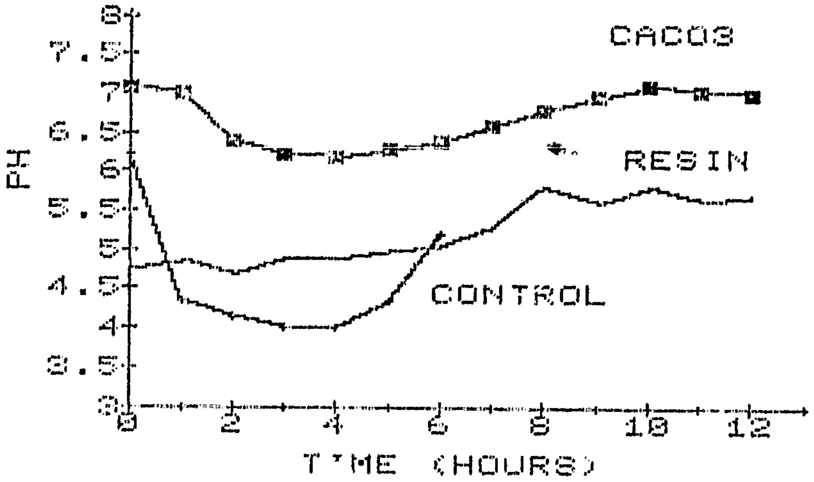
Fig. 8



Assimilation of Mixed-N Sources, NO_3^-, NO_2^-, 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 — NO_3^-. NO_2^-, 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 NO_3^-, NO_2^-, 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 NO_2^- were determined by HPLC (Thayer and Huffaker, 1980), and 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 NO_2^- on NO_3^- assimilation (Tables I and II)

In light, the reduction of NO_3^- decreased about 17% as NO_2^- was increased in concentration in the external solution (Table I, line E). Ninety-nine percent of the NO_2^- absorbed from the external solution and the NO_2^- resulting from NO_3^- reduction was inhibited (line H). Since essentially all the NO_2^- in the plant is reduced, the total N reduced from both sources (line 1) increased as NO_2 increased in the external solution. In darkness, NO_2^- had little effect on NO_3^- reduction (Table II, line E). Again, all of the NO_2^- in the plant was further reduced (line H), whereas about 40% of the NO_3^- was reduced (line G). The presence of NO_2^- increased the total N reduced in darkness (37 to 76%, line I).

Effect of NO_3^- on NO_2^- assimilation (Tables III and IV)

Increasing NO_3^- in the substrate solution had no effect on NO_2^- assimilation in light or darkness (line H). Again 98% of the NO_2^- taken up from the external solution and that formed from NO_3^- reduction was reduced. Because less NO_3^- is reduced than NO_2^-, the total N reduced decreased as NO_3^- increased in the substrate solution (line I).

Effect of NH_4^+ on NO_3^- assimilation (Tables V and VI)

As NH_4^+ was increased in the external nutrient solution, NO_3^- reduction was somewhat decreased in both light and dark (line G). In light a constant amount of NH_4^+ was utilized (Table V, line H), whereas in darkness, NH_4^+ utilization increased as NH_4^+ increased (Table VI, line H). In light the presence of external NH_4^+ slightly decreased the total utilization from both sources, whereas in darkness, the presence of NH_4^+ increased the total utilization of both sources (line I). Since the leaf NO_2^- concentration was negligible, essentially all NO_3^- reduced was converted

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to NH_4^+. The extent of utilization of NO_3^- and NH_4^+ was more complete in light than darkness.

Effect of NO_3^- on NH_4^+ assimilation (Tables VII and VIII)

As external NO_3^- increased, more NH_4 was assimilated (line H), although the total N assimilated was affected little in light (line I). In darkness, NH_4^+ assimilation also increased in the presence of NO_3^- (line H); however, overall assimilation of total N decreased somewhat as NO_3^- increased (Table VIII, line I). because of low assimilation of NO_3^-. More NH_4^+ was assimilated than NO_3^-.

Effect of NH_4^+ on NO_2^- assimilation (Tables IX and X)

Increasing NH_4^+ did not affect 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 NH_4^+.

Effect of NO_2^- on NH_4^+ assimilation (Tables XI and XII)

Almost all NO_2^- absorbed was reduced (96% or more, line G). Total utilization of NH_4^+ increased in the presence of NO_2^-, from 64 to 82% in light and 59 to 81% in darkness, as NO_2^- increased in the external solution (line H). Total assimilation from both N sources (line I) increased as external NO_2^- increased.

Effect of urea on NO_3^- assimilation (Tables XIII and XIV)

Urea, supplied at a similar concentration to NO_3^- or NH_4^+, caused a 3-fold increase in both NO_3^- and NH_4^+ concentrations, respectively, and inhibited NO_3^- reduction about 24% in light (Table XIII, lines B, H, and C, respectively). Urea was metabolized to NH_4^+ very rapidly inside the leaves (line F); the resulting 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 NO_3^- reduction by 88% (lines C and D) at one-half equimolar concentration, and it totally inhibited NO_3^- reduction at an equimolar concentration (Table XIV). Urea was completely metabolized (line F); the resulting NH_4^+ was also efficiently assimilated (lines K, L). The great decrease in NO_3^- reduction in the presence of urea may be due to its rapid conversion to NH_4^+, which would produce an NH_4^+ concentration equal to 2 times that of NO_3^-. Because more urea and NH_4^+ were assimilated than NO_3^-, the presence of urea increased the percentage of total assimilation from all N sources (line M).

Effect of NO_3^- on urea assimilation (Tables XV and XVI)

NO_3^- had no effect on urea metabolism (line F) or the resulting NH_4^+ assimilation in light (lines G, H, I, J) (Table XV). NO_3^- also had no effect on the percentage of total N assimilation from any N source (line K).

In darkness NO_3^-, also had no effect on either urea or NH_4^+ assimilation (Table XVI). As NO_3^- concentration increased, the percentage of N metabolized from the total available amount decreased (line L) because of total inhibition of NO_3^- reduction in the presence of urea and NH_4^+ (line C).

B. Wheat Roots

Effect of NH_4^+ on assimilation of NO_3^- (Table XVII)

The presence of NH_4^+ at equimolar concentration with NO_3^- in the external solution inhibited NO_3^- uptake 50% (line A) but only slightly decreased the NO_3^- reduced as a percentage of that taken up (line D). Hence, the major effect of NH_4^+ on NO_3^- assimilation was inhibition of NO_3^- uptake. Because more NH_4^+ was assimilated than NO_3^-, the total utilization from both N sources increased as NH_4^+ increased (line L).

Effect of NO_2^- on NO_3^- assimilation (Table XVIII)

NO_2^- greatly inhibited both NO_3^- uptake (by 50% at one-half the concentration of NO_3^- and by 75% at equimolar concentration in the nutrient solution (Line A). NO_3^- reduction was totally inhibited at the lowest concentration of NO_2^- in the external solution (line C). Because 94 to 100% of the NO_2^- was assimilated (line K), total N utilization increased as NO_2^- increased in the nutrient solution (line L).

Effect of NO_2^- on NH_4^+ assimilation (Table XIX)

Nitrite at one-half the concentration of NH_4^+ in the external solution inhibited NH_4^+ uptake 99.9% (line A); hence, essentially no NH_4^+ from the external solution was utilized by the plant in the presence of a 1/2 equimolar concentration of nitrite.

Effect NO_3^- on NH_4^+ assimilation (Table XX)

Nitrate in the external solution slightly enhanced the utilization of NH_4^+ (lines I and K) because it had no effect on NH_4^+ uptake and slightly increased the percentage of NH_4^+ assimilated.

Effect of NO_3^- on NO_2^- assimilation (Table XXI)

External NO_3^- had no effect on NO_2^- uptake (line A) or NO_2^- reduction (lines H, J). Since NO_2^- totally inhibited NO_3^- reduction (line E), the percentage of N used from all N sources decreased (line K) as NO_3^- increased in the external solution.

Effect of NH_4^+ on NO_2^- assimilation (Table XXII)

The presence of NH_4^+ in the external solution inhibited 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 NH_4^+ uptake (line F) was totally inhibited and NO_2^- reduction (lines D, E) was somewhat inhibited.

The effects of NO_2^- and NH_4^+ on urea metabolism and, conversely, the effect of urea on NO_2^- and 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 NO_3^-, NO_2^-, and NH_4^+ in roots in this phase of the study. It will be interesting to determine whether NO_2^- will inhibit the absorption of urea as it does NH_4^+.

IV. Summary

In leaves, the presence of NO_2^- in the external solution increased the overall utilization of mixed N sources because of the great efficiency with which leaves reduce NO_2^-. Although NO_3^- reduction decreased as NO_2^- increased, the great utilization of NO_2^- more than made up for the decreased NO_3^- reduction in both light and darkness. As NO_3^- increased in the external solution in the presence of NO_2^-, the overall utilization of N decreased, because a smaller portion of the NO_3^- absorbed was reduced. The assimilation of NH_4^+ was increased as NO_3^- increased in the external solution in both light and darkness whereas NH_4^+ tended to inhibit NO_3^- reduction. NH_4^+ had no effect on NO_2^- assimilation in either light or dark; NO_2^-, on the other hand, increased the overall NH_4^+ utilization in both light and dark. The leaves preferentially reduce NO_2^-, perhaps because of its toxicity.

The cereal leaves metabolized urea and the resulting NH_4^+ extremely efficiently. Urea influenced NO_3^- assimilation then in essentially the same way NH_4^+ did in light. In darkness, urea inhibited NO_3^- reduction almost totally. By contrast, NH_4^+ inhibited NO_3^- reduction only slightly. The greater effect by urea may have been due to the systems having to assimilate twice as much NH_4^+ per molecule of urea; nevertheless, the steady-state NH_4^+ concentration was almost the same in the presence or absence of urea; so, the reasons for the great inhibition of NO_3^- reduction by urea in darkness remain to be worked out.

The major effect of NH_4^+ on NO_3^- assimilation in wheat roots was an inhibition of NO_3^- uptake. The amount of NO_3^- reduced compared with the amount absorbed was affected only slightly by NH_4^+. The presence of NO_2^- in external solutions containing NO_3^- or NH_4^+ had greatly affected on the assimilation of NO_3^- or NH_4^+.

NO_2^- substantially inhibited NO_3^- uptake and totally inhibited NO_3^- reduction at half the concentration of NO_3^-. Nitrite at half the concentration of NH_4^+ totally inhibited NH_4^+ uptake. Low levels of NO_2^- may have devastating effects on assimilation of mixed-N nources that must be taken up by roots. The presence of NO_3^-, on the other hand, had little effect on the metabolism of either NO_2^- or NH_4^+.

Since NH_4^+ assimilation by roots was much more efficient than NO_3^- assimilation, the efficiency of utilization from these mixed sources increased as NH_4^+ increased.

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Table I. Effect of NO_2^- on NO_3^- Assimilation in Light (µmoles/g × 8 h except for % columns)

Treatment NO_2^- (mM)	Treatment NO_3^- (mM)	NO_3^- uptake A	NO_2^- uptake B	NO_3^- in leaves C	NO_2^- in leaves D	NO_3^- reduced E	Total NO_2^- reduced F	% NO_3^- reduced G	% NO_2^- reduced H	$\frac{\text{N reduced (\%)}}{\text{N available}}$ I
0	5	19.5	0	6.0	0.05	13.5	13.5	69	100	69
2.5	5	23.0	16.9	9.9	0.18	13.1	29.8	57	99	75
5	5	19.4	29.8	9.3	0.53	10.1	39.4	52	99	80
		A	B	C	D	E=A–C	F=B+E–D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table II. Effect of NO_2^- on NO_3^- Assimilation in Darkness (µmoles/g × 12 h except for % columns)

Treatment NO_2^- (mM)	Treatment NO_3^- (mM)	NO_3^- uptake A	NO_2^- uptake B	NO_3^- in leaves C	NO_2^- in leaves D	NO_3^- reduced E	Total NO_2^- reduced F	% NO_3^- reduced G	% NO_2^- reduced H	$\frac{\text{N reduced (\%)}}{\text{N available}}$ I
0	5	3.8	0	2.4	0.03	1.4	1.4	37	100	37
2.5	5	4.2	6.8	2.6	0.05	1.6	8.4	38	100	76
		A	B	C	D	E=A–C	F=B+E–D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

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Table III. Effect of NO_3^- on NO_2^- Assimilation in Light (μmoles/g × 8 h except for % columns)

Treatment NO_2^- (mM)	Treatment NO_3^- (mM)	NO_3^- uptake	NO_2^- uptake	NO_3^- in leaves	NO_2^- in leaves	NO_3^- reduced	Total NO_2^- reduced	% NO_3^- reduced	% NO_2^- reduced	N reduced (%) / N available
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
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table IV. Effect of NO_3^- on NO_2^- Assimilation in Darkness (μmoles/g × 12 h)

Treatment NO_2^- (mM)	Treatment NO_3^- (mM)	NO_3^- uptake	NO_2^- uptake	NO_3^- in leaves	NO_2^- in leaves	NO_3^- reduced	Total NO_2^- reduced	% NO_3^- reduced	% NO_2^- reduced	N reduced (%) / N available
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
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

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Table V. Effect of NH_4^+ on NO_3^- Assimilation in Light (μmoles/g × 8 h)

Treatment NO_3^- (mM)	Treatment NH_4^+ (mM)	NO_3^- uptake	NH_4^+ uptake	NO_3^- in leaves	NH_4^+ in leaves	NO_3^- reduced	NH_4^+ utilized	% NO_3 reduced	% NH_4^+ utilized	$\frac{\text{N reduced (\%)}}{\text{N available}}$
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	E=A–C	F=B+E–D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table VI. Effect of NH_4^+ on NO_3^- Assimilation in Darkness (μmoles/g × 12 h)

Treatment NO_3^- (mM)	Treatment NH_4^+ (mM)	NO_3^- uptake	NH_4^+ uptake	NO_3^- in leaves	NH_4^+ in leaves	NO_3^- reduced	NH_4^+ utilized	% NO_3 reduced	% NH_4^+ utilized	$\frac{\text{N reduced (\%)}}{\text{N available}}$
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	E=A–C	F=B+E–D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

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Table VII. Effect of NO_3^- on Assimilation of NH_4^+ in Light (μmoles/g × 8 h)

Treatment NO_3^- (mM)	Treatment NH_4^+ (mM)	NO_3^- uptake	NH_4^+ uptake	NO_3^- in leaves	NH_4^+ in leaves	NO_3^- reduced	NH_4^+ utilized	% NO_3 reduced	% NH_4^+ utilized	N reduced (%) / N available
0	5	0	8.7	0	2.7	0	6.0	0	69	69
2.5	5	5.5	11.5	1.8	2.5	3.8	12.8	69	84	75
5	5	13.6	12.2	3.9	2.6	9.7	19.3	71	88	75
		A	B	C	C	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table VIII. Effect of NO_3^- on Assimilation of NH_4^+ in Darkness (μmoles/g × 12 h)

Treatment NO_3^- (mM)	Treatment NH_4^+ (mM)	NO_3^- uptake	NH_4^+ uptake	NO_3^- in leaves	NH_4^+ in leaves	NO_3^- reduced	NH_4^+ utilized	% NO_3 reduced	% NH_4^+ utilized	N reduced (%) / N available
0	5	0	2.5	0	0.91	0	1.6	0	64	64
2.5	5	1.3	1.8	0.9	0.74	0.4	1.5	31	66	47
5	5	2.3	2.9	1.7	0.82	0.6	2.7	26	77	52
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

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Table IX. Effect of NH_4^+ on Assimilation of NO_2^- in Light (μmoles/g fr wt × 8 h)

Treatment NO_2^- (mM)	Treatment NH_4^+ (mM)	NO_2^- uptake	NH_4^+ uptake	NO_2^- in leaves	NH_4^+ in leaves	NO_2^- reduced	NH_4^+ utilized	% NO_2^- reduced	% NH_4^+ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
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
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table X. Effect of NH_4^+ on Assimilation of NO_2^- in Darkness (μmoles/g × 12 h)

Treatment NO_2^- (mM)	Treatment NH_4^+ (mM)	NO_2^- uptake	NH_4^+ uptake	NO_2^- in leaves	NH_4^+ in leaves	NO_2^- reduced	NH_4^+ utilized	% NO_2^- reduced	% NH_4^+ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
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
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table XI. Effect of NO_2^- on Assimilation of NH_4^+ in Light (μmoles/g × 8 h)

Treatment (mM)		NO_2^- uptake	NH_4^+ uptake	NO_2^- in leaves	NH_4^+ in leaves	NO_2^- reduced	NH_4^+ utilized	% NO_2^- reduced	% NH_4^+ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
NO_2^-	NH_4^+									
0	5	0	7.0	0.05	2.5	0	4.5	0	64	64
2.5	5	4.2	5.4	0.08	2.4	4.1	7.1	98	75	74
5	5	7.5	4.7	0.28	2.2	7.2	9.7	96	82	80
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table XII. Effect of NO_2^- on Assimilation of NH_4^+ in Darkness (μmoles/g × 12 h)

Treatment (mM)		NO_2^- uptake	NH_4^+ uptake	NO_2^- in leaves	NH_4^+ in leaves	NO_2^- reduced	NH_4^+ utilized	% NO_2^- reduced	% NH_4^+ utilized	$\frac{\text{N reduced (100)}}{\text{N available}}$
NO_2^-	NH_4^+									
0	5	0	2.9	0.005	1.2	0	1.7	100	59	59
2.5	5	1.7	1.7	0.007	1.0	1.7	2.4	100	71	71
5	5	2.7	2.1	0.009	0.9	2.7	3.9	100	81	81
		A	B	C	D	E=A−C	F=B+E−D	$G=\frac{E(100)}{A}$	$H=\frac{F(100)}{B+E}$	$I=\frac{F(100)}{A+B}$

Table XIII. Effect of Urea on NO_3^- Assimilation in Light by Excised Cereal Leaves (µmoles/g × 8 h except for % columns)

Treatment NO_3	urea (mM)	NO_3^- uptake	NO_3^- in leaves	NO_3^- reduced	% NO_3^- reduced	Urea uptake	Urea in leaves	Urea used	NH_4^+ in leaves at T_0	NH_4^+ in leaves at T_{8h}	Total NH_4^+ available from NO_3^- and urea	NH_4^+ used	% NH_4^+ used	$\frac{\text{N used (100)}}{\text{N available}}$
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
		A	B	C=B−A	$D=\frac{C(100)}{A}$	E	F	G=E−F	H	I	J=C+2G+H	K=I−J	$L=\frac{K(100)}{I}$	$M=\frac{K(100)}{A+2E+H}$

Table XIV. Effect of Urea on NO_3^- Assimilation by Excised Cereal Leaves in Darkness (µmoles/g × 12 h)

Treatment NO_3	urea (mM)	NO_3^- uptake	NO_3^- in leaves	NO_3^- reduced	% NO_3^- reduced	Urea uptake	Urea in leaves	Urea used	NH_4^+ in leaves at T_0	NH_4^+ in leaves at T_{8h}	Total NH_4^+ available from NO_3^- and urea	NH_4^+ used	% NH_4^+ used	$\frac{\text{N used (100)}}{\text{N available}}$
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
		A	B	C=B−A	$D=\frac{C(100)}{A}$	E	F	G=E−F	H	I	J=C+2G+H	K=I−J	$L=\frac{K(100)}{I}$	$M=\frac{K(100)}{A+2E+H}$

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Table XV. Effect of NO_3^- on Assimilation of Urea by Excised Cereal Leaves in Light (µmoles/g × 8 h)

Treatment NO_3 (mM)	Treatment urea (mM)	NO_3^- uptake	NO_3^- in leaves	NO_3^- reduced	% NO_3^- reduced	Urea uptake	Urea in leaves	Urea used	NH_4^+ in leaves at T_o	NH_4^+ in leaves at T_{8h}	Total NH_4^+ available from NO_3^- and urea	NH_4^+ used	% NH_4^+ used	$\frac{\text{N used (100)}}{\text{N available}}$
5	0	0	0	0	0	8.71	0	8.71	1.22	3.01	18.64	15.63	84	84
5.	2.5	3.94	1.76	2.18	55	10.8	0	10.8	1.22	3.32	25.0	21.68	87	81
5	5.0	8.71	3.13	5.58	64	9.51	0	9.51	1.22	3.53	25.82	22.29	86	77
		A	B	C=B−A	$D=\frac{C(100)}{A}$	E	F	G=E−F	H	I	J=C+2G+H	K=J−I	$L=\frac{K(100)}{J}$	$M=\frac{K(100)}{A+2E+H}$

Table XVI. Effect of NO_3^- on Assimilation of Urea by Excised Cereal Leaves in Darkness (µmoles/g × 12 h)

Treatment NO_3 (mM)	Treatment urea (mM)	NO_3^- uptake	NO_3^- in leaves	NO_3^- reduced	% NO_3^- reduced	Urea uptake	Urea in leaves	Urea used	NH_4^+ in leaves at T_o	NH_4^+ in leaves at T_{8h}	Total NH_4^+ available from NO_3^- and urea	NH_4^+ used	% NH_4^+ used	$\frac{\text{N used (100)}}{\text{N available}}$
0	5	0	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	0	3.26	0	3.26	0.7	1.27	7.22	5.95	82.4	72.6
5.0	5	3.09	4.04	0	0	3.59	0	3.59	0.7	1.40	7.88	6.48	82.2	59.0
		A	B	C=B−A	$D=\frac{C(100)}{A}$	E	F	G=E−F	H	I	J=C+2G+H	K=I−J	$L=\frac{K(100)}{I}$	$M=\frac{K(100)}{A+2E+H}$

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Table XVII. Effect of NH_4^+ on Assimilation of NO_3^- by Excised (Yecora Rojo) Wheat Roots (μmoles/g × 8 h)

Treatment NO_3^- NH_4^+ (mM)		NO_3^- uptake	NO_3^- in roots	NO_3^- reduced	% NO_3^- reduced	NH_4^+ uptake	NH_4^+ in roots at t_o	NH_4^+ in roots at t_{8h}	Total NH_4^+ available	NH_4^+ used	% NH_4^+ used	Total N available in roots	$\frac{\text{N used (100)}}{\text{N available}}$
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	3.6[illegible]	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
		A	B	C=A-B	$D=\frac{C(100)}{A}$	E	F	G	H=C+E+F	I=H-G	$J=\frac{I(100)}{G}$	K=A+E+F	$L=\frac{I(100)}{K}$

Table XVIII. Effect of NO_2^- on Assimilation of NO_3^- by Yecora Rojo Wheat Roots (μmoles/g × 8 h)

Treatment NO_3^- NO_2^- (mM)		NO_3^- uptake	NO_3^- in roots	NO_3^- reduced	% NO_3^- reduced	NO_2^- uptake	NO_2^- in roots at t_o	NO_2^- in roots at t_{8h}	Total NO_2^- available	Total N NO_2^- reduced	Total N available in roots from NO_3^-	% NO_2^- reduced of total NO_2^- avaliable	$\frac{\text{N used (100)}}{\text{N available}}$
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
		A	B	C=A-B	$D=\frac{C(100)}{A}$	E	F	G	H=C+E+F	I=H-G	$J=\frac{I(100)}{G}$	K=A+E+F	$L=\frac{I(100)}{K}$

Table XIX. Effect of NO_2^- on uptake and utilization of NH_4^+ by Excised Yecora Rojo Wheat Roots (μmoles/g × 8 h)

Treatment NO_2^- NH_4^+ (mM)		NH_4^+ uptake	NH_4^+ in roots		NO_2^- uptake	NO_2^- in roots		NO_2^- reduced	% NO_2^- reduced	Total NH_4^+ available in roots	NH_4^+ used	Total N available in roots	% NH_4^+ used	$\frac{\text{N used (100)}}{\text{N available}}$
			at t_o	at t_{8h}		at t_o	at t_{8h}							
0	1.0	41.24	1.95	8.95	---	0.0	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	9.14	90	11.16	8.21	12.15	74	68
1.0	1.0	0	1.97	3.03	8.64	0.0	1.28	7.36	85	9.34	6.31	10.62	68	59
		A	B	C	D	E	F	G=D−F	$H=\frac{G(100)}{D}$	I=A+B+G	J=I−C	K=A+B+D	$L=\frac{J(100)}{I}$	$M=\frac{J(100)}{K}$

Table XX. Effect of NO_3^- on Uptake and Utilization of NH_4^+ by Excised Yecora Rojo Wheat Roots (μmoles/g × 8 h)

Treatment NO_3^- NH_4^+ (mM)		NH_4^+ uptake	NH_4^+ in roots		NO_3^- uptake	NO_3^- in roots	NO_3^- reduced	% NO_3^- reduced	Total NH_4^+ available in roots	NH_4^+ used	Total N available in roots	% NH_4^+ used	$\frac{\text{N used (100)}}{\text{N available}}$
			at t_o	at t_{8h}									
0	1.0	32.12	1.61	11.05	---	---	---	---	33.73	22.68	33.73	67	67
0.5	1.0	33.71	1.61	8.54	11.55	9.3	2.24	19	37.56	29.02	46.86	77	62
1.0	1.0	32.08	1.61	8.7	13.78	11.2	2.58	19	36.27	27.57	57.57	76	58
		A	B	C	D	E	F=D−E	$G=\frac{F(100)}{D}$	H=A+B+F	I=H−B	J=A+B+D	$K=\frac{I(100)}{H}$	$L=\frac{I(100)}{J}$

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Table XXI. Effect of NO_3^- on Uptake and Utilization of NO_2^- by Excised Yecora Rojo Wheat Roots (µmoles/g × 8 h)

Treatment NO_3^- (mM)	Treatment NO_2^- (mM)	NO_2^- uptake	NO_2^- in roots at t_o	NO_2^- in roots at t_{8h}	NO_3^- uptake	NO_3^- in roots	NO_3^- reduced	% NO_3^- reduced	Total NO_2^- available in roots	Total NO_2^- reduced	Total N available in roots	% NO_2^- reduced of available NO_2^-	N used (100) / N available
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	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	0	18.07	16.48	30.55	96.7	57.2
		A	B	C	D	E	F	G	H=A+F	I=A+B+F-C	J=A+B+D	$K=\frac{I(100)}{H}$	$L=\frac{I(100)}{J}$

Table XXII. Effect of NH_4^+ on Uptake and Utilization of NO_2^- by Excised Yecora Rojo Wheat Roots (µmoles/g × 8 h)

Treatment NO_2^- (mM)	Treatment NH_4^+ (mM)	NO_2^- uptake	NO_2^- in roots at t_o	NO_2^- in roots at t_{8h}	NO_2^- reduced	% NO_2^- reduced	NH_4^+ uptake	NH_4^+ in roots at t_o	NH_4^+ in roots at t_{8h}	Total NH_4^+ available in roots	NH_4^+ used	% NH_4^+ used of total available	Total N available in roots	N used (%) / N available
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
		A	B	C	D=A-C	$E=\frac{D}{A}$	F	G	H	I=D+F+G	J=I-H	$K=\frac{J(100)}{I}$	L=A+B+F+G	$M=\frac{J(100)}{L}$

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