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Effects of Nitrification Inhibitors on Efficiency of Nitrogen Utilization and Crop Productivity

**R.H. Hageman, F.E. Below, J. Hagin, R.G. Hoeft,
A. Shaviv**

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EFFECTS OF NITRIFICATION INHIBITORS ON EFFICIENCY OF NITROGEN
UTILIZATION AND CROP PRODUCTIVITY

PRINCIPAL INVESTIGATOR: R. H. Hageman
COOPERATING INVESTIGATORS: F. E. Below, J. Hagin, R. G. Hoeft, and A. Shaviv
PRINCIPAL INSTITUTION: Department of Agronomy
University of Illinois
1102 South Goodwin Avenue
Urbana, IL 61801
COOPERATING INSTITUTION: Technion-Israel
Institute of Technology
Haifa, Israel

SIGNATURE OF PRINCIPAL INVESTIGATOR:

Ronald H. Hageman

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Highlights of the Complete Project

1. Professor Hagin and associates established that the nitrification inhibitor (dicyandiamide) could be effectively utilized to maintain N in the ammoniacal form at the desired and predetermined levels for two soil types.
2. Dr. Below and coworkers found that durum wheat (cv Inbar) grown in nutrient solutions that contained mixtures of ammonium and nitrate produced 80% more grain than plants grown on nitrate alone. This data permits the conclusion that this cultivar could not obtain sufficient N for maximum growth and grain yield when nitrate was the sole source of N. In a similar experiment with maize, the N mixtures increased grain yield by only 8 to 14%.
3. These studies indicate that plant species and cultivars respond differently to the presence and availability of ammonium.
4. When durum wheat (cv Inbar) was grown in soils (similar studies were done at Haifa, Israel and Urbana, IL) that contained nitrate, ammonium or various mixtures of the two forms of N, the increases in grain yield in response to the N mixtures, relative to nitrate alone, were small (seldom significant) when grown in Israel and nil when grown in Illinois.
5. The failure of the mixed N treatments to increase grain yield, of the same cultivar, to the same degree obtained in hydroponic culture poses a challenge of practical importance for future work. The reasons for the minimal or lack of yield response to the presence of ammonium in soils are not clear. The following possibilities are suggested: (a) In the greenhouse the soil conditions were unfavorable for ammonium uptake and plant development (aeration root temperature, water); and (b) In soils ammonium is bound while nitrate remains mobile. This situation may

in some way minimize the availability of ammonium when nitrate is present above some given level.

6. The use of plants as a sampling tool to determine the availability (absorption by) of ammonium to the plant shows some promise but its potential use was not fully exploited. The potential use of this concept is indicated by the fact that in all experiments where the rooting medium contained five levels of ammonium the increases in total reduced N concentration in the plant or plant part were proportional to the increases in ammonium in the rooting zone. The higher vegetative reduced N content obtained with mixed N nutrition for experiments conducted at both Israel and Illinois indicates that maintenance of N in the ammonical form has the potential to improve the efficiency of fertilizer N use.

Chapter 1

1. Nitrification inhibition

Introduction

Maintenance of predetermined ammonium concentrations in soils is a precondition for any experiments on the influence of ammonium to nitrate ratios on plant growth and composition. The use of nitrification inhibitors was recently reviewed by Hauck (1980) and Hoeft (1984). Dicyandiamide (DCD) was used as a nitrification inhibitor in several investigations. Amberger (1986) reviewed the use of DCD and states that the compound $\left(\begin{array}{c} \text{NH}_2 \\ \diagup \text{C} = \text{NH} \\ \diagdown \text{NH}-\text{C}\equiv\text{N} \end{array} \right)$ specifically inhibits oxidation of ammonium to nitrate by *Nitrosomonas* and that it is a bacteriostatic and not a bactericidal agent. DCD is soluble and at 30°C (47g dissolves in 100g water and 131g dissolves in 100g liquid ammonia.) The effectiveness of DCD lasts for about 1-3 months, depending on temperature, soil moisture, organic matter, pH and probably other factors. The material is decomposed in its final stage into NH_3 , CO_2 and H_2O . Amberger (1986) states that DCD is essentially non-toxic to plants, but when used in large doses it can be taken up by plants, transported in the xylem and exuded at leaf margins, where minor necroses may occur. Work of Reeves and Touchton (1986) indicates that DCD-N concentrations of about 10% in nitrogen fertilizers present a minimal toxicity risk if broadcasted, but may have a toxic effect when banded.

Experiments described in this chapter aimed at defining conditions for maintaining ammonium and predetermined ratios of ammonium to nitrate in soils.

Methods and Materials

Some of the characteristics of soils used in incubation experiments are given in table 1.1.

Table 1.1

Some characteristics of experimental soils

Sampling location	Texture	Field capacity %	pH	EC mmhos/cm	CaCO ₃ %	Org. C %
Sharon	Loamy sand	15.7	6.8	0.3	0.5	0.5
Lakhish	Sandy clay	28.9	7.2	1.1	21.7	0.4
Beerli	Sandy loam	18.4	8.1	0.4	12.0	0.3
Georgia, USA	Silt loam	22.3	5.2	1.0	-	1.3
Popayan, Colombia	Organic soil	37.1	5.2	1.6	-	7.5

incubation experiments

In 10g air dry soil samples were placed in 150 ml loosely stoppered vials. Nitrogen was added as an ammonium sulfate (AS) solution. In one experiment a calcium nitrate (CAN) solution was also used. The nitrogen solutions were prepared to contain 4g N/l. Nitrification inhibitor used was Dicyandiamide (DCD). A solution of DCD was prepared to contain 0.4g N/l and added to the soil sample. Amounts of AS and DCD added were such to give 0, 5, 10 and 20 percent of N as DCD-N. Water was added with mixing to bring the soil moisture to field capacity. Soil samples with or without applied ammonium sulfate and DCD were incubated at 30°C for various length of time. At predetermined incubation times duplicate samples were extracted in a 1M KCl solution. Moisture and ammonium and nitrate nitrogen contents of the samples were determined. Ammonium nitrogen was determined by distillation with MgO and nitrate + ammonium nitrogen by reducing the nitrate with De Warda reagent and continued distillation.

In a series of incubation experiments pelleted organic manure was tested as a source of mixed ammonium and nitrate nitrogen. Some of the properties of manures are tabulated in table 1.2.

Table 1.2

Some characteristics of pelleted organic
manures

Percentage calculated on dry matter

No.	Source	N	P	C	moisture
1	cow manure	4.1	1.9	31.0	10.4
2	cow manure	2.3	1.0	23.2	13.1
3	50% cow + 50% poultry manure	2.8	1.6	20.7	16.3

In an incubation experiment organic manure pellets (No. 1) were placed into the Sharon soil and incubated as described for ammonium sulphate. Pellets added supplied 200 mg total N per kg soil.

In another series of experiments pellets of organic manure were tried under leaching conditions. It was assumed that the pellets should act as slow release nitrogen sources, maintaining both ammonium and nitrate in the soil. 27g of soil were placed into Buchner funnels, covered by filter paper and on top of the paper another portion of 15g soil containing organic manure pellets was placed. Amounts of organic manure added to soil samples are stated in tables presenting results. The soil was wetted to field capacity and incubated at 30°C. At several incubation times, the soil in the funnel was leached with 100 ml of water and by suction brought again to its original moisture content. Concentrations of nitrates and ammonium, pH and electrical conductivity were determined in leachates and in soil samples at termination of incubation times.

Results

Results obtained with incubation of ammonium sulphate with varying ratios of nitrification inhibitor DCD in several soils are presented in the following.

Ammonium and nitrate concentrations measured at various times of incubation in soil samples that did not receive applied nitrogen were subtracted from concentrations found in samples with added AS and DCD nitrogen. Percentage of nitrogen recovered as ammonium or nitrate were calculated on air dry soil basis. Results obtained in Sharon and Lakhish soil with 200 mg N.kg^{-1} soil and with 0, 5 and 10 percent DCD-N are presented in figures 1.1 and 1.2. Best fitting free hand curves were drawn. In the presented nitrate and ammonium nitrogen measurements, about 5-10 percent of applied nitrogen are not accounted for.

Results for the coarse textured, neutral Sharon soil are plotted in figure 1.1. In samples not amended with DCD after a lag period of about 4 days, nitrification proceeds at a fast rate, about half of the ammonium is nitrified after 13 days and oxidation of most of the ammonium applied is completed after 25 days. In samples with 5 percent of N as DCD the lag period is prolonged to about 6 days and half of the applied nitrogen is nitrified after 21 days. Samples incubated with AS and 10% DCD-N show some variability in first weeks of incubation but after 85 days about 80% of ammonium is preserved and only very small amounts of nitrate are found.

In the sandy clay calcareous Lakhish soil (figure 1.2) nitrification in samples not amended with DCD proceeds without a lag period, nitrate and ammonium curves cross at day 11 and nitrification is almost completed at day 18. Samples amended with 5% DCD-N have a nitrification lag period of about 18 days and ammonium and nitrate curves cross at day 47. In samples containing 10% DCD-N nitrification was inhibited and at end of 85 days 80%-90% of added ammonium are maintained and very small amounts of nitrate were measured.

Samples of Sharon and Lakhish soil were incubated with ammonium sulphate and 20% DCD-N and results calculated in the same manner as for figures 1.1 and 1.2. In these samples nitrification was inhibited effectively, very little variation was found between sampling dates and only part of the calculated results are given in table 1.3.

Table 1.3

Percentage of recovered $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ from samples receiving 200 mg $\text{N}\cdot\text{kg}^{-1}$ soil, 20% of it as DCD-N, after some incubation periods.

Incubation time, days	N recovered, % of applied			
	Sharon soil		Lakhish soil	
	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$
8	90	-	90	-
29	92	-	90	-
85	87	-	85	-

Nitrification measurements in incubated Beerli soil, a calcareous sandy loam, were done in a similar way to those described for the first two soils. Since the results do not differ in principal from those presented earlier, only some are presented in table 1.4.

Table 1.4

Percentage of recovered $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ from samples of Beerli soil receiving 200 mg $\text{N}\cdot\text{kg}^{-1}$, 0 and 10% of it as DCD-N, after some incubation periods.

Incubation time, days	N recovered, % of applied			
	0 DCD - N		10% DCD - N	
	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$
8	78	11	81	5
22	44	44	75	2
43			83	11
85			80	14

Incubation experiments with AS and DCD on two additional soils imported from abroad, an acid silt loam soil collected in Georgia, USA and an organic soil from Popayan, Colombia were not successful. In both soils nitrification did not proceed at all. In the silt loam soil ammonium nitrogen concentrations measured throughout a period of 120 days stayed approximately constant and according to the amounts added initially. In the control samples of the organic soil, ammonium concentrations were about $200 \text{ mg N} \cdot \text{kg}^{-1}$ and nitrate concentrations about $50 \text{ mg N} \cdot \text{kg}^{-1}$. Thus the amount of ammonium added in AS was smaller than that present initially. The lack of nitrification may be due to sterilization done on these soils at the port of entry.

Incubation experiments in soils with ammonium sulphate and DCD described until now indicate that addition of DCD to AS in a ratio, based on N content, of about 1:10 can maintain most of the nitrogen added in an ammoniacal form, at least 85 days. Other means for maintaining a desired ammonium to nitrate ratio were tried.

In one incubation experiment with Sharon soil organic manure pellets amended with DCD were tested as sources of ammonium and nitrate. Results of the experiment are given in table 1.5.

Results in table 1.5 show that pelleted manure with or without DCD is not a very reliable source of predetermined ammonium to nitrate ratios.

Table 1.5

Percentage of recovered $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ from samples of Sharon soil receiving 200 mg N in manure per kg soil, and DCD-N relative to manure N, after some incubation periods.

Incubation time, days	N recovered		% of manure applied		N	
	0	DCD	5%	DCD	10%	DCD
	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$
1	9	1	6	1	5	1
3	7	2	9	1	9	-
8	9	2	11	-	9	-
28	16	7	39	5	11	-
36	7	13			19	24

In another experiment, soil samples incubated with organic manure pellets (OM) were leached several times. Ammonium and nitrate nitrogen concentrations, electrical conductivity and pH were measured in leachates and in soils. Results are presented in tables 1.6, 1.7 and 1.8.

Table 1.6

Analysis of leachates and of soil incubated with organic manure pellets (OM) No. 2 and 3. Sharon soil.

Days of incubation	pH	EC mmhos/cm	NH ₄ - N mg.kg ⁻¹	NO ₃ - N mg.kg ⁻¹
OM No. 2 0.44(0.04)g / sample				
leachate				
0	6.6 (0.0)	0.2 (0.0)	2.4 (0.1)	2.4 (0.1)
1			3.8 (0.4)	1.4 (0.0)
7			2.0 (0.2)	2.0 (0.1)
14	7.8 (0.1)	0.1 (0.0)	1.8 (0.2)	2.5 (0.1)
soil				
0	8.0 (0.0)	0.3 (0.0)	23.5 (0.5)	8.5 (2.0)
1	8.3 (0.0)	0.1 (0.0)	28.0 (2.0)	9.0 (0.0)
7	7.9 (0.0)	0.2 (0.0)	31.5 (1.5)	5.7 (0.7)
14	8.3 (0.0)	0.1 (0.0)	23.7 (0.7)	7.5 (0.0)
OM No. 3 0.41(0.03)g / sample				
leachate				
0	6.7 (0.1)	0.2 (0.0)	2.9 (0.2)	2.5 (0.2)
1	6.9 (0.1)	0.2 (0.0)	2.1 (0.5)	1.7 (0.3)
7	6.2 (0.2)	0.1 (0.0)	1.4 (0.1)	2.2 (0.2)
soil				
0			8.8 (0.3)	10.2 (4.3)
1			13.2 (0.4)	5.9 (0.5)
7			13.6 (0.6)	4.6 (0.5)

Table 1.7

Analysis of leachates and of soil incubated with organic manure pellets (OM) No. 1210 without and with 200 µg N-DCD/sample Sharon soil.

Days of incubation	pH	EC mmhos/cm	NH ₄ - N mg.kg ⁻¹	NO ₃ - N mg.kg ⁻¹
OM No. 1 0.40(0.00)g/sample without DCD				
leachate				
0	6.8 (0.1)	0.3 (0.0)	3.3 (0.2)	1.1 (0.0)
1			2.2 (0.1)	0.8 (0.1)
7			1.6 (0.0)	1.5 (0.1)
14	5.3 (0.0)	0.1 (0.0)	1.9 (0.7)	2.0 (0.2)
soil				
0	7.8 (0.0)	0.2 (0.0)	6.7 (0.0)	2.8 (0.8)
1	8.2 (0.1)	0.1 (0.0)	7.8 (0.0)	1.5 (0.0)
7			7.3 (0.5)	2.9 (0.2)
14	8.0 (0.0)	0.1 (0.0)	4.1 (0.4)	0.5 (0.0)
OM No. 1 0.40(0.00)g/sample with DCD (200µgN/sample)				
leachate				
0	7.3 (0.5)	0.3 (0.0)	1.9 (0.2)	0.7 (0.0)
1			4.2 (0.1)	1.8 (0.0)
7			3.7 (0.5)	2.7 (0.2)
14	7.3 (0.0)	0.1 (0.0)	1.2 (0.2)	1.6 (0.2)
soil				
0	8.0 (0.0)	0.2 (0.0)	7.3 (0.5)	4.6 (0.0)
1	8.1 (0.0)	0.1 (0.0)	11.8 (0.0)	4.4 (1.8)
7	7.5 (0.0)	0.1 (0.0)	15.1 (0.3)	10.3 (1.2)
14	8.0 (0.1)	0.1 (0.0)	3.8 (1.2)	1.8 (0.0)

Table 1.8

Analysis of leachates and of soil incubated with organic manure pellets No. 3, 0.37(0.02)g manure/sample.

Georgia soil

Days of incubation	pH	EC mmhos/cm	NH ₄ - N mg.kg ⁻¹	NO ₃ - N mg.kg ⁻¹
leachate				
0	5.4 (0.1)	0.3 (0.0)	8.7 (2.2)	16.4 (1.7)
1	5.8 (0.1)	0.1 (0.0)	4.8 (0.5)	1.8 (0.2)
7	6.4 (0.1)	0.1 (0.0)	2.8 (0.2)	0.9 (0.1)
soil				
0			36.5 (0.5)	12.5 (0.5)
1			43.0 (1.0)	11.0 (0.0)
7			35.0 (0.0)	11.0 (0.0)

According to results in tables 1.6, 1.7 and 1.8 addition of organic manure pellets to soil under leaching conditions is instrumental in maintaining a higher level of ammonium nitrogen in soil than that of nitrate nitrogen. However, the results indicate that it would be difficult to predict the level of mineral nitrogen in the soil. That would be dependent among other factors on the source of organic manure.

According to incubation results, the most promising technique for maintaining approximately constant levels of ammonium in the soil is by adding the nitrogen fertilizer with DCD at the beginning of the experimental period in a ratio of ammonium - N : DCD-N = 10 : 1.

In an additional incubation experiment conditions similar to those planned for a greenhouse experiment were simulated. Ammonium (AS) nitrogen with DCD and nitrate (CAN) nitrogen were applied in five ratios, at two levels of nitrogen application and to two soils. Results presented in table 1.9 confirm that a mixed application of ammonium with DCD and nitrate nitrogen can maintain reasonably well for at least six weeks a predetermined ratio of ammonium to nitrate in soils. Although not all the nitrogen applied was recovered in those measurements.

Table 1.9

Effectiveness of DCD in maintaining ammonium to nitrate ratios in soils amended with ammonium sulphate (AS) and calcium nitrate (CAN). Incubation at 30°C. AS : DCD = 10:1.

Incubation days			N		form							
	NH ₄	NO ₃	NH ₄	NO ₃	NH ₄	NO ₃	NH ₄	NO ₃	NH ₄	NO ₃	NH ₄	NO ₃
	mg N / kg soil											
Sharon soil												
0*	200	0	150	50	100	100	50	150	0	200		
7	171	13	148	38	96	71	62	92	14	132		
14	180	14	113	43	81	75	54	100	17	132		
42	166	14	133	42	83	74	53	101	4	141		
ANOVA	F	30	37									
	l.s.d.	29	10									
Lakhish soil												
0*	400	0	300	100	200	200	100	300	0	400		
7	351	10	273	65	195	127	129	173	28	265		
14	334	14	275	75	220	126	118	179	33	256		
42	343	11	279	71	179	128	110	203	7	274		
ANOVA	F	305	36									
	l.s.d.	9	22									
Lakhish soil												
0*	200	0	150	50	100	100	50	150	0	200		
7	189	38	142	72	101	91	76	119	30	142		
14	179	32	143	62	111	100	78	122	11	172		
42	174	38	135	70	86	92	60	119	1	166		
ANOVA	F	16	150									
	l.s.d.	20	6									
Lakhish soil												
0*	400	0	300	100	200	200	100	300	0	400		
7	357	27	264	91	183	142	106	207	52	249		
14	351	31	268	94	197	144	107	203	23	262		
42	358	28	262	91	201	143	102	206	9	288		
ANOVA	F	100	45									
	l.s.d.	17	19									

SUMMARY

Incubation experiments with ammonium sulphate (AS), DCD, a nitrification inhibitor, calcium nitrate (CAN) and with pelleted organic manure (OM) were performed in several soils. The experiments aimed at finding ways for maintenance of predetermined ratios of ammonium to nitrate in soils for several weeks. Ratios of AS-N to DCD-N of 20:1, 10:1 and 5:1 were tested. The ratio of 10:1 was sufficient for preventing nitrification for several weeks. OM with or without DCD was not reliable enough for supplying predictable ratios of ammonium to nitrate in soils. Applications to soils of AS with DCD and CAN mixtures could maintain reasonably well predetermined ammonium to nitrate ratios.

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Figure 1.1

Percentage of applied N as $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$
versus time of incubation

Sharon soil.

Figure 1.2

Percentage of applied N as $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$

versus time of incubation

Lakhish soil.

Chapter 2. Effects of $\text{NO}_3^-:\text{NH}_4^+$ Ratios in the Nutrient Medium on Metabolites and Yields of Maize.

Part A. Metabolites:

INTRODUCTION

As outlined in the original proposal, previous work has established that relative to plants grown only on nitrate, increasing amounts of ammonium ions in the nutrient medium causes most plant species to accumulate more amides, total organic N and less organic acids (especially malate) and cations (especially Ca^{++} and Mg^{++} and sometimes K^+). Changes in the level of the organic constituents result from the amounts of NO_3^- and NH_4^+ absorbed and metabolized by the plants. Beyond affecting the absorption of ions from the rooting medium, the ratios of $\text{NO}_3^-:\text{NH}_4^+$ in the rooting medium should have no effect on the level of the above listed organic constituents. Although laborious, the total amounts of NO_3^- and NH_4^+ present in the rooting zone can be determined. This approach is considered a valid way to estimate the presence and duration of NH_4^+ and nitrification inhibitors in the rooting medium. However, the presence of a given $\text{NO}_3^-:\text{NH}_4^+$ ratio in the rooting medium does not establish that the plant is absorbing these ions at the measured ratio. Further the ratio in the soil is dynamic. The establishment that changes in amides, total organic N, organic acids, as cations of the plant or plant part is proportional to the ratio of $\text{NO}_3^-:\text{NH}_4^+$ in the solution culture medium would permit an approach to determining the amounts of NO_3^- and NH_4^+ available to (absorbed by) the plant grown in any medium. The lack of a limited change in these specific metabolites in plants supplied with NH_4^+ salts, relative to a nitrate control, would indicate that the amounts of NH_4^+ absorbed by the plant was nil or low.

The original objective was to show that the effectiveness and duration of nitrification inhibitors in the rooting medium can be assessed by following changes in certain metabolites of the plant as affected by the relative proportions of NO_3^- and NH_4^+ available to the plant from the rooting medium.

To achieve the objective a three phase approach was outlined: (a) to establish standard changes in concentration of amides, total organic N, NO_3^- -N or malate in corn plants grown on nutrient solutions containing the following ratios of $\text{NO}_3^-:\text{NH}_4^+$, 100:0, 75:25, 50:50, 25:75 and 0:100 under greenhouse conditions; (b) to establish standard changes as in (a) when plants were grown in soil that contained varying ratios of $\text{NO}_3^-:\text{NH}_4^+$ with the NH_4^+ levels stabilized by nitrifications inhibitors; and (c) to evaluate the effectiveness of the standards by assessing, at intervals during the growing season, the level of the selected metabolites in plants grown on field plots that were supplied with different ratios of $\text{NO}_3^-:\text{NH}_4^+$ (as above) with and without a nitrification inhibitor.

MATERIALS AND METHODS

Kernels of maize (cv. B73xMol7) were germinated and grown for 30 days in hydroponic culture in the greenhouse. Hydroponic culture vessels were 5 liter polyethylene pots painted black and covered with aluminum foil. Natural lighting was supplemented with metal halide lamps to provide 14 hours of illumination. Greenhouse temperatures were maintained at 26°C during the light cycle and 18°C during the dark cycle. Six seeds per pot were germinated directly into one-quarter strength nutrient solution by the use of a collar-seed-wick assemblage inserted into holes (1.5 cm) on the perimeter of the culture vessel lid. The collar-wick system involved enclosing the seed within a collar (a 3 cm section of plastic tubing cut lengthwise and filled with vermiculite) which was connected to a wick (an 8 cm section of cotton string

inserted in the overlap of the collar). The wick served to wet the vermiculite which supplied the imbibing seed with water. This technique allows for undisturbed growth on the newly emergent radicle and results in excellent seedling uniformity. Two days after germination the wicks between the collar and the solution were removed and the plants thinned to three per pot.

Treatments consisted of five ratios NO_3^- -N to NH_4^+ -N (100/0, 75/25, 50/50, 25/75, and 0/100) arranged in a randomized complete block design with five replications. The concentration of total nitrogen in full strength solution was 10 mM. Other nutrients were at levels according to Hoagland and Arnon (Calif. Agric. Exp. Sta. Circ. 347, 1950) and iron was added as daily additions of ferrous sulfate (Hageman et al., Agron. J. 53:175-180, 1961). Sulfur was allowed to vary in order to achieve the best possible balance between the ion concentrations. For aeration and pH control of the solution a recirculating ion exchange column was added to each culture vessel. This column was loaded with 75 ml of the appropriate mixture of H^+ and Ca^{2+} forms of Amberlite IRC 50 cation exchange resin to maintain a pH of 5.0 ± 0.5 (Harper and Nicholas, Physiol. Plant. 38:24-28, 1971). When the solution pH deviated more than 0.5 unit the exchange column was replaced. The frequent replacement of the nutrient media and the daily assays for NO_3^- and NH_4^+ uptake insured that the desired levels of $\text{NO}_3^-:\text{NH}_4^+$ were maintained throughout the experimental period.

At 30 days after planting, leaf punches from the fifth fully expanded leaf were removed and analyzed for malate by modification of the procedure of Holzer and Soling (In: Methods and Enzymatic Analysis. Ed. H. U. Bergmeyer. Dekker, New York. pp. 332-334, 1963). Although total organic acids were not measured, the method described by Kirkby and Mengel (Plant

Physiol. 42:6-14, 1967) would be appropriate. Amides were not measured because of their lability and because the method is too laborous to use for large numbers of samples. Total organic N, which includes the amide fraction, is considered a more convenient procedure. The remaining plant matter was harvested, divided into root and shoot, and analyzed for dry matter, total N, and nitrate-N.

The experiment was repeated three times during the winter and spring of 1984. Experiment 1 was planted on February 25, experiment 2 on March 29, and experiment 3 on April 4. For each experiment and averaged over experiments, associations between the concentration of chemical constituents in the vegetation and percent of N in the nutrient solution as ammonium were tested with regression using chemical constituents as dependent variables and ammonium level as the independent variable.

RESULTS

With the possible exception of the all NH_4^+ treatment, dry weights of the roots remained relatively unchanged. As the ratio of $\text{NO}_3^-:\text{NH}_4^+$ in the nutrient solution (10 mM N in all cases) was changed from all NO_3^- to all NH_4^+ , by 25% increments (Table 2.1). The reason for the variation within and among experiments is not clear. Average weights (all three experiments) of plants grown on solutions where NH_4^+ comprised 25, 50, 75 and 100% of the total N were 109, 117, 106 and 85% of the average weight of the plants grown on 100% nitrate.

The relationship of the metabolites selected as standards in response to the ratio of $\text{NO}_3^-:\text{NH}_4^+$ in the nutrient media are shown in Table 2.2. The regression equation was obtained by regressing the concentration of the

Table 2.1. The effect of varying ratios of $\text{NO}_3^-:\text{NH}_4^+$ in the nutrient solution on growth of maize seedlings. Total N was supplied at 10 mM for all treatments. Plants were harvested 30 days after planting.

Percent NH_4^+ in the $\text{NO}_3^-/\text{NH}_4^+$ ratio	Expt.	Root	Shoot	Total	Shoot/Root Ratio
---g/plant part or plant---					
0	1	1.2	4.2	5.4	3.5
	2	0.6	3.0	3.6	5.0
	3	1.0	4.2	5.2	4.2
	X	0.9	3.8	4.7	4.2
25	1	1.2	4.5	5.9	3.8
	2	0.8	3.6	4.4	4.0
	3	1.1	4.1	5.2	3.7
	X	1.0	4.1	5.1	4.1
50	1	1.2	4.5	5.7	3.8
	2	0.9	3.9	4.8	4.3
	3	1.4	4.6	6.0	3.3
	X	1.2	4.3	5.5	3.6
75	1	1.1	4.3	5.4	3.9
	2	0.8	3.5	4.3	4.4
	3	1.1	4.3	5.4	3.9
	X	1.0	4.0	5.0	4.0
100	1	1.1	3.4	4.5	3.1
	2	0.6	2.7	3.3	4.5
	3	0.9	3.2	4.1	3.6
	X	0.9	3.1	4.0	3.4
LSD (0.05)	1	NS	0.6	0.6	
	2	NS	0.3	0.4	
	3	0.4	0.2	0.5	
	X	0.4	0.4	0.5	

Table 2.2 Regression of the concentrations of reduced N, NO_3^- -N and malate of leaves and shoots of maize seedlings against percent of N in the nutrient solutions as NH_4^+ . Other details as in Table 1 and Methods.

Range in Percentage of NH_4^+ Supplied				
Experiment	-----0 to 50%-----		-----0 to 100%-----	
	Y as a function of X	r^2	Y as a function of X	r^2
Malate* (5th leaf from bottom)				
1	$-0.026X + 7.7$	.99	$-0.020X + 7.6$	.92
2	$-0.030X + 7.2$	.85	$-0.018X + 7.0$	.82
3	$-0.014X + 7.0$	.75	$-0.015X + 7.0$	.81
X	$-0.024X + 7.3$	.92	$-0.018X + 7.2$	.94
Reduced $\text{N}^\dagger$ (5th leaf from bottom)				
1	$0.060X + 48.0$	.99	Not calculated because above 50% NH_4^+ reduced N values did not increase.	
2	$0.072X + 46.5$	.83		
3	$0.104X + 47.6$	.92		
X	$0.078X + 47.4$	.93		
Reduced $\text{N}^\dagger$ (whole shoot)				
1	$0.016X + 27.6$	.66	$0.053X + 27.0$	.88
2	$0.084X + 25.9$	.92	$0.097X + 25.6$	.97
3	$0.070X + 27.0$	.99	$0.080X + 26.8$	.99
X	$0.056X + 26.9$	.97	$0.077X + 26.4$	.98
NO_3^- (whole shoot)				
1	$-0.018X + 5.8$	.67	$-0.053X + 6.5$	.87
2	$-0.032X + 9.0$	.98	$-0.083X + 10.0$	.88
3	$-0.036X + 7.8$	.98	$-0.077X + 8.5$	.93
X	$-0.028X + 7.5$	.99	$-0.071X + 8.4$	.90

*Expressed as mg dm^{-2} of leaf.

† Expressed as mg g^{-1} dry weight.

selected metabolites against the $\text{NO}_3^-:\text{NH}_4^+$ the ratio values (0 to 100% range). The r^2 values show that the concentrations of leaf malate or whole shoot NO_3^- decreased and the concentration of leaf or whole shoot total organic N increased in proportion to increase of NH_4^+ in the nutrient medium. These values are as predicted and indicate these metabolites are of use in estimating the relative amounts of NO_3^- and NH_4^+ available to (taken up) by the plant.

Although the r^2 values for organic N were slightly lower for the fifth leaf than for the whole shoot, the values are great enough to permit leaf sampling to be used in preparation of a standard. Leaf sampling is simple, rapid, and nondestructive and would permit measurements to be made at intervals from plants at a given location. It is suggested that alternate leaves should be sampled (e.g. leaf 5, 8, 12) and that each leaf be sampled at a time that it achieves full expansion. There is one objection to leaf sampling, namely the leaves had an upper limit to the amount of organic N that they can accumulate in response to increased NH_4^+ supply.

Additional direct efforts to establish standard patterns of the effect of increased supply of NH_4^+ , relative to NO_3^- , on organic acids, organic N and ion levels in maize were not continued for the following reasons: (a) Because of the unlimited supply of water and nutrients in hydroponic cultures, the concentrations of organic N and other ions in the plant exceed those levels found in field grown plants. This observation indicates that standards (changes in selected constituents in response to changes in ratios of $\text{NO}_3^-:\text{NH}_4^+$) should be established with plants grown in soil rather than nutrient culture; (b) To facilitate cooperation within the BARD project, we agreed to conduct greenhouse studies with wheat (Triticum durum cv. Inbar) that was used by Professor Hagin in his experiments; (c) Because divergent

opinions exist over the effects of increasing and maintaining NH_4^+ (with nitrification inhibitors) at levels higher than normally found in production plots and fields, it was decided to determine the effects of a 50:50 $\text{NO}_3^-:\text{NH}_4^+$ mixture versus all NO_3^- on grain yields under field hydroponic culture.

CONCLUSIONS

Because only a limited amount of effort was expended on Part A, only tentative conclusions can be drawn. The tentative conclusions that are presented are supported by our work done with wheat and work done at the Technion in Haifa. These data are presented in other chapters of this report and certain aspects will be amplified in Chapter 6.

1. In response to increasing ratios of $\text{NO}_3^-:\text{NH}_4^+$ in nutrient cultures from 0 to 100% (in 25% increments), proportional decreases in malate, and NO_3^- -N and increases in organic N were observed in the leaves and shoots of maize. Significant linear regressions were obtained when the concentrations of these constituents were plotted against increases in the percentage of NH_4^+ .
2. The plant can be used as a sampling tool to estimate the relative proportion of NH_4^+ available to (absorbed by) the plant, regardless of the rooting media.
3. Other conclusions and clarifying comments will be presented in the concluding discussion.

Part B. Yield.INTRODUCTION

Although maize can effectively utilize either NO_3^- or NH_4^+ , when growing nutrient cultures, NO_3^- grown plants usually amass more dry weight than NH_4^+ grown plants. In soils and under production conditions, NO_3^- is considered to be the predominant form of N available to the plant, because NH_4^+ is rapidly nitrified in soils suitable for maize production. As reported elsewhere in this report, soils supplied only with NH_4^+ salts plus a nitrification inhibitor produced smaller plants than when the plants grown in soil supplied only with NO_3^- . There is an increasing number of articles that indicate that mixtures of NO_3^- and NH_4^+ are more effective, than either NO_3^- or NH_4^+ alone in producing more growth for several plant species (4, 5, 6, 8). Maize plants grown on sandy soil and provided with supplemental N (ammonium salts, or urea) produced significantly more grain and stover than plants supplied only with nitrate (11). For maize plants grown in solution culture, Schrader et al. (15) found that the plants grew more rapidly and absorbed more N where they were provided with mixtures of NO_3^- and NH_4^+ than when supplied with either form separately. These results imply that, at least, some plant species cannot attain their full growth potential when supplied only with nitrate. We found no reports in the literature where hydroponic cultures had been used to establish the effect of varying ratios of $\text{NO}_3^-:\text{NH}_4^+$ on plant mass and yield at maturity among maize genotypes.

The objective was to compare the effects of a mixture of $\text{NO}_3^-:\text{NH}_4^+$ (50:50) versus all NO_3^- , in hydroponic culture on the dry grain yield and yield components and on the final accumulation, and partitioning of dry mass, N, P, and K for two maize hybrids.

MATERIALS AND METHODS

The maize hybrid Farm Service brand 854 was chosen for study because of its high productivity potential and its response to high soil fertility; FS854 was the hybrid used by Herman Warsaw of Saybrook, IL to achieve a world record maize yield of 370 bu/ac in 1985, and a 15-year average of 274 bu/ac (13). Because of the high level of organic matter in Warsaw's soil, we hypothesized that ammonification may be supplying substantial levels of ammonium throughout the growing season, and that FS854 responded to this ammonium. The other hybrid chosen to compare with FS854 was B73xLH51. This hybrid was selected because it is high yielding and widely grown, and because it has a much different N acquisition pattern than FS854 (our unpublished data).

Seed of each hybrid were planted on 3 May, 1986 in individual peat pots (2") containing a sterilized soil mix (by volume, two parts Drummer soil, one part peat moss, and one part sand). Seeds were germinated and grown in the greenhouse (80°F with natural lighting) to the three leaf stage. Seedlings were then transplanted (14 May) to a field-gravel-hydroponic system (see Figure 2.1). The system consists of two independent four-pot units that are situated adjacent to each other in a small field plot. Each unit consists of a below-ground holding tank (57 liter) with a sump pump, an above-ground reservoir, four culture pots (114 liter gal plastic barrel) filled with quartz gravel, and the appropriate plumbing to connect the various parts of the system (Fig. 2.1). At intervals (controlled by a timer), the solution in the holding tank is pumped into the reservoir from which it flows by gravity to the four culture pots. From the culture pots the excess solution drains back into the holding tank. The solution volume in the holding tank is maintained by additions of deionized water, and the pH controlled at 5.5 by frequent titrations with acid or base. The arrangement of the units and pots is such

Figure 1. Design and arrangement of the field-gravel-hydroponic system used to grow maize.

that a standard 76 cm row spacing can be achieved by growing border plants in the surrounding soil. This system has the capability of growing maize plants to maturity while maintaining a high level of fertility and a relatively constant nitrate/ammonium ratio.

For N treatments, one unit contained an all NO_3^- nutrient solution, while the other unit contained nutrient solution with the same amount of N provided by equal proportions of NO_3^- and NH_4^+ . Three plants of a given hybrid were planted into four culture pots (two pots of each hybrid for each N treatment). The two hybrids were also planted in the surrounding soil to give an overall stand density (calculated as the total number of plants, soil or hydroponic, in the land area occupied by the hydroponic system) equivalent to 39,536 plants/ha. Because the design of the hydroponic system necessitated assigning N treatments to individual units, analysis of variance procedures to test for treatment effects and treatment interactions could not be performed. To provide an estimate of experimental error a standard error of the mean was calculated from the two pots of each hybrid in each N treatment. A conscientious effort was made to assure that environmental and cultural conditions were similar for both units.

The concentrations of macronutrients in the full strength nutrient solution for the N treatment solutions are shown in Table 2.3. In these solutions, S, K, Ca, and Mg are all allowed to vary (largest fluctuation in S) in order to achieve the best possible balance between the ion concentrations. Iron was supplied as Chel-330 (Ciba-Geigy Corp., Greensboro, NC) at a concentration of 5 ppm Fe, and an additional 1 ppm Fe was added daily in the ferrous form by additions of 0.03 M FeSO_4 (9). A full complement of micronutrients (Cu, Zn, B, Mn, and Mo) was included at concentrations according to Hoagland and Arnon (10), except for Mn which was four times

Table 2.3. The concentration of macronutrient elements in the nutrient solutions used for hydroponic culture of maize.

Element	$\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$	
	100/0	50/50
	-----mg Liter ⁻¹ -----	
$\text{NO}_3\text{-N}$	216	108
$\text{NH}_4\text{-N}$	--	108
P	24	24
K	280	240
Mg	58	40
Ca	180	132
S	80	240

higher. Under these conditions, color and morphological development of plants were similar to soil grown plants.

Nitrate and ammonium ions in the culture solution were monitored throughout the experimental period, and the solutions were renewed when the N concentration (either ion) dropped below half its initial level. The nutrient concentrations were gradually increased in a stepwise manner as the plant developed. Times of change in solution strength are as follows: 20% of full strength until 7 days after transplanting (DAT), 30% of full strength between 7 and 27 DAT, 40% of full strength between 27 and 33 DAT, 60% of full strength between 33 and 40 DAT, 80% of full strength between 40 and 61 DAT, full strength from 61 to 84 DAT, and 80% of full strength from 84 DAT until physiological maturity.

Upon attainment of physiological maturity (approximately 124 DAT for both hybrids and N treatments), above ground plant material was harvested and separated into leaves, stalk (including leaf sheaths), husk, shank, and ears. Leaves and stalks were weighed and passed through a mechanical chopper, and subsamples (200 g stalks, 100 g leaves) were dried to constant weight at 80°C in a forced draft oven. After reweighing, the dried portions were mechanically ground (20-mesh screen) prior to chemical analysis. The ears, husk, and shank were dried by 80°C to constant weight prior to shelling of the ear and combining the cob with the husk and shank. Kernels and the combined husk-cob-shank fraction were redried, weighed, ground, and stored for chemical analysis.

For each plant fraction, tissue was analyzed for total N, nitrate N, and P, as described previously (7). Acid digests used to determine total N and P were also analyzed for K by atomic absorption. Reduced N was determined by subtracting nitrate N from total N. Whole plant constituents were obtained by summing the values for the plant parts and do not include roots.

RESULTS AND DISCUSSION

Compared to plants receiving only nitrate, mixed N nutrition increased grain yield by 14% for FS854 and by 8% for B73xLH51 (Table 2.4). For both hybrids, this increase in grain yield was due to an increase in kernel number because kernel dry weight was similar for both treatments.

Relative to nitrate, the mixed N treatment caused small increases in total shoot dry matter accumulation at maturity (2% for FS854 and 8% for B73xLH51) (Table 2.5). For B73xLH51, the mixed N treatment increased the N, P and K contents of the whole plant 34, 41 and 13% respectively over the nitrate treatment. For FS854, the comparable increases in N, P and K was 10, 50 and 0% respectively. FS854 was more effective in accumulating N from the NO_3^- only medium than B73xLH51, while the reverse was true when the medium contained mixed N. For both hybrids, the additional N (2.8 g for B73xLH51 and 1 g for FS854) acquired when the medium contained NH_4^+ was associated with small but proportional increases in shoot dry weight (e.g. 45 and 15 g, respectively).

The mixed N treatment caused marked increases in the concentration of N and P for B73xLH51 and P for FS854 and a minor increase in N for FS854 (Table 2.5). In contrast, the K concentration was similar for both treatments and hybrids. Greater amounts of P uptake, or higher vegetative P concentrations, as a function of NH_4^+ versus NO_3^- nutrition have been reported previously for several crop species (2, 3, 6).

Regardless of N treatment, both hybrids produced more total dry shoot mass, grain and had higher N contents (Tables 2.4 and 2.5) than field plants grown at similar plant densities. For example, when B73xMol7 was grown in 1980 on an adjacent fertile field the total shoot dry mass, grain yield and N

Table 2.4 Effect of a mixture of nitrate and ammonium versus nitrate alone on the yield and yield components of two maize hybrids. Values are the average of two observations $\pm$ standard error of the mean.

Genotype	$\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$	Grain weight	Kernel weight	Kernel number
	ratio	g plant^{-1}	mg kernel^{-1}	no. plant^{-1}
B73 x LH51	100/0	254 ± 1	369 ± 10	688 ± 21
	50/50	275 ± 22	361 ± 10	768 ± 21
FS854	100/0	277 ± 17	339 ± 16	821 ± 89
	50/50	315 ± 10	315 ± 25	1006 ± 46

Table 2.5 Effect of a mixture of nitrate and ammonium versus nitrate alone on whole shoot dry weight and reduced N, phosphorus, and potassium contents and concentrations at physiological maturity for two maize hybrids. Values are the average of two observations $\pm$ standard error of the mean.

Genotype	$\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$	Dry weight	Reduced N	Phosphorus	Potassium
	ratio	-----g plant ⁻¹ -----			
B73 x LH51	100/0	528 $\pm$ 12	8.3 $\pm$ 0.6	1.7 $\pm$ 0.04	11.6 $\pm$ 0.03
	50/50	573 $\pm$ 16	11.2 $\pm$ 0.5	2.4 $\pm$ 0.01	13.1 $\pm$ 0.31
FS854	100/0	540 $\pm$ 20	9.8 $\pm$ 0.6	1.4 $\pm$ 0.12	13.2 $\pm$ 0.35
	50/50	555 $\pm$ 18	10.8 $\pm$ 0.3	2.1 $\pm$ 0.18	13.0 $\pm$ 0.63
		-----%-----			
B73 x LH51	100/0		1.6	0.32	2.2
	50/50		2.0	0.42	2.3
FS854	100/0		1.8	0.26	2.4
	50/50		1.9	0.38	2.3

content were 466, 250, and 7 g, respectively (1). Under nonirrigated commercial production conditions where higher plant densities are used, plant mass grain yield and nutrient content are even lower than previously noted for B73xMol17. Because even the lowest content and concentration values of these three elements of plants grown on NO_3^- (Table 2.5) were high relative to high yielding commercially produced maize and because grain crude protein in all cases exceeded 14%, it is difficult to visualize a direct beneficial effect on yield from the increased levels of N and K resulting from mixed N nutrition. These data would indicate that it is the availability of nutrients in the soil that limits growth and nutrient content rather than a plant deficiency.

The partitioning of dry weight to the grain of B73xLH51 was the same for both N treatments while the presence of NH_4^+ in the rooting medium increased the partitioning of dry matter to the grain of FS854 (Table 2.6). Less dry weight was partitioned to the stalks of both hybrids grown on mixed N medium and this decrease in stalk dry matter was apparently associated with the increase in dry weight of grain of FS854 and husks, shanks and cobs of B73xLH51. B73xLH51 plants developed full second ear shoots that failed to develop kernels.

FS854 plants grown on mixed N senesced 7 to 10 days earlier, and lodged to a greater extent the FS854 plants grown on NO_3^- . Whether the earlier senescence and stalk lodging of these plants was caused by the decrease in stalk dry weight is not known. FS854 is known to have poor stalk quality and lodges excessively under production conditions (13, 16). These data indicate that NH_4^+ nutrition may exacerbate this problem.

Table 2.6 Effect of a mixture of nitrate and ammonium versus nitrate alone on partitioning of dry matter, reduced N, phosphorus, and potassium among plant parts at physiological maturity for two maize hybrids.

Genotype	Nitrate/Ammonium ratio	Plant part			
		Leaves	Stalk	Husk, Cob	Grain
				Shank	
		-----% of whole shoot-----			
Dry Matter					
B73 x LH51	100/0	10	31	11	48
	50/50	11	27	14	48
FS854	100/0	11	24	14	51
	50/50	11	17	15	57
Reduced N					
B73 x LH51	100/0	11	15	4	70
	50/50	14	19	4	63
FS854	100/0	13	12	5	70
	50/50	15	11	5	69
Phosphorus					
B73 x LH51	100/0	7	31	5	57
	50/50	15	37	8	40
FS854	100/0	9	14	6	71
	50/50	16	16	6	62
Potassium					
B73 x LH51	100/0	17	55	13	15
	50/50	17	48	21	14
FS854	100/0	14	55	17	14
	50/50	16	43	22	19

These data show the mixed N nutrition altered the partitioning of dry matter between the stalk and grain of FS854 (a prolific hybrid) and between stalk and reproduction tissues of B73xLH51 (a predominately single eared hybrid). For both hybrids, the presence of NH_4^+ in the rooting media increased kernel number (Table 2.4) which implies that increased reduced N concentration plays a role in achieving maximum kernel number. Because of the high content and concentration of N in all vegetative parts and the exceptionally high grain crude protein (excess of 14%) percentage in both hybrids when grown on NO_3^- , it is difficult to visualize a direct role for excess N in enhancing reproductive development. Because other work has indicated that prolific hybrids are more responsive to NH_4^+ nutrition than single-eared hybrids, (14), it is suggested that a prolific hybrid with good stalk quality should show maximum yield in response to mixed N nutrition.

In contrast to dry matter, the partitioning of N among the parts of FS854 plants was not altered by N treatment (Table 2.6). For B73xLH51 plants, the extra whole plant N that resulted from utilization of N mixtures was partitioned preferentially to the leaves and stalk, while less than normal amounts were partitioned to the grain. Because the partitioning of N to the grain or reproductive support tissues remained unchanged or decreased for plants grown on mixed N, while partitioning of dry matter was increased, these data can be interpreted to imply the availability of carbohydrate, and not N, was the major yield limiting factor for both hybrids. The high content and concentration of N in all plant parts also supports this view. These data are in contrast to field studies which generally indicate that the ability of the vegetation to supply the ear with carbohydrate is greater than the ability to supply the ear with N (1, 16).

Relative to NO_3^- grown plants, mixed N nutrition decreased the partitioning of P to the grain of both hybrids, especially B73xLH51 (Table 2.6). Regardless of treatment, FS854 distributed a greater proportion of the total P to the grain than B73xLH51. However the P content of the grain of both hybrids was about 1 g/plant when NO_3^- was the sole source of N while on mixed N, the P content of the grain was 1 g/plant for B73xLH51 and 1.3 g/plant for FS854. When grown on mixed N, both hybrids partitioned more P to the leaves than when the plants were grown on NO_3^- . Expressed on a content basis, leaves of both hybrids had 2.8-fold more P in response to the mixed N treatment. Regardless of N treatment, B73xLH51 partitioned about twice as much of the total plant P to stalks than did FS854, and the presence of NH_4^+ in the media increased the distribution only to the stalks of B73xLH51. The reasons for these differences in partitioning of P and the role and effect of the P ion in the different plant parts in affecting yield is not clear.

The pattern of K distribution among plant parts was similar to dry matter as N mixtures resulted in a decrease in the proportion of whole plant K partitioned to stalks and an increase in that partitioned to grain (FS854) or reproductive support tissue (B73xLH51) (Table 2.6). These data suggest an association between dry matter and K partitioning that is influenced by the form of N being utilized. A role for K in facilitating translocation and partitioning of assimilates has been reported (12).

The final partitioning of K among the various plant parts was relatively similar for both hybrids (Table 2.6). For all treatments, the majority (43 to 55%) of whole plant K was present in the stalk at physiological maturity, with comparatively low percentages partitioned to the grain.

CONCLUSIONS

Both the prolific hybrid, FS854, and the single eared hybrid, B73xLH51 produced more grain (14% and 8%, respectively) when grown on a mix of equal portions of $\text{NO}_3^-:\text{NH}_4^+$ than when grown on NO_3^- alone. The increase in grain yield was the result of an increase in the numbers of kernels per plant. Only when grown on mixed N, did the single eared hybrid produce a full sized (shank, cob and husk) second ear, however these ears developed few kernels (less than 50). We suggest that the increased concentration of reduced N in the plant supplied with $\text{NO}_3^-:\text{NH}_4^+$ mixture was, in some manner, associated with the increased reproductive development and kernel number.

For FS854, the yield increase resulting from mixed N nutrition was the result of alteration in dry matter partitioning and not an increase in total dry matter production (i.e. harvest index increased). In contrast for B73xLH51, the yield increase was associated with a similar increase in total dry matter production as harvest indices were the same for both N treatments.

When grown on NO_3^- both hybrids had exceptionally high contents and concentrations of N, P and K and the levels of N and P were substantially higher for both hybrids grown on mixed N. FS854 accumulated more N per plant than B73xLH51 when the hybrids were cultured on NO_3^- , while the reverse was true where the nutrient medium contained NO_3^- and NH_4^+ . This observation indicates that these two hybrids differ in their ability to accumulate NO_3^- and NH_4^+ from nutrient cultures. B73xLH51 plants accumulated more K when cultured on mixed N than on NO_3^- while FS854 accumulated similar amounts of K for both treatments.

The reasons for the differential partitioning of the various constituents among the plant parts of the two hybrids or the effect of these partitioning patterns on grain yield is not clear.

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Chapter 3. Effect of Mixtures of Nitrate and Ammonium on Nitrogen Utilization and Productivity of Wheat: Hydroponic and Soil Cultures.

INTRODUCTION

The question as to which form of N, nitrate or ammonium, is superior for obtaining maximum crop productivity remains unanswered. Although both forms of N can be absorbed and metabolized by plants, under field conditions nitrate is usually the predominant N form available. This situation arises because ammonium is rapidly converted to nitrate by microorganisms in well-aerated warm soils. Because the nitrate ion can be subsequently lost from the rooting zone via leaching or denitrification, the maintenance of N in the ammoniacal form could lead to improvements in N fertilizer management.

It has been well documented that mixtures of nitrate and ammonium often support better plant growth than either form alone. For young wheat (Triticum aestivum L.) plants grown in solution culture, Cox and Reisenauer (1973) observed a 74% increase in vegetative yield when ammonium was supplied as 17% of the total N. Gashaw and Mugwira (1981), found that seedlings of wheat, triticale (Triticosecale) and rye (Secale cereale L.) produced higher dry matter with N mixtures containing 50 to 75% ammonium than with 100% nitrate. Weissman (1951) reported that dry weights, total protein content and protein concentration were all higher in leaves of wheat grown on ammonium plus nitrate than either form alone. For wheat, Spratt and Gasser (1970) and Leyshon et al., (1980) found ammonium-grown plants produced more tillers than plants receiving only nitrate although the enhanced tillering was not always associated with increased yield.

Enhanced growth and yield from utilization of N mixtures has also been reported for other crop species. Schrader et al. (1972) found a 69% increase in dry matter yield of 47-day-old maize (Zea mays L.) plants grown on a 50/50 mixture of nitrate and ammonium as compared to an all nitrate treatment. When grown on sandy soils, maize plants provided with supplemental ammonium produced significantly more grain and stover than plants supplied only with nitrate (Jung et al., 1972). For rice (Oryza sativa L.) grown under hydroponic conditions, mixed N nutrition increased plant tillering which was associated with higher grain yields (Mori et al., 1985). For tomato (Lycopersicum esculentum Mill), a nearly 100% increase in shoot dry matter was observed for 7-week-old plants grown on a 50/50 $\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$ mixture, compared to all nitrate (Ganmore-Neumann and Kafkafi, 1980). Increases in growth by utilization of mixed N nutrition have also been reported for soybean (Glycine max L.) (Rufty et al., 1983), rye (Nittler and Kenny, 1976), sunflower (Helianthus annuus L.) (Weissman, 1964) and orchard grass (Dactylis glomerata L.) (Drouineau and Blanc, 1960). Collectively, these results can be interpreted to imply that under certain conditions some plant species cannot obtain adequate N for maximal growth when supplied only with nitrate.

With regard to a physiological explanation for enhanced growth from utilization of both nitrate and ammonium, experiments with a number of plant species have shown that the form of N supply exerts a pronounced effect on the chemical composition of the plant and on the rhizosphere due to the necessity of maintaining cation-anion balance. Ammonium nutrition typically results in lower concentrations of K^+ , Ca^{2+} , Mg^{2+} and organic acids, and higher concentrations of inorganic anions and amides compared to nitrate nutrition (Blair et al., 1970; Harada et al., 1968; Ikeda and Yamada, 1984; Kirkby, 1968). The reason for the decreased concentrations of cations for ammonium-

grown plants is considered to be increased competition in the uptake process. The difference in organic acids has been proposed to be caused by differential internal pH that results from unbalanced cation:anion uptake and the metabolic reactions associated with the assimilation of nitrate and ammonium (Hageman, 1984). To maintain electrical neutrality and provide some degree of pH control the plant synthesizes (nitrate assimilation) or catabolizes (ammonium assimilation) organic acids.

It has been proposed that simultaneous uptake of nitrate and ammonium contributes to maintaining the proper pH of the cytoplasm which promotes the rapid conversion of inorganic N to organic N (Ikeda and Yamada, 1984; Raven and Smith, 1976). Alternatively, under soil conditions, the change in pH near the root caused by ammonium or nitrate uptake may influence the availability of other ions such as P, Al and Fe. The decreased pH as a result of ammonium absorption could have a beneficial effect on Fe availability, but could also lead to toxic levels of available Al (Reisenauer, 1978). These observations provide a possible explanation for the enhanced growth and nutrient absorption that has been reported for plants grown on mixtures of nitrate and ammonium.

An alternative advantage for the utilization of both N forms has been proposed by Tsai et al. (1978, 1985). These authors reported that maize plants fertilized with ammonium (and a nitrification inhibitor) incorporated more sugars into kernels than did plants receiving the same level of fertilizer without the nitrification inhibitor. It was suggested that ammonium played an important physiological role by enhancing sucrose transport from the leaves and thereby increasing photosynthetic efficiency.

Because of the technical difficulty of maintaining specific nitrate/ammonium ratios in soils, the majority of studies which demonstrate a clear affect of mixed N nutrition on plant growth have utilized solution

culture techniques. Consequently, similar increases in growth as a result of mixed N nutrition, have not been consistently obtained for soil-grown plants. In fertile soils, plants generally have an adequate supply of nitrate and variable quantities of ammonium. Only under unusual circumstances, such as strongly acid or anaerobic soils, are all-ammonium systems ever encountered (Reisenauer, 1978).

The lack of a yield increase in soil may arise from the fact that the level of nutrients at the root surface differs from the level in the soil because nutrients must engage plant roots to be absorbed. Engagement of nutrients by plant roots can be through mass flow and/or diffusion. Elements such as Ca^{2+} , Mg^{2+} and NO_3 are supplied primarily by mass flow, while roots usually engage NH_4^+ , PO_4^{2-} and K^+ by diffusion. Rates of diffusion through the soil solution vary with the nutrient, and the degree to which it is adsorbed by the soil. Ammonium is strongly adsorbed to negatively charged clay minerals because of its cationic properties. Specifically, it can be bound rather selectively to 2:1 clay minerals, such as illites, vermiculites and montmorillonites. (Mengel and Kirkby, 1978). Therefore, the rate of absorption of the relatively immobile ammonium ion from soil is also influenced by the rate at which roots grow to the areas of ammonium concentration.

The difference in results from hydroponic and soil studies illustrate the need to further evaluate the effect of ammonium versus nitrate utilization on crop productivity. A review of the literature revealed no studies that directly compared the effects of nitrate/ammonium ratios in soils and hydroponics. Therefore the objective of this study was to compare the effects of varying nitrate/ammonium ratios in hydroponic and soil culture on the growth and yield of spring wheat. An additional objective was to identify

factors associated with differences in productivity resulting from either N treatment or culture method.

MATERIALS AND METHODS

PLANT CULTURE AND TREATMENTS

Durum spring wheat plants (Triticum durum cv. Inbar) were grown to maturity in the greenhouse in both soil and hydroponic culture during the winter of 1986. Natural lighting was supplemented with metal halide lamps (approximately $300 \text{ } \mu\text{mol/m}^2/\text{s}$, between 400 and 700 nm wavelength) to provide 14 h of illumination. Greenhouse temperatures were maintained at 24°C during the light cycle and 18°C during the dark cycle. Under these conditions, color and morphological development of soil- and hydroponically-grown plants were similar to field-grown plants.

Soil versus hydroponic culture were the main plots of a split plot design while N treatments were subplots. There were five replications of each culture method-N treatment combination. The N treatments consisted of three ratios of NO_3^- -N to NH_4^+ -N: 100/0, 75/25 and 50/50. Soil and hydroponic pots were arranged so that each occupied the same bench area ($0.212 \text{ m}^2/\text{pot}$) and border effects were similar.

For hydroponic culture, four plants were grown in 7 L culture vessels to give a stand density equivalent to 125 plants/m^2 . Details of germination, growth and nutrient solutions used for hydroponic culture are discussed elsewhere (see Chapter 6). For soil culture, plants were grown in 20 L pots that contained 16 kg of a soil mixture consisting of (by volume) six parts soil (unsterilized Drummer silty clay loam), two parts peat, one part perlite, and one part sand. Plantings were made on 11 January and stands subsequently thinned to 19 plants per pot to give a stand density equivalent to 300

plants/m². A greater number of plants per pot was used in the soil culture to partially compensate for the anticipated greater tillering of plants grown hydroponically (our preliminary unpublished data) and to be more applicable to production conditions.

Throughout the life cycle each pot received the amounts of mineral nutrients listed in Table 3-1. Sixty-two percent of the total fertilizer application was applied by thoroughly mixing the fertilizer salts with the soil just prior to planting. The remaining 38% was supplied as a nutrient solution in six sequential additions made during the life cycle. With all N additions, to maintain the nitrate/ammonium ratios, the nitrification inhibitor, dicyandiamide, was added at a rate of 5% of the total N. Pots were watered with tap water as needed.

SAMPLING

Physiological maturity (estimated visually by complete loss of green color from glumes, Hanft and Wych, 1982) occurred at 112 DAE for soil-grown plants and 134 DAE for hydroponic-grown plants. At physiological maturity, plants were harvested, divided into leaves, stems, roots and heads and each part dried to constant weight at 80°C in a forced-draft oven (Heberer et al. 1984).

Dried samples were weighed and mechanically ground to pass a 20-mesh screen. Dried heads were threshed and grain was redried, weighed and mechanically ground as above. Prior to chemical analysis, tissue was redried for 72 h at 60°C.

CHEMICAL ANALYSIS

Total N A 100 mg sample of dried, ground tissue was weighed into a Folin-Wu digestion tube to which 4 mL of 18 M H₂SO₄ was added. After standing for 15 h, 100 mg of sodium thiosulfate was added to each tube and the tubes were

Table 3-1. The total amount of macronutrients supplied to the soil to evaluate the effect of mixtures of nitrate and ammonium versus nitrate alone on growth and yield of spring wheat.

Element	$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$		
	100/0	75/25	50/50
	----- g/pot -----		
N (NO_3^-)	2.43	1.82	1.22
N (NH_4^+)	0	0.61	1.22
P	2.16	2.16	2.16
K	2.65	2.65	2.65
Mg	1.20	1.26	1.32
Ca	2.63	2.71	2.78
S	1.32	2.38	3.44

heated to 220°C over a 3 h period. This predigestion procedure was used to facilitate the conversion of nitrate N to reduced N (Nelson and Sommers, 1973). After predigestion, the tissue was digested at 220°C with dropwise additions of H_2O_2 (8.8 mol/L) until the solution became clear. The digests were then diluted to 50 mL with dionized water, mixed, and aliquots analyzed for $\text{NH}_4^+\text{-N}$ (Cataldo et al., 1974). Reduced N was determined by subtracting the $\text{NO}_3^-\text{-N}$ concentration from the total-N concentration. For grain, reduced N concentration was converted to protein using the conversion factor 5.65 (Tkuchuk, 1969).

Total P Aliquots (0.5 to 1.0 mL) were removed from the total N digests, and total P was determined using the method of Chen et al. (1956).

Nitrate N A 100 mg sample of dried, ground tissue was autoclaved (60 min; 103 kPa) in 25 mL of 0.02 mol/L benzoic acid. After filtering, aliquots (300 to 1000 μL) were removed from the extracts and analyzed for $\text{NO}_3^-\text{-N}$ using dissimilatory nitrate reductase as described by McNamara et al. (1971).

Chlorophyll Selected flag leaves (three per pot) were harvested at 1 and 40 days after flowering (DAF) for chlorophyll determination. Compositated leaves were chopped into approximately 1 cm^2 sections and 0.5 g fresh tissue was placed in a glass bottle to which 10 mL of 95% (v/v) ethanol was added. Bottles were capped and agitated in the dark at ambient temperature for 20 h. Aliquots (0.5 mL) were removed from the bottles and diluted with 4.5 mL of 95% ethanol. Chlorophyll concentrations were calculated from absorbance values at 649 and 665 nm according to Holden (1976).

STATISTICAL ANALYSIS

Data were analyzed by analysis of variance procedures. Because of extreme differences between soil and hydroponic pots in cultural conditions and plant

growth, the data for each was analyzed separately as two different experiments. For each culture method the LSD ($P \leq 0.05$) was calculated to compare N treatment means.

RESULTS

YIELD AND YIELD COMPONENTS

For hydroponically-grown wheat, both N mixtures resulted in significantly higher grain yield (85 and 70% higher for the 75/25 and 50/50 N treatments, respectively) compared to plants grown with all nitrate (Table 3-2). In contrast, for soil-grown plants, grain yield was similar for all N treatments. Hydroponically-grown plants produced more grain than similarly treated soil-grown plants when expressed on either a per plant or a space area basis (Table 3-2). However, because of differences in initial plant density, expressing the data on a space area basis provides a more valid comparison for hydroponic versus soil grown plants. When expressed on a space area basis, hydroponically-grown plants produced 30 (nitrate grown) to 130% (grown with either N mixture) more grain than soil grown-plants.

In contrast to grain yield, N treatments or culture conditions had no significant effect of grain yield components (Table 3-2). For this variety, each fertile tiller produced approximately 27 kernels which weighed 52 mg/kernel and contained about 20% crude protein.

TILLERING

For hydroponically-grown plants, the first visual response to the N treatments was an increase in plant tillering for plants receiving either N mixture (data not shown). This difference was evident as early as 12 days after emergence (DAE) and was markedly noticeable by 30 DAE. At physiological

Table 3-2. The effect of mixtures of nitrate and ammonium versus nitrate alone on grain yield components for hydroponically- and soil-grown wheat.

$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$	Grain yield		Kernel weight	Kernel number	Grain protein
	g/plant	g/m ²	mg/kernel	no./tiller	g/kg
----- Hydroponic -----					
100/0	20	377	49	28	200
75/25	37	698	52	30	210
50/50	34	642	51	29	220
LSD	6	42	NS	NS	NS
----- Soil -----					
100/0	3	269	53	26	200
75/25	3	269	52	26	190
50/50	3	269	53	26	200
LSD	NS	NS	NS	NS	NS

maturity, hydroponically-grown plants receiving all nitrate had 15 grain bearing tillers as compared to 23 for plants receiving N mixtures (53% increase) (Table 3.3). In hydroponic culture, plants continued to develop tillers throughout the life cycle, although many of later formed tillers were barren (developed heads but no grain).

For soil-grown plants, there was no difference in tillering as plants in all treatments produced approximately 2.5 grain bearing tillers per plant (Table 3-3). When calculated on an area basis, hydroponic grown plants produced from 26 to 93% more grain bearing tillers than did similarly treated soil-grown plants (Table 3-3).

WHOLE PLANT ACCUMULATION

In hydroponics, plants receiving mixtures of nitrate and ammonium produced 80 and 75% (75/25 and 50/50, respectively) more dry matter than plants grown with 100% nitrate (Table 3-4). In contrast, the dry matter production of soil-grown plants were similar regardless of treatment. Compared to soil-grown plants, hydroponically-grown plants produced 44 to 150% more total plant mass per square meter of space area.

By maturity, plants grown in hydroponics with mixed N nutrition contained twice as much whole plant reduced N as nitrate grown plants (Table 3-4). The 2-fold difference in whole plant reduced-N content corresponds to a 2-fold difference in cumulative N uptake from solution for these same plants (see Chapter 6). The higher reduced-N accumulation by plants receiving N mixtures was the result of both an increase in phytomass and an increase in the concentration of plant N (Table 3-4).

Similar to whole plant reduced-N accumulation, hydroponically-grown plants receiving N mixtures accumulated approximately 70% more total P by maturity

Table 3-3. The effect of mixtures of nitrate and ammonium versus nitrate alone on the number of grain bearing and barren tillers at physiological maturity for hydroponically- and soil-grown wheat. Values in parentheses represent number of tillers/m².

$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$	Grain bearing		Barren*	
	Hydroponic	Soil	Hydroponic	Soil
	----- no./plant -----			
100/0	15 (283)	2.5 (224)	10 (188)	0
75/25	23 (434)	2.5 (224)	14 (264)	0
50/50	23 (434)	2.5 (224)	9 (170)	0
LSD	2 (40)	NS	NS	

*Barren tillers had developed heads but no grain.

Table 3-4. The effect of mixtures of nitrate and ammonium versus nitrate alone on the accumulation of dry matter, N and P by whole plants at physiological maturity for hydroponically- and soil-grown wheat. Values in parentheses represent concentrations of constituents expressed as g/kg.

$\text{NO}_3^- - \text{N} / \text{NH}_4^+ - \text{N}$	Dry matter		N	P
	g/plant	g/m ²	----- mg/plant -----	
	----- Hydroponic -----			
100/0	48	906	1174 (25)	345 (7)
75/25	86	1623	2441 (28)	593 (7)
50/50	84	1585	2356 (28)	584 (7)
LSD	12	226	250 (1.4)	68 (NS)
	----- Soil -----			
100/0	7	627	151 (22)	24 (3)
75/25	7	627	139 (21)	24 (3)
50/50	7	627	146 (22)	24 (3)
LSD	NS	NS	8 (NS)	NS

than did nitrate-grown plants (Table 3-4). However, in contrast to reduced-N accumulation, the increase in whole plant P content was entirely the result of greater phytomass, as hydroponically-grown plants in all treatments had similar concentrations of plant P. In contrast, for soil-grown plants, N treatments had no effect on the contents or concentrations of either whole plant reduced N or whole plant P (Table 3-4).

Regardless of treatment, plants cultured hydroponically had a substantially greater N and P status than soil grown plants (Table 3-4). Hydroponically-grown plants had 20 to 30% higher reduced-N concentration, and 130% higher P concentration, than soil-grown plants.

DISTRIBUTION AMONG PLANT PARTS

Dry matter Regardless of culture media, the various N treatments had no effect on the distribution of final plant mass among the various plant parts (Table 3-5). Despite the greater phytomass production of hydroponically-compared to soil-grown plants, the partitioning of dry matter among the plant parts was similar. At maturity, plants had partitioned approximately 11% of their total dry mass to the leaves, 20% to the stems, and 7% to the roots. Because hydroponically-grown plants produced substantial amounts of barren vegetation (6 to 9% of the total dry matter), the partitioning of dry matter to the grain was less than for soil-grown plants.

Reduced N For soil-grown plants, the content and concentration of reduced-N compounds at maturity within a given plant part was similar, regardless of treatment (Table 3-6). In contrast, for hydroponically-grown plants, accumulation of reduced N within various plant parts differed with N treatment. The largest difference occurred in the leaves where the content of reduced N was 4 to 5-fold higher and the concentration 67 to 93% higher when

Table 3-5. The effect of nitrate and ammonium versus nitrate alone on distribution of dry matter among plant parts at physiological maturity for hydroponically- and soil-grown wheat.

NO ₃ ⁻ -N/NH ₄ ⁺ -N	Plant part				
	Leaves	Stems	Roots	Grain	Barren tillers
	----- % of total -----				
	----- Hydroponic -----				
100/0	9	20	7	43	9
75/25	12	20	5	43	9
50/50	13	20	8	41	6
	----- Soil -----				
100/0	11	20	5	49	-
75/25	11	20	4	52	-
50/50	11	19	8	49	-

Table 3-6. The effect of mixtures of nitrate and ammonium versus nitrate alone on reduced-N content in various plant parts at physiological maturity for hydroponically- and soil-grown wheat. Values in parentheses represent concentrations expressed as g/kg.

$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$	Plant part				
	Leaves	Stems	Roots	Grain	Barren tillers
----- mg/plant -----					
----- Hydroponic -----					
100/0	64 (15)	74 (8)	81 (25)	727 (36)	227 (47)
75/25	250 (25)	185 (11)	134 (29)	1376 (38)	495 (65)
50/50	312 (29)	244 (13)	124 (29)	1337 (39)	359 (62)
LSD	61 (6)	59 (3)	37 (NS)	190 (NS)	137 (10)
----- Soil -----					
100/0	12 (16)	10 (7)	6 (16)	123 (36)	-
75/25	10 (14)	7 (6)	5 (16)	117 (34)	-
50/50	11 (14)	9 (7)	8 (16)	119 (36)	-
LSD	NS	NS	NS	NS	

plants were grown with N mixtures. A similar increase in the content of reduced-N for plants grown on mixed N nutrition was observed for stems (2.9-fold), roots (1.6-fold), grain (1.8-fold) and barren vegetation (1.4-fold). However, the greater content of reduced N in roots and grain was entirely the result of more plant mass as there was no difference in concentration of reduced N in these plant parts. Similar to leaves, the higher reduced-N content of stems and barren vegetation of plants receiving mixed N nutrition was the result of both increased N concentration and increased dry matter.

Regardless of culture media, all plants contained 34 to 39 g/kg reduced N in the grain, and 6 to 13 g/kg reduced N in the stems at PM (Table 3-6). In contrast, hydroponically-grown plants contained approximately 80% higher concentration of reduced N in the root than did soil-grown plants. Either culture media resulted in a similar leaf N concentration when plants were grown with all nitrate, while for N mixtures in hydroponic culture leaf N concentration was nearly 100% higher than soil-grown plants.

For soil-grown plants, the distribution of reduced N among the various plant parts was similar regardless of N treatment (Table 3-7). In hydroponics, the accumulation of total plant reduced N differed in response to N treatment (Table 3-4), and the distribution of N to the various plant parts was also affected by treatment. Growth on N mixtures resulted in increased partitioning of reduced N to the leaves (130%) and stems (50%), decreased (28%) allocation to the roots, and had no effect on partitioning to the grain (Table 3-7).

Grain contained approximately 82% of the whole plant reduced N for soil-grown plants and approximately 58% for hydroponically-grown plants (Table 3-7). Barren vegetation produced in hydroponic grown plants contained from 14

Table 3-7. The effect of mixtures of nitrate and ammonium versus nitrate alone on distribution of reduced N among plant parts at physiological maturity for hydroponically- and soil-grown wheat.

$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$	Plant part				
	Leaves	Stems	Roots	Grain	Barren tillers
	----- % of total -----				
	----- Hydroponic -----				
100/0	5	6	7	62	19
75/25	10	8	5	56	19
50/50	13	10	5	57	14
	----- Soil -----				
100/0	8	7	4	81	-
75/25	7	5	4	84	-
50/50	7	6	5	82	-

to 19% of the whole plant reduced N. Distribution of reduced N among stems, roots and leaves, also varied between culture media. All soil-grown plants partitioned reduced N to the roots similar to hydroponic plants grown with N mixtures. In contrast, reduced N partitioning to stems of soil grown plants was similar to hydroponic plants grown with all nitrate (Table 3-7).

Phosphorus Regardless of N treatment, for soil-grown plants, both the P contents and the P concentrations within a given plant part were similar (Table 3-8). In contrast, for hydroponically-grown plants, the content of P within leaves, stems and grain, varied with N treatment. As with accumulation of reduced N, the largest difference occurred in the leaves where the content of P was 140% higher when plants were grown with N mixtures (Table 3-8). For both stems and grain, growth on mixed N nutrition resulted in approximately 68% higher content of P. In roots and barren vegetation, the accumulation of P (both content and concentration) was similar regardless of N treatment. For leaves, stems and grain of hydroponically-grown plants, the increase in P was entirely the result of a greater phytomass, as concentration of P in these plant parts was similar.

For soil-grown plants, the distribution of P among the various plant parts was similar regardless of N treatment (Table 3-9). Similar to reduced N, distribution of whole plant P to the various plant parts was effected by N treatment when plants were grown in hydroponics. Growth on mixed N nutrition resulted in greater partitioning of P to the leaves (33 to 50%), decreased allocation (27%) to roots, and had no effect on partitioning to stems or grain (Table 3-9).

Of the whole plant P at maturity, approximately 74% was present in the grain of soil-grown plants and approximately 33% in the grain of hydroponically-grown plants (Table 3-9). In hydroponically-grown plants,

Table 3-8. The effect of mixtures of nitrate and ammonium versus nitrate alone on P content in various plant parts at physiological maturity for hydroponically- and soil-grown wheat. Values in parentheses represent concentrations expressed as g/kg.

NO ₃ ⁻ -N/NH ₄ ⁺ -N	Plant part				
	Leaves	Stems	Roots	Grain	Barren tillers
----- mg/plant -----					
----- Hydroponic -----					
100/0	63 (15)	82 (8)	38 (12)	113 (5)	49 (10)
75/25	144 (14)	132 (8)	47 (10)	198 (5)	71 (9)
50/50	158 (15)	143 (9)	46 (10)	183 (5)	54 (10)
LSD	21 (NS)	26 (NS)	NS	34 (NS)	NS
----- Soil -----					
100/0	3 (4)	2 (2)	0.6 (2)	18 (5)	-
75/25	3 (5)	2 (2)	0.5 (2)	18 (5)	-
50/50	3 (4)	2 (2)	0.8 (2)	17 (5)	-
LSD	NS	NS	NS	NS	-

Table 3-9. The effect of mixtures of nitrate and ammonium versus nitrate alone on distribution of P among plant parts at physiological maturity for hydroponically- and soil-grown wheat.

$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$	Plant part				
	Leaves	Stems	Roots	Grain	Barren tillers
	----- % of total -----				
	----- Hydroponic -----				
100/0	18	24	11	33	14
75/25	24	22	8	33	12
50/50	27	24	8	31	9
	----- Soil -----				
100/0	13	9	2	75	-
75/25	14	9	2	75	-
50/50	14	9	3	71	-

barren vegetation contained 9 to 14% of the whole plant P. Hydroponically-grown plants also had a higher percentage of whole plant P remaining in leaves (40 to 90%), stems (160%) and roots (2.6 to 5.5-fold) than soil-grown plants (Table 3-9).

Chlorophyll For both soil- and hydroponically-grown plants there was no effect of N treatment on the concentration of leaf chlorophyll (Table 3-10). At 1 DAF, all plants, regardless of culture media, had similar concentrations of leaf chlorophyll. Between 1 and 40 DAF, concentration of chlorophyll in leaves of soil grown plants decreased more than 50%, while in hydroponically-grown plants there was a 41 to 54% increase (Table 3-10). Visual observations support the chlorophyll data, as leaves of soil-grown plants senesced about 3 weeks earlier than comparable leaves of hydroponically-grown plants (data not shown).

DISCUSSION

The data are in agreement with previous studies with wheat which showed an increased yield of grain and whole plant dry matter when plants were grown on mixtures of nitrate and ammonium (Cox and Reisenauer, 1973; Gashaw and Mugwira, 1981; Spratt and Gasser, 1970). For plants in hydroponic culture, mixed N nutrition resulted in 70 to 85% higher grain yield (Table 3-2) and 75 to 80% higher nongrain plant mass (Table 3-4) than the all nitrate treatment. The increased grain yield for plants grown on N mixtures was not due to larger kernels or more kernels per tiller, rather it was the result of an increase in the number of grain bearing tillers per plant (Table 3-3). For soil-grown plants, N mixtures had no effect on growth, tillering or grain yield relative to nitrate-grown plants.

On a space area basis, hydroponically-grown plants produced substantially

Table 3-10. The effect of mixtures of nitrate and ammonium versus nitrate alone on the concentration of chlorophyll at 1 and 40 DAF for hydroponically- and soil-grown wheat.

$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$	Culture medium	
	Hydroponic	Soil
----- mg/g fresh weight -----		
1 DAF		
100/0	2.7	3.0
75/25	2.6	3.0
50/50	2.6	2.9
LSD	NS	NS
40 DAF		
100/0	3.8	1.4
75/25	4.0	1.4
50/50	3.9	1.4
LSD	NS	NS

more grain (30 to 130%) (Table 3-2), and nongrain plant mass (44 to 150%) (Table 3-4) than similarly treated soil-grown plants. The increase in grain yield resulted from enhanced tillering as yield components were similar between soil- and hydroponically-grown plants (Table 3-2). Although a much greater total dry mass and grain yield was observed for hydroponically-, as compared to soil-grown plants, the partitioning of dry matter between the various plant parts was similar for plants grown with either culture media.

For soil-grown plants, N treatment had no effect on the contents or concentration of either whole plant reduced N or whole plant P (Table 3-4). While by physiological maturity, plants grown in hydroponics with mixed N nutrition contained significantly more whole plant N and P than nitrate-grown plants. For hydroponically-grown plants, N mixtures increased reduced-N content of all plant parts and reduced-N concentration of leaves and stems (Table 3-6). Mixed N nutrition also altered the distribution of whole plant reduced N among the various plant parts (Table 3-7). The higher concentration of reduced N in the leaves of plants grown on N mixtures was associated with the high rates of N absorption by these plants (see Chapter 6).

Although there was a greater concentration of reduced N in most plant parts of hydroponically- as compared to soil-grown plants, the concentration of reduced N in the grain at maturity was similar. These data indicate that despite large differences in total N absorption, a constant proportion of dry matter to reduced N was deposited in the grain. These data can be interpreted to imply that in all instances sufficient N was available for attainment of maximum grain reduced N concentration, and when in excess, additional N was stored in the leaves (Table 3-6).

For hydroponically-grown plants, the distribution of reduced N among plant parts is indicative of substantial post anthesis N uptake and assimilation, as

almost 20% of the whole plant N was partitioned to barren tillers formed after anthesis. However, the high concentration of reduced N in barren tillers (47 to 65 g/kg) indicates that availability of N was probably not a factor limiting seed set. In contrast, the distribution of reduced N among plant parts of soil-grown plants, indicates that some of the grain N was derived from remobilization and that post anthesis N uptake was limited.

For hydroponically-grown plants, an effect of N treatment was observed for the accumulation and partitioning of whole plant P. At maturity, mixed N nutrition resulted in 70% more whole plant P, 68% higher P content of stems and grain, and 140% higher P content in leaves. Contrary to N absorption, the increased whole plant P for N mixtures was entirely due to the increase in total plant dry mass as the concentration of P within any plant part was similar for all N treatments..

As with grain reduced N, all treatments had similar concentrations of P in the grain regardless of plant P status. In order to meet grain P requirements, soil-grown plants appeared to rely on redistribution from vegetation, while hydroponically-grown plants absorbed P during seed fill. This conclusion is based on the large difference in percentage of the whole plant P partitioned to the grain of soil compared to hydroponic plants (33 versus 75%)

Although soil-grown plants absorbed less N and P per plant than hydroponically-grown plants, the efficiency of utilization of these elements (calculated as grain weight/whole plant content of the element, Moll et al., 1982) was up to 47% higher for N and 100% higher for P. Moll et al. (1982) also found that plants were less efficient in N use at high levels of N supply.

Comparison of results from hydroponic and soil culture must consider the variation in levels of nutrients in the rhizosphere due to differences in rates of supply to the root. In hydroponic culture, nutrients are continuously available, while in the soil, nutrients must move to the root by mass flow and/or diffusion, or the root must grow to the nutrient. Because the ammonium ion is relatively immobile in the soil, (due to binding by soil colloids and immobilization by microorganisms), it is likely to be positionally unavailable to plant roots. Given this case, soil plants would be utilizing primarily nitrate, which may explain the lack of difference between N treatments. Alternatively, the Drummer soil used in this study may have supplied the crop with adequate amounts of ammonium through mineralization, such that differences between the all nitrate and N mixture treatments were masked.

The similar concentration of reduced N in leaves of soil-grown plants and hydroponically-grown plants receiving all nitrate (Table 3-6), indicates that soil-grown plants are utilizing predominately nitrate and supports the idea that in the soil ammonium was positionally unavailable to plant roots. Soil assays showed that the nitrification inhibitor, dicyandiamide, was reasonable effective in maintaining the appropriate nitrate/ammonium ratios in the soil.

Visual observations indicated that soil-grown plants senesced approximately 3 weeks earlier than hydroponically-grown plants. However, data for chlorophyll concentration indicated that this difference was not a function of N treatment (Table 3-10). Despite the difference in leaf area duration, all plants had similar times of grain maturity. This data indicates that leaf senescence of wheat can be independent of grain development. The delayed leaf senescence of hydroponic plants may be associated with the continued accumulation of N during the later stages of grain fill (see Chapter 6).

The difference in response to N mixtures for the two culture conditions indicates the difficulty in direct comparisons of results from soil and hydroponic experiments. In view of the complexity of N interactions and transformations in soils it is not surprising that the growth promoting potential of mixed N nutrition may not be readily realized under soil conditions. However, the results of the hydroponic experiment demonstrate the potential for substantial increases in wheat productivity by utilization of N mixtures, and indicate that further research is needed to elucidate the growth promoting mechanisms associated with mixed N nutrition.

CONCLUSIONS

Hydroponic and soil culture conditions were utilized to determine the effect of 100% nitrate and mixtures of nitrate and ammonium (ratios of 75/25 and 50/50) on the growth and grain yields of durum spring wheat (Triticum durum cv. Inbar). From this data the following conclusions were drawn.

1. The hydroponically-grown plants produced more total dry mass and grain (about 80%) and developed more tillers (40%) when grown on either N mixtures than when grown solely on nitrate.
2. The increased grain yield of the ammonium-cultured plants was due primarily to increased tiller formation and kernel number as kernel weight remained relatively constant regardless of N treatment.
3. Plants cultured a mixed N accumulated more total reduced N (2-fold) and P (1.7-fold) than plants grown on nitrate alone. Consequently, at maturity the concentrations of N were slightly higher while concentrations of P were similar.
4. At maturity the concentration of reduced N in both leaves and stems increased progressively (nearly linear) with increased amounts of NH_4^+

ammonium (0 to 50%) in the nutrient solution.

5. The wheat cultivar Inbar was unable to absorb sufficient N for maximum plant development when the nutrient solution contained only nitrate.

6. For soil grown plants, N mixtures caused no significant differences in growth, grain yield, tillering, and contents of reduced N and P relative to nitrate as the sole source of N.

7. The reason for the lack of plant response to the mixed N treatment is not clear, however these data support the conclusion that the plants grown on soil fortified with mixed N were not absorbing any more ammonium than the plants grown on soil fertilized only with nitrate. (The effectiveness of the nitrification inhibition was verified by control pots without plants).

8. When the N source was mixed N, the concentrations of reduced N in the vegetative parts at maturity of the hydroponically-grown plants was higher (1.8 to 20-fold than for soil grown plants. When the N source was nitrate, the concentrations of reduced N of the vegetative parts were similar regardless of rooting media.

9. Regardless of culture method or treatment, the partitioning of dry matter among plant parts was similar. In contrast, the distribution of N and P among plant parts differed for soil - and hydroponically-grown plants and the distribution was altered by N treatments for hydroponically grown plants.

10. The hydroponic experiment shows that mixtures of nitrate and ammonium markedly increases productivity of the wheat cultivar Inbar. Thus there is a potential for increasing wheat production by supplying supplemental ammonium. The soil experiment shows that there are unknown factors that apparently prevented the absorption of ammonium from this support medium.

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Chapter 4. The Effect of Various Mixtures of Ammonium and Nitrate on the Growth and Yield of Wheat and the Growth of Millet - Soil Experiment.

4. Wheat and millet greenhouse experiment 1984/5

Introduction

The ability to maintain a portion of the soil N in the ammoniacal form throughout the growing season could provide a practical approach to increasing crop yields. In a review article Olsen (1986) cited several studies where addition of ammonium to an all-nitrate system resulted in increased corn yields. Hageman (1980, 1984) reviewed the effects of ammonium - and nitrate - nitrogen nutrition on plant growth and cited several experiments indicating that higher crop yields were obtained with a mixture of nitrate and ammonium than with either source alone. Ganmore-Neumann and Kafkafi (1983), working with nutrient solutions varying in nitrate and ammonium concentrations, obtained optimal growth for strawberries with an equal ratio of nitrate to ammonium. Under field conditions, Israeli et al. (1985) obtained maximum yield of bananas when equal ratios of ammonium to nitrate were present in the soil extracts.

In the experiments presented here an attempt was made to maintain the applied ratio of ammonium to nitrate nitrogen in the soil by addition of dicyandiamide (DCD). The influence of chosen ammonium-to-nitrate ratios on plant growth, content of reduced nitrogen and cation uptake in wheat and millet were investigated.

Methods and Materials

The soil for the greenhouse experiment was collected in the Sharon area of Israel and its characteristics are given in table 4.1.

TABLE 4.1
Some characteristics of soil used in
greenhouse experiment

Location of sampling	Sharon (Ilanot)
Texture	Loamy sand
Electrical conductivity mmhos/cm	0.3
pH	6.95
CaCO ₃ , %	2.1
C organic %	0.6
N, total, %	0.04
Field capacity moisture %	15.7
Iseael soil classific.*	Hamra
U.S. soil taxonomy*	typic Rhodoxeralf

* Dan and Koyumdjiski (1979)

In greenhouse experiments plants were grown in 3-liter pots containing 3kg of soil, with 8 replicates per treatment. The N fertilizer treatments were 0, 0.5 and 1.0g N per pot. At each level of added N, five ratios of NH₄-N to NO₃-N (100/-, 75/25, 50/50, 25/75 and -/100) were obtained by applying the proper proportions of ammonium sulphate (AS) (21% N) and calcium nitrate (CAN) (12% N). All AS treatments contained dicyandiamide (DCD), a nitrification inhibitor (0.1g DCD per 1.0g AS). Superphosphate was applied at the rate of 1g P per pot to all pots. The fertilizers were mixed with the whole soil volume in each pot. Wheat (*Triticum durum*, cv Inbar) was planted on November 1, 1984 and thinned to 15 plants per pot. Water was applied to bring the soil to field capacity as frequently as needed. Duplicate pots were harvested December 27 (beginning of flowering) and January 29, 1985. Exudates were collected from the cut stems. Dry matter green yield was determined. The final 4 replicates were harvested April 1, 1985, when the grain had matured.

Leaf elongation was measured for three consecutive days, each day and night, on the youngest leave of six replicates, between December 16 and 19

1984. The leaves were measured on 7.45 a.m. (night elongation) and 2.30 p.m. (day elongation). Stomata opening was measured with a porometer at 8.00 and 11.00 a.m. and 1.00 p.m., but no significant differences between treatments were found.

Evapotranspiration rates in the wheat experiment were measured by weighing 6 pots of one treatment after and before the next irrigation, 6 times between the 12.18.84 and 1.11.85. In the millet experiment pots were weighed at 3 dates.

In an additional greenhouse experiment wheat was grown on nitrate or ammonium only both with DCD.

The millet (*Setaria italica*) experiment was planted on May 10, 1985 and thinned to 18 plants per pot. Duplicate pots from each treatment were harvested on June 2 and 9, 1985 during the vegetative phase. The remaining 4 pots per treatment were harvested on June 23, 1985 at the onset of flowering. Roots were separated from the soil after harvesting the millet at the three harvest dates. This was done in single pots and only in some treatments. Soil type and experimental treatments were as for wheat. Soil was sampled from the wheat and millet experiments at intervals to determine the effectiveness of the DCD treatments in the presence of plants. Ammonium and nitrate N were determined in these samples as described for incubation experiments in chapter 1. Applied N fertilizer with endogenous soil nitrogen could generally be accounted for at a precision level of ~ 10 percent.

N, P, Ca, Mg, K and Na concentrations in shoots were determined on composite samples and therefore no statistical analysis of results was performed. The analytical procedure which was carried out in duplicates gave very good reproducibility. Shoot content of reduced nitrogen, as an indicator of proteinaceous materials, was determined by the Kjeldahl procedure without prior reduction of nitrate. For cations and P determinations the plant material was digested in concentrated HNO_3 and

HC10₄ solution. Cation concentration was measured by atomic absorption and P determined in an autoanalyzer by the molybdic blue method.

Results

Wheat

A small greenhouse experiment with wheat was laid out to test whether DCD has a yield depressing effect. In one treatment AS was applied and in the other CAN, both at a level of 1g N/pot. Both treatments received DCD in the same ratio to nitrogen as in the main experiment and all other treatments parallel to that, in four replicates. Wheat was sown on February 12, 1985 and whole plants cut on April 1, 1985. Results are given in Table 4.2.

TABLE 4.2

Results of DCD test experiment with wheat

mg N appl. per pot as NH ₄ /NO ₃	Dry matter yield g/pot	N in soil at harvest	
		NH ₄	NO ₃
		mg/kg	mg/kg
1000/0	19.6	172 (25)	8 (3)
0/1000	27.8	11 (1)	107 (4)
Analysis of variance			
	F	91**	mean (s.e.)
	s	0.6	
	D	2.7	

The results in table 4.2 show that yields were depressed in the ammonium treatment relative to the nitrate treatment, although both were amended with DCD.

TABLE 4.3

Ammonium and nitrate nitrogen in soil samples
taken at intervals from pots used for growing wheat.

mg N appl. per pot as NH ₄ /NO ₃	Soil N levels at indicated dates					
	---12/27---		----1/29---		---4.1---	
	NH ₄	NO ₃	NH ₄	NO ₃	NH ₄	NO ₃
	-----mg N / pot-----					
0/0	0	3	30	6	18	6
500/0	150	3	75	6	27	3
375/125	150	45	54	6	21	3
250/250	249	96	45	6	21	6
125/375	39	156	48	21	21	3
0/500	30	201	33	51	1	3
ANOVA F	38	92	121	314	290	322
1.s.d.	8	4	2	1	1	0.4
1000/0	630	9	801	9	222	6
750/250	486	129	516	60	75	6
500/500	264	300	249	180	93	60
250/750	171	570	138	408	51	174
0/1000	9	690	45	558	21	237
ANOVA F	27	38	29	42	67	52
1.s.d.	6	10	11	15	9	8

In the main experiment with wheat soil samples were taken during the growth period. Soil analyses of the amounts of ammonium and nitrate present at various stages of plant development indicate that ammonium oxidation was effectively inhibited by the DCD treatments (Table 4.3). After 4 months at the 0.5g N/pot application rate the mineral nitrogen was nearly completely depleted from the pots, irrelevant of whether it was applied as ammonium or nitrate. At the 1.0g N/pot application rate roughly 25 percent of applied nitrogen remained in mineral form in the soil.

TABLE 4.4

Results of greenhouse experiment with wheat
Dry matter yield and number of ears and stalks

mg N appl. per pot as NH_4/NO_3	Yield, g/pot, by:				Number of	
	12.27	1.29	4.1			
			grain	straw	ears/pot	stalks/pot
0/0	4.7	7.8	3.8	7.2	15	15
500/0	7.0	16.7	12.6	18.6	27	28
375/125	9.3	19.2	15.6	17.6	22	25
250/250	9.4	22.4	16.4	18.7	19	21
125/375	10.0	21.0	18.5	18.6	19	20
0/500	8.6	20.2	18.0	18.7	17	22
1000/0	5.9	11.8	9.0	17.4	27	33
750/250	8.3	15.3	13.6	18.5	24	30
500/500	9.6	18.2	15.4	20.3	28	30
250/750	9.4	19.5	16.3	20.6	21	30
0/1000	7.9	19.0	16.1	19.4	22	30
ANOVA F:	19**	63**	136**	127**	20**	14**
l.s.d.	1.2	1.6	1.0	0.9	2.6	3.8

Fig. 4.1: Dry matter accumulation by wheat plants (above ground parts) in soil containing varying ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$, at three intervals. Treatment ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ are in paranthesis. F - value and standard error from analysis of variance.

Dry matter production by wheat during vegetative and reproductive development was measured and the data (Table 4.4 and Figure 4.1) show a significant response of wheat to nitrogen application. During the early vegetative growth period mixed N sources with ammonium-to-nitrate (N) ratios of 50/50 and 25/75 seem to be optimal. In all cases 100 percent ammonium nitrogen nutrition produces the lowest yields. A 25/75 NH_4/NO_3 (N) ratio produces the highest yields at maturity, though they are not statistically different from yields produced with 100% NO_3 (N) application.

Number of ears per pot, representing fertile tillers per pot increases with increasing proportion of ammonium in fertilizers applied (Table 4.4). Regressions and correlation coefficients were calculated and averages plotted in figure 4.2. At the 0.5gN/pot rate of application of nitrate only, nearly no tillering was observed, while treatments containing increasing proportions of ammonium induced tillering, up to nearly doubling the number of ears ($r=0.80$). The significantly increased number of fertile ears is not reflected in significant yield increases, probably due to the dense stand in the pots.

Treatments receiving 1.0g N/pot show the same trend, but data are quite dispersed around the regression line (correlation coefficient $r=0.59$).

Leaf elongation was measured in some treatments. Results are presented in table 4.5.

Fig. 4.2: Number of ears of wheat related to applied ammonium to nitrate ratio.

TABLE 4.5

Average leaf elongation between December 16 and 19 1984
and average evapotranspiration between 12.18.84 and 1.11.85

mg N appl. per pot as NH_4/NO_3	Average leaf elongation mm/hour (s.d.)		Average evapotranspiration
	day	night	g/24 hours (s.d.)
0/0	0.9 (0.3)	0.6 (0.1)	57 (17)
500/0	0.9 (0.3)	1.3 (0.2)	89 (26)
250/250	1.6 (0.6)	1.3 (0.2)	104 (33)
0/500	1.8 (0.4)	1.1 (0.2)	99 (26)

Leaf elongation during day time in the ammonium only treatment is as low as in the not fertilized treatment. The 50/50 ammonium/nitrate treatment and the only nitrate treatment have higher leaf elongation rates and the differences between the two is not significant. Rate of leaf elongation by day exceeds that by night. For the last treatment that may indicate osmotic stress by day. The control, not fertilized treatment gives lowest rate of growth by night. This may be used, perhaps, as an indication for nitrogen availability.

Data for evapotranspiration (Table 4.5) show that the control treatment had the lowest evapotranspiration rate. The highest was observed in the NH_4/NO_3 : 50/50 treatment, although it did not differ significantly from the evapotranspiration rates in the only nitrate or only ammonium treatments.

Volume of exudates from cut plants and their ammonium and nitrate concentrations in some treatments are given in table 4.6.

TABLE 4.6
Average exudate volumes and ammonium and nitrate
nitrogen concentrations in exudates.
0.5g N/pot applied

mg N appl. per pot as NH ₄ /NO ₃	E x u d a t e s				
	Volume	N concent.		N output	
	μl/plant	μgN/ml		μgN/plant	
		NH ₄	NO ₃	NH ₄	NO ₃
		Sampled 12.27.84			
0/0	7	259	0	1.8	0.0
500/0	16	628	38	10.0	0.6
250/250	29	332	124	9.6	3.6
0/500	17	349	175	5.9	3.0
		Sampled 1.29.85			
500/0	9	1377	8	12.4	0.1
250/250	10	267	33	2.7	0.3
0/500	8	144	122	1.2	1.0

Results in table 4.6 show that at the vegetative stage the treatment receiving NH₄/NO₃ ratio of 50/50 has the highest quantity of N per plant and at both vegetative and reproductive stages the largest exudate volume per plant. The ammonium only treatment at the reproductive stage gives the highest amount of nitrogen. Most nitrogen leaves the root in reduced form, whatever fertilizer treatment is given. Although an increase in nitrate concentration and in most cases in nitrate output with increased applied nitrate proportion is observed.

TABLE 4.7
Reduced nitrogen concentration and accumulation
in dry matter of wheat

mg N appl.	N, %	by			N accum. mg/pot, by				
per pot as	12.27	1.29	4.1		12.27	1.29	4.1		
NH ₄ /NO ₃	straw grain				straw grain total				
0/0	1.5	1.0	0.6	2.0	70	78	43	76	119
500/0	4.6	3.8	1.3	3.6	322	635	242	454	696
375/125	4.9	3.6	1.2	3.3	456	691	211	515	726
250/250	4.0	2.8	1.0	3.0	376	627	187	492	679
125/375	3.9	3.0	0.8	3.3	390	630	149	611	760
0/500	3.6	2.7	0.7	3.0	310	545	131	540	671
1000/0	4.8	6.2	4.3	4.6	283	732	748	414	1162
750/250	4.2	5.2	4.0	4.1	349	796	740	558	1298
500/500	4.1	4.4	2.7	4.0	394	801	548	616	1164
250/750	4.0	4.6	2.1	5.3	376	897	433	864	1297
0/1000	3.4	3.1	1.5	3.8	263	589	291	612	903

Data for reduced nitrogen concentration and accumulation in wheat shoots (Table 4.7) indicate that plants provided with 0.5g total N per pot absorbed most of the added fertilizer N by Jan. 29. Between Jan. 29 and April 1, the shoots of these plants only accumulated an additional 12% (average of all treatments) of the total N accumulated by maturity. In contrast, when plants were provided with 1.0g N per pot, the shoots accumulated 35% of the total N accumulated by maturity between Jan. 29 and April 1, (average of all treatments). The increased accumulation of N by the shoots of plants receiving 1.0g N per pot (regardless of ammonium to nitrate ratio) during the last sampling period did not result in increased

dry weight of straw or grain (Figure 4.1). This observation indicates that this late season accumulation of N did not stimulate photosynthetic activity. Moreover in the 100% ammonium treatment it may have depressed photosynthetic activity. Shoot accumulation of reduced N was always relatively higher when the N fertilizer was applied as a mixed N source (Table 4.7). Reduced N accumulation in the shoots was lowest when NO_3 only was the N fertilizer source.

P percentages and accumulation by wheat are given in table 4.8. P percentages decrease somewhat with time. All pots received equal applications of phosphorus, however, when 1.0g N was applied per pot, the shoots of the 100% nitrate plants accumulated less P at all sampling dates than did the shoots of plants receiving 25% or higher NH_4 . Thus for the 100% ammonium treatments, the average of accumulations by the mature shoots were 72 and 87 mg P per pot for the 0.5g and 1.0g N treatments respectively, whereas for the 100% nitrate treatment the values were 65 and 58 mg P per pot.

TABLE 4.8
Phosphorus concentration and accumulation
in dry matter of wheat

mg N appl.	P _i	%	by		P accum. mg/pot by				
per pot as	12.27	1.29	4.1		12.27	1.29	4.1		
NH ₄ /NO ₃	straw grain				straw grain				total
0/0	0.38	0.33	0.34	0.06	18	26	24	2	26
500/0	0.56	0.40	0.28	0.07	39	67	52	9	61
375/125	0.60	0.39	0.53	0.04	56	75	93	7	100
250/250	0.44	0.26	0.21	0.08	41	58	39	13	52
125/375	0.49	0.36	0.33	0.07	49	76	61	13	74
0/500	0.48	0.37	0.30	0.05	41	75	56	9	65
1000/0	0.62	0.63	0.49	0.09	36	75	85	8	93
750/250	0.51	0.54	0.43	0.07	43	83	79	9	88
500/500	0.54	0.46	0.36	0.09	52	84	73	16	89
250/750	0.43	0.41	0.33	0.07	40	80	68	11	79
0/1000	0.43	0.29	0.25	0.06	34	55	48	10	58

Concentrations of cations in dry matter plant tissues are given in table 4.9.

TABLE 4.9
Cation concentration in dry matter of wheat
percentages

mg N appl. per pot as NH ₄ /NO ₃	Ca	Mg	K	Na	Date	of	sampling	Ca	Mg	K	Na
					12.27					1.29	
0/0	0.40	0.34	2.86	0.07				0.30	0.32	1.80	0.06
500/0	0.42	0.39	2.90	0.06				0.70	0.37	1.54	0.02
375/125	0.46	0.46	3.00	0.07				0.42	0.43	1.74	0.04
250/250	0.58	0.56	3.00	0.12				0.35	0.43	1.39	0.07
125/375	0.55	0.59	2.84	0.10				0.47	0.52	1.51	0.09
0/500	0.75	0.61	3.02	0.13				0.51	0.47	1.44	0.05
1000/0	0.35	0.39	3.25	0.02				0.30	0.36	2.00	0.04
750/250	0.40	0.35	2.35	0.13				0.45	0.43	1.85	0.15
500/500	0.49	0.47	2.46	0.06				0.54	0.49	1.80	0.10
250/750	0.75	0.59	2.29	0.09				0.62	0.56	1.50	0.08
0/1000	0.91	0.72	2.71	0.11				0.67	0.57	1.48	0.10
4.1											
		straw						grain			
0/0	0.42	0.42	1.90	0.07				0.14	0.38	0.50	0.02
500/0	0.46	0.35	1.35	0.03				0.12	0.35	0.45	0.02
375/125	0.52	0.45	1.64	0.14				0.08	0.34	0.58	0.04
250/250	0.58	0.45	1.25	0.12				0.04	0.33	0.50	0.04
125/375								0.07	0.33	0.52	0.04
0/500	0.91	0.48	1.25	0.08				0.22	0.32	0.59	0.00
1000/0	0.54	0.42	1.62	0.10				0.13	0.38	0.54	0.04
750/250	0.65	0.49	1.31	0.06				0.07	0.30	0.51	0.04
500/500	0.84	0.56	1.20	0.10				0.08	0.34	0.57	0.07
250/750	0.97	0.68	1.29	0.10				0.10	0.38	0.53	0.05
0/1000	1.19	0.74	1.40	0.14				0.14	0.37	0.50	0.03

These concentrations were also calculated in mg. eq. and subsequently accumulation (uptake) in mg. eq. was calculated. These results are presented in figure 4.3 and table 4.10.

For both levels of total N, the accumulation of Ca+Mg by the mature shoots decreased in proportion to the increase in NH_4 supplied (Figure 4.3). However, when the plants were sampled at Dec. 27 and Jan. 29, the ratio of NH_4 had to be 50% or higher before marked depressions of Ca+Mg contents of the shoots were evident. Between Jan. 29 and April 1, the shoots of the plants supplied with 1.0g N per pot, nearly doubled their Ca+Mg contents, regardless of the $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratio. When 0.5g N was applied per pot, the average increase in Ca+Mg of the shoots during this same period was about 50% with the NO_3 only treatment exhibiting the smallest increase.

At both levels of N, the shoots of plants applied only with NH_4 tended to accumulate less K (and Na) than shoots with the other forms of N treatment (Table 4.10).

TABLE 4.10

Potassium and sodium accumulation by shoots of wheat plants grown
in soil that contained varying amounts and ratios of NH_4 and $\text{NO}_3\text{-N}$
at three sampling dates

mg N appl. per pot as NH_4/NO_3	K accumulation by			Na accumulation by		
	12/27	1/29	4.1 [*]	12/27	1/29	4.1 [*]
	-----meq./pot-----			-----meq./pot-----		
0/0	3.4	3.6	2.8	0.14	0.20	0.17
500/0	5.2	6.6	7.9	0.18	0.15	0.35
375/125	7.1	8.5	9.7	0.28	0.33	1.33
250/250	7.2	8.0	8.1	0.49	0.68	1.26
125/375	7.3	8.1	-	0.43	0.82	-
0/500	6.6	7.4	8.7	0.49	0.44	0.65
1000/0	4.9	6.0	8.4	0.05	0.21	0.92
750/250	5.0	7.3	8.0	0.11	1.00	0.72
500/500	6.0	8.4	8.5	0.25	0.79	1.09
250/250	5.5	7.5	9.0	0.37	0.68	1.25
0/1000	5.5	7.2	9.0	0.38	0.83	1.39

* straw + grain

Fig. 4.3: Ca+Mg accumulation by wheat plants (above ground parts) in soil containing varying ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$, at three intervals. Treatment ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ are in paranthesis.

TABLE 4.11

Ratio of N uptake to (Ca+Mg) and (Ca+Mg+K+Na) uptake

mg N appl. per pot as NH_4/NO_3	N m.eq./pot: (Ca+Mg)m.eq/pot				N m.eq./pot: (Ca+Mg+K+Na)m.eq/pot			
	by		by		by		by	
	12.27	1.29	4.1		12.27	1.29	4.1	
	straw		grain		straw		grain	
0/0	2.2	1.9	0.7	3.6	0.8	0.8	0.4	2.5
500/0	6.2	4.2	1.8	7.3	2.5	2.6	1.0	5.3
375/125	5.8	4.6	1.4	7.4	2.5	2.5	0.8	4.8
250/250	3.9	3.9	1.1	7.4	1.8	2.2	0.7	4.9
125/375	3.6	3.2		7.7	1.8	1.9		5.2
0/500	2.9	3.0	0.6	5.8	1.5	1.9	0.4	4.1
1000/0	6.9	9.8	5.0	8.8	2.6	4.5	2.8	6.2
750/250	6.1	6.6	3.9	10.3	2.6	3.4	2.6	6.8
500/500	4.6	4.6	2.2	9.0	2.2	2.7	1.6	5.7
250/750	3.3	4.3	1.4	10.5	1.9	2.8	1.1	7.5
0/1000	2.3	2.8	0.9	7.3	1.3	1.8	0.7	5.1

TABLE 4.12
Ratio of N uptake to P uptake

mg N appl. per pot as NH_4/NO_3	N m.eq./pot : P m.eq./pot		by	
	12.27	1/29	4.1	
			straw	grain
-0/0	8	7	4	50
500/0	18	20	10	107
375/125	18	20	5	185
250/250	21	24	10	88
125/375	11	18	5	110
0/500	17	16	5	130
1000/0	17	22	20	100
750/250	18	21	21	133
500/500	16	21	16	88
250/750	21	25	14	155
0/1000	17	28	14	147

A ratio between N uptake by the plants to (Ca+Mg) and to (Ca+Mg+K+Na) taken up, in mg. eq. was calculated. Results are presented in table 4.11. The ratio between N taken up to P taken up is tabulated in table 4.12.

The ratio of N taken up by the plants to (Ca+Mg) taken up as related to nitrogen forms applied is graphically presented in figure 4.4. For the 1.0g N/pot application, nitrogen in the ammonium form induces the highest N/(Ca+Mg) uptake ratio (5-10) with a relative decrease of N uptake with increased applied nitrate nitrogen form (2.5). For the 0.5g N/pot application the same trend may be seen, but with smaller differences.

Figure 4.4

Ratio of N uptake to (Ca+Mg) uptake

Uptake, m.e.
N/(Ca+Mg)

Millet

Ammonium and nitrate nitrogen concentrations in soil were monitored during the growing period of millet (Table 4.13). The data show that here, as in the wheat experiment, the ammonium form was retained in the soil. Depletion of both ammonium and nitrate apparently commenced between June 2 and 9 (vegetative stage) and continued until flowering (June 23) when practically the whole amount of applied nitrogen at the 0.5g N/pot level was exhausted.

TABLE 4.13

Ammonium and Nitrate Nitrogen Recovered from Soil Samples Taken at Intervals from the Pots Used for Millet.

mg N appl. per pot as NH ₄ /NO ₃	June 2		June 9		June 23	
	NH ₄	NO ₃	NH ₄	NO ₃	NH ₄	NO ₃
	-----mg N / pot-----					
0/0	27	9	24	9	18	6
500/0	480	3	303	3	30	6
375/125	471	114	267	105	27	6
250/250	234	213	123	183	24	24
125/375	117	405	54	267	21	18
0/500	270	477	24	321	21	36
ANOVA F	525	135	52	20	15	6
l.s.d.	4	8	8	14	0.8	3
1000/0	1161	3	1122	3	609	3
750/250	897	258	825	180	384	132
500/500	543	525	390	444	234	252
250/750	318	774	264	735	111	297
0/1000	45	1218	45	894	30	489
ANOVA F	665	28	340	329	170	54
l.s.d.	8	42	11	10	12	17

TABLE 4.14

Dry matter yield and evapotranspiration rate of millet shoots.

mg N appl. per pot as NH ₄ /NO ₃	Dry matter yield g/pot, by			Evapotranspiration ml/24h per pot		
	6.2	6.9	6.23	6.7	6.10	6.18
0/0	1.7	3.0	5.7	76(8)	83(3)	105(6)
500/0	2.5	6.6	18.3	171(7)	179(5)	233(17)
375/125	2.6	6.6	18.4	139(18)	159(10)	230(24)
250/250	2.7	6.5	19.5	133(11)	160(20)	235(13)
125/375	2.9	6.8	19.8	169(12)	165(20)	240(8)
0/500	2.6	6.0	18.7	151(10)	148(10)	245(6)
1000/0	2.0	4.3	14.0	118(7)	163(6)	233(13)
750/250	2.2	5.2	15.6	132(6)	158(10)	218(5)
500/500	2.3	5.9	16.6	120(12)	140(8)	215(13)
250/750	2.5	5.6	16.6	120(15)	126(11)	205(10)
0/1000	2.2	5.0	15.7	123(8)	149(3)	180(14)
ANOVA F	19**	35**	208**			
l.s.d.	0.2	0.5	0.6			

The effects of the amounts and ratios of NH₄-N and NO₃ on dry matter accumulation by millet were similar but not identical to those for wheat (Table 4.14 and Figure 4.5). At the final harvest for the 0.5g N per pot

Fig. 4.5: Dry matter and nitrogen accumulation by millet plants (above ground parts) in soil containing varying ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$, at two intervals. Treatment ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ are in paranthesis. Standard error from analysis of variance.

treatment, the shoots of plants grown with 25/75 and 50/50 mixtures accumulated more dry weight than the 0/100, 75/25 and 100/0 shoots. Relative to the 0.5g total N per pot treatment, all 1.0g N treatments decreased dry matter accumulation and these decreases were most marked at the June 23 harvest. Regardless of N level or time of harvest, the 100% NO_3 treatment produced more shoot dry matter than the 100% NH_4 treatment. At the final harvest for the 1.0g N per pot treatment, the rankings with respect to dry matter accumulation were: 25/75 = 50/50 > 0/100 = 75/25 > 100/0.

Evapotranspiration was measured at three dates (table 4.14), but the results do not show a distinct trend, except that it is lower in the treatment that did not receive nitrogen. Dry matter of roots was related to dry weight of the harvested above-ground plant parts. Results are given in table 4.15. The ratio of shoots to roots is about 8 at the earlier growth and about 12 at the flowering stage. Percentage of nitrogen in roots is also given in table 4.15.

TABLE 4.15
Dry weight of roots, percentage of N in roots
and shoot/root ratios of millet

mg N appl.	6.2		6.9		6.23		
per pot as	Dry weight	<u>shoots</u>	Dry weight	<u>shoots</u>	Dry weight	<u>shoots</u>	N in
NH_4/NO_3	roots	roots	roots	roots	roots	roots	roots,
	g/pot		g/pot		g/pot		%
500/0	0.3	8.3	0.8	8.3	1.5	12.1	1.2
0/500	0.3	8.7	0.8	7.5	1.7	11.0	1.2
1000/0	0.2	10.0	0.4	10.7	1.0	14.0	2.4
0/1000	0.3	7.3	0.8	6.3	1.1	14.3	1.5

Nitrogen concentration in millet plants was determined at three dates during the experiment and nitrogen uptake calculated. Results are given in table 4.16 and in figure 4.5.

TABLE 4.16
Nitrogen concentrations in plants (dry matter)
and nitrogen uptake, Millet

mg N appl.	N ,	% ,	by	N uptake, mg/pot, by		
per pot as	6.2	6.9	6.23	6.2	6.9	6.23
NH ₄ /NO ₃						
0/0	1.3	0.8	0.7	22	25	42
500/0	4.3	2.8	1.6	107	187	285
375/125	3.6	3.2	1.5	93	209	269
250/250	3.1	3.6	1.2	84	233	228
125/375	2.8	1.9	1.0	181	133	202
0/500	2.5	1.9	1.0	66	112	184
1000/0	4.9	4.0	2.9	97	172	412
750/250	4.2	3.3	2.6	94	172	479
500/500	3.4	3.6	1.8	78	211	308
250/750	3.3	2.3	1.5	80	128	250
0/1000	2.7	1.8	1.2	59	91	195

At the first harvest, the shoots of plants grown on 0.5g N per pot had accumulated more reduced N than shoots of plants grown at 1.0g N treatment (Figure 4.5). Nitrogen accumulation by the shoots increased in proportion to the increase in level of NH₄ and at 100% NH₄ the shoots had 1.8 fold more reduced N than at 100% NO₃. At the second harvest, with the exception of 100% NH₄ in the 1.0g N per treatment, the accumulation of

reduced N by the shoots also increased proportionally to increased amounts of NH_4 supplied (Figure 4.5).

Percentage of nitrogen from applied N taken up by millet at the flowering stage and found in mineral form in soil was calculated and presented in table 4.17. Nitrogen in roots was not accounted for, but its share is relatively small.

TABLE 4.17

Percentage of nitrogen from total mineral applied, taken up by millet and found in mineral form in soil samples, at the flowering stage

mg N appl. per pot as NH_4/NO_3	Percentage Taken up by plants	N of Residual mineral in soil	applied Unaccounted
500/0	57	7	36
375/125	54	7	39
250/250	46	10	44
125/375	40	8	52
0/500	37	11	52
1000/0	41	61	0
750/250	48	52	0
500/500	31	49	20
250/750	25	41	34
0/1000	19	52	29

Results in table 4.17 show that a greater share of nitrogen is unaccounted in treatments receiving a higher proportion of nitrate in the ammonium-nitrate mixture. This may indicate that some nitrate was lost by denitrification.

Phosphorus concentration in millet plants and P accumulation (uptake) are presented in table 4.18 and figure 4.6.

TABLE 4.18
Phosphorus concentration in millet (dry matter)
and phosphorus accumulation

mg N appl. per pot as NH_4/NO_3	P ,	% ,	by	P uptake, mg/pot, by		
	6.2	6.9	6.23	6.2	6.9	6.23
0/0	0.34	0.33	0.27	5.8	9.9	15.6
500/0	0.41	0.37	0.30	10.1	24.6	54.8
375/125	0.43	0.37	0.29	11.2	24.5	53.4
250/250	0.37	0.36	0.26	10.1	23.5	50.6
125/375	0.41	0.31	0.22	11.8	21.2	43.6
0/500	0.38	0.32	0.22	9.8	19.1	41.3
1000/0	0.44	0.41	0.35	8.8	17.6	49.0
750/250	0.42	0.36	0.33	9.3	18.9	51.3
500/500	0.39	0.38	0.32	8.9	22.4	53.2
250/750	0.41	0.36	0.29	10.2	20.3	48.0
0/1000	0.40	0.30	0.24	8.7	15.1	37.7

At the 0.5g N level, phosphorus accumulation was increased in rough proportion to the amount of NH_4 supplied, especially for the second harvest (Figure 4.6). At the 1.0g N levels, shoots of plants receiving ammonium treatments, accumulated more P than those receiving only nitrate.

Fig. 4.6: P accumulation by millet plants
(above ground parts) in soil containing
varying ratios of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$, at two
intervals. Treatment ratios of
 $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ are in paranthesis.

Cations concentrations and accumulation by millet are presented in tables 4.19 and 4.20.

Magnesium uptake, especially at the flowering stage, increases with increasing proportion of nitrate in the nitrogen mix at both levels of N application and calcium uptake at the higher level of N application. No consistent trend is observed for other cations. The findings for millet are consistent with the results for wheat.

TABLE 4.19

Percentage of cations in dry green matter of millet

mg N appl. per pot as NH_4/NO_3	Ca	Mg % 6.2	K by	Na	Ca	Mg % 6.9	K	Na
0/0	0.9	1.0	6.8	0.09	0.8	0.7	6.2	0.10
500/0	0.6	0.8	4.8	0.05	0.5	0.8	3.2	0.04
375/125	0.4	1.0	5.0	0.06	0.6	1.0	3.1	0.04
250/250	0.7	1.1	5.1	0.12	0.6	1.3	3.3	0.07
125/375	0.9	1.5	4.6	0.07	0.5	1.7	3.0	0.07
0/500	0.9	2.1	4.7	0.09	0.9	2.3	3.0	0.10
1000/0	0.6	0.7	4.3	0.07	0.5	0.7	3.3	0.05
750/250	0.8	0.9	4.3	0.10	0.5	0.9	3.3	0.11
500/500	0.8	1.0	4.6	0.13	0.7	1.1	3.0	0.05
250/750	0.6	1.2	4.3	0.07	0.7	1.5	2.9	0.08
0/1000	1.1	2.1	4.8	0.09	0.8	2.3	2.7	0.08
		% by 6.23						
0/0	0.5	0.5	4.2	0.09				
500/0	0.4	0.8	1.6	0.08				
375/125	0.5	1.0	1.9	0.05				
250/250	0.4	1.3	1.7	0.05				
125/375	0.4	1.4	1.7	0.07				
0/500	0.4	1.7	1.8	0.10				
1000/0	0.3	0.6	2.0	0.04				
750/250	0.5	1.0	1.9	0.04				
500/500	0.6	1.2	1.7	0.10				
250/750	0.6	1.4	1.7	0.08				
0/1000	0.8	1.8	1.5	0.08				

TABLE 4.20
Cations uptake by millet

mg N appl. per pot as NH ₄ /NO ₃	C a t i o n s u p t a k e m.e / p o t							
	Ca	Mg	K	Na	Ca	Mg	K	Na
	----m.eq. by 6.2----				-----m.eq. by 6.3----			
0/0	0.9	1.4	2.9	0.07	1.2	0.6	4.8	0.12
500/0	0.7	1.7	3.0	0.05	2.0	4.7	5.3	0.13
375/125	0.5	2.1	3.4	0.08	2.0	4.6	5.3	0.13
250/250	1.1	2.5	3.6	0.14	2.0	4.6	5.2	0.20
125/375	1.4	3.7	3.4	0.09	2.1	4.1	5.5	0.21
0/500	1.3	4.4	3.1	0.10	3.0	3.0	4.8	0.24
1000/0	0.6	1.2	2.2	0.06	1.3	2.2	3.4	0.09
750/250	0.9	1.6	2.4	0.09	1.6	2.6	4.2	0.26
500/500	0.9	1.8	2.7	0.14	2.4	3.5	4.7	0.12
250/750	0.7	2.5	2.7	0.07	2.3	3.4	4.0	0.17
0/1000	1.3	3.7	2.6	0.09	2.0	2.0	3.5	0.15
	m.eq. by 6.23							
0/0	1.7	2.3	6.3	0.2				
500/0	3.7	12.8	7.3	0.5				
375/125	5.5	14.7	9.2	0.4				
250/250	3.9	21.4	7.8	0.4				
125/375	4.0	23.8	7.9	0.6				
0/500	3.8	26.3	9.4	0.8				
1000/0	2.8	7.0	7.0	0.3				
750/250	4.7	12.4	7.8	0.3				
500/500	5.0	15.0	6.7	0.7				
250/750	5.0	19.9	6.6	0.5				
0/1000	6.3	22.0	6.3	0.5				

Conclusions

Addition of a nitrification inhibitor (DCD) to various mixtures of ammonium and nitrate fertilizers maintains the desired and predetermined ratio of NH_4 to NO_3 in the soil during incubation and plant growth experiments.

A significant response of wheat and millet dry matter accumulation to forms of nitrogen maintained in soil is observed. An ammonium-only supply produces lower yields than combined supplies of ammonium and nitrate nitrogen. Supplying only nitrate nitrogen produces a somewhat (statistically insignificant) lower yield than the mixtures. Supplying nitrate alone results in the lowest accumulation of reduced nitrogen compounds in wheat and millet plants, whereas mixed ammonium and nitrate supplies result in higher plant accumulation of reduced N compounds (protein). These results may be partially explained by the fact that ammonium-only nutrition has a toxic effect on plants, whereas nitrate-only nutrition may be relatively more energy consuming (Hageman, 1984). Controlling the ratio of ammonium-to-nitrate in soil leads to differences of up to 300 percent in total accumulation of magnesium and calcium in plant tops.

In conclusion, our results indicate that coapplication of a nitrification inhibitor with nitrogen fertilizers may be used to regulate the ammonium-to-nitrate ratio in soil for prolonged periods and thus to regulate plant growth and accumulation of reduced nitrogen compounds.

Summary

Incubation and greenhouse experiments were conducted with a loamy sand soil having a pH of 6.9 and a 0.6% organic C content. Preliminary soil incubation studies established that the nitrification inhibitor, Dicyandiamide (DCD), could maintain the ratio of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ at predetermined levels. When one part DCD was mixed with 10 parts of the ammonium fertilizer prior to incorporation with the soil, nitrification was inhibited for at least six weeks. In a greenhouse experiment wheat was grown to maturity and millet to the flowering stage in pots each containing 3 kg of the soil. N fertilizers were applied at two levels (0.5 and 1.0 gN/pot) and at five $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratios (100/0, 75/25, 50/50, 25/75 and 0/100). The ammonium fertilizers were treated with DCD as in the incubation studies. Soil analyses during the plant growth period indicated that ammonium oxidation in soil was effectively inhibited. Plants of both species exposed to ammonium only with DCD produced lower yields than those exposed to a mixture of nitrate and ammonium nitrogen with DCD. Plants supplied with nitrate-only gave somewhat lower yields than the mixtures. The nitrate-only treatments resulted in the lowest accumulation of reduced nitrogen compounds in shoots of both species. Magnesium uptake by millet and calcium and magnesium uptake by wheat were reduced as the proportion of ammonium in soil was increased.

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Chapter 5. The Effect of Total Amount, Form and Ratio of Forms on Growth and Grain Yield of Wheat (cv. Inbar) Grown on Two Soil Types.

5. Wheat greenhouse experiment 1985/6

Introduction

In a greenhouse experiment with wheat and millet, (Chapter 4 of this report) plants receiving a mixed ammonium and nitrate nitrogen nutrition, accumulated more reduced nitrogen and more divalent cations than plants receiving mainly nitrate nitrogen. Plants grown on ammonium-nitrate mixtures showed a tendency for producing a slightly higher growth than those on nitrate only. Ammonium only nutrition depressed growth. In view of these findings another greenhouse experiment was laid out. In this experiment it was aimed to obtain yield curves and therefore additional N levels were applied. On the other hand the ammonium-only and 75/25 ammonium to nitrate ratio treatments were not included. Preplanting fertilizer applications were on two soils and on one soil nitrogen fertilizers were applied in portions with irrigation water.

Methods and Materials

Two soils were used in a greenhouse experiment in 3 liter pots. Some characteristics of experimental soils are given in table 5.1.

TABLE 5.1

Some characteristics of soils used in
greenhouse experiment

Location of sampling	Sharon (Ilanot)	Negev (Beeri)	
Texture	Loamy sand	Silt loam	
Electrical cond. mmhos/cm	0.3	0.4	
pH	6.9	8.1	
CaCO ₃ , %	2.1	12.0	
C organic, %	0.6	0.3	
N, total, %	0.04	0.03	
Field capacity moisture, %	15.7	22.0	
Israel soil class.*	Hamra	Loessial arid brown	
U.S. soil taxonomy*	Typic Rhodoxeralf	Typic Xerofluvent	
*	Dan and Koyumdjiski, 1979 (See references Chapter 4)		

Ammonium was applied as ammonium sulphate (AS) and nitrate as calcium nitrate (CAN). Nitrification inhibitor was dicyandiamide (DCD) applied in a 1:10 ratio to ammonium sulphate. Rates of N application and ratios of ammonium to nitrate are listed in table 5.2. In addition to the listed treatments DCD was added to CAN at the 1.0g N/pot level and yields and plant composition were compared to the parallel treatment but without DCD application. Addition of DCD did not cause yield or nutrient uptake depressions. These results are not reported here. All pots received 1g P/pot as superphosphate. Water was added frequently to field capacity by weight. Wheat (*Triticum durum*) cv. Inbar was sown mid December 1985 and thinned to 15 plants per pot. It flowered in the second half of February 1986 and ripened mid April 1986. In the Beeri soil the nitrogen fertilizers were mixed with the whole soil volume prior to planting. In the Ilanot soil the same was done in part of the treatments, while in other treatments the nitrogen fertilizers without DCD were applied in 15 portions in irrigation water. The experiment with preplant fertilizer application started with 6 replicates per treatment. 3 of them were cut at flowering stage and 3 left to the ripening stage. Treatments with portional fertilizer application were in 3 replicates and cut when ripe. Soil samples were taken at times of cutting the plants.

Soil and plant analytical procedure were as described in chapter 1 and

4.

Results

Yields of wheat and tillering in Beeri soil are presented in table 5.2. Free hand drawn yield curves relating yield obtained with various ammonium to nitrate ratios at flowering and at ripeness are given in figure 5.1.

TABLE 5.2

Dry matter yield and tillering in Beeri soil

N ratio NH_4/NO_3 appl.	N appl. gM/pot	Yield, g/pot at			No. per pot of	
		Flowering	R i p e n e s s		tillers	heads
			Straw	Grain		
		5.3	7.1	1.5	19	15
	0.25	13.1	16.3	11.4	16	15
	0.50	16.0	18.9	16.8	15	15
-/100	0.75	16.0	21.0	15.2	15	15
	1.00	15.9	21.0	15.6	17	16
	0.25	12.8	17.0	10.5	19	15
	0.50	15.5	19.8	14.6	16	15
25/75	0.75	17.4	23.4	13.7	18	15
	1.00	17.6	23.1	14.8	17	16
	0.25	12.1	17.0	9.0	19	15
	0.50	15.4	20.5	13.0	17	15
50/50	0.75	16.4	23.7	13.6	18	17
	1.00	16.3	22.9	11.5	18	17
ANOVA	F	136***	78***	49***	N.S.	N.S.
	l.s.d.	0.8	1.0	1.6		

*** significant at 0.005 level.

5.4
Figure 5.1

Yield of wheat, dry matter Beerli soil

Results show response of wheat to nitrogen fertilization. The 25/75 ammonium to nitrate mixture produced significantly more vegetative growth than nitrate-only at flowering and at maturity, while the 50/50 mixture was more effective only at maturity. Grain yields at the 1.0g N/pot level were lower in the 50/50 mixture than in others. The yield data indicate that the 25/75 ammonium to nitrate mixture was a more effective source of nitrogen than nitrate-only for vegetative growth and that it was similar to nitrate for grain production.

Levels of application and forms applied did not influence significantly the number of tillers and number of heads produced. This may be due to the dense stand of plants in pots.

Results for treatments with fertilizers applied before planting on Ilanot soil are similar to those obtained on Beeri soil and they are presented in table 5.3 and figure 5.2. Again 25/75 ammonium to nitrate mixture was a more effective source of nitrogen than nitrate-only for vegetative growth but not for grain production.

Experiments with fertilizer application before planting and with portional application during the growing period in Ilanot soil were performed at the same time and place and therefore one statistical analysis was done for both. Results for treatments receiving nitrogen fertilizers in portions with irrigation water in Ilanot soil are given in table 5.4 and figure 5.3. At the 1.0g N/pot level of application the 50/50 mixture produced a slightly higher statistical significant vegetative growth than the other two treatments. At the same level of N application the 25/75 mixture produced slightly more grain than found in other treatments.

TABLE 5.3
Dry matter yield and tillering in Ilanot soil
Fertilizers applied before planting.

N ratio NH_4/NO_3 appl.	N appl. g/pot	Yield, g/pot at			No. per pot of	
		Flowering	R i p e n e s s		tillers	heads
			Straw	Grain		
	-	4.6	5.6	2.1	19	15
	0.25	14.5	15.3	12.6	15	15
	0.50	16.4	18.7	16.4	17	15
-/100	0.75	16.7	20.5	17.6	17	16
	1.00	16.4	18.2	15.4	16	16
	0.25	15.1	15.9	12.4	15	15
	0.50	18.1	19.1	16.2	16	16
25/75	0.75	18.4	20.6	15.9	19	16
	1.00	17.9	21.1	15.1	20	17
	0.25	14.4	16.3	12.1	16	15
	0.50	16.5	17.8	15.7	17	15
50/50	0.75	17.1	19.0	16.2	17	16
	1.00	16.2	20.7	11.8	24	17
ANOVA	F	194***	46***	31***	N.S.	N.S.
	l.s.d.	0.7	1.4	1.5		

*** significant at 0.005 level.

Figure 5.2

Yield of wheat, dry matter. Ilanot soil
Fertilizer applied before planting

TABLE 5.4

Dry matter yield and tillering at ripeness in Ilanot soil
Fertilizers applied portionally in irrigation water

N ratio NH_4/NO_3	N appl. g/pot	Yield, g/pot		No. per pot of	
		Straw	Grain	tillers	heads
-/100	0.25	15.9	13.3	15	15
	0.50	19.2	14.8	17	16
	0.75	20.9	15.9	17	17
	1.00	20.1	14.8	16	15
25/75	0.25	16.0	13.8	15	15
	0.50	19.0	16.5	16	15
	0.75	20.0	16.1	17	15
	1.00	20.3	16.2	17	16
50/50	0.25	15.6	12.4	15	15
	0.50	18.0	16.8	15	15
	0.75	20.4	16.6	17	17
	1.00	22.0	13.5	20	18
ANOVA	F	46***	31***	N.S.	N.S.
	l.s.d.	1.4	1.5		

*** significant at 0.005 level

Ammonium and nitrate nitrogen concentrations in soils receiving preplanting fertilizers were measured at the flowering stage and at maturity. It was assumed from previous experience that the nitrification inhibitors are effective shortly after application and thus the applied ratio of ammonium to nitrate is maintained during earlier stages of growth. Results (tables 5.5 and 5.6) show that at the flowering stage at the higher levels of application the predetermined ammonium to nitrate nitrogen ratio was approximately maintained, although a relatively large portion of nitrogen was already exhausted. At the maturity stage most of the nitrogen was exhausted, but still at the highest level of nitrogen application some of the applied ammonium was maintained. Statistical

Figure 5.3
Yield of wheat, dry matter, Ilanot soil
Fertilizer applied portionally in irrigation water

analysis of results in Ilanot soil was done together for both parts of the experiment, preplant and portional fertilizer application.

In treatments in Ilanot soil receiving portional fertilization in irrigation water, the soils were sampled only at harvest time (Table 5.7). Most of the mineral nitrogen was exhausted at that time, even more so than in soils that received fertilizers before planting.

TABLE 5.5
Ammonium and nitrate nitrogen concentrations in Beeri soil

N ratio NH_4/NO_3 appl.	N appl. g/pot	mg N per kg soil at			
		Flowering		Ripeness	
		NH_4	NO_3	NH_4	NO_3
-	-	5	3	6	1
	0.25	7	4	9	3
	0.50	6	4	8	3
-/100	0.75	7	48	8	3
	1.00	9	159	8	39
	0.25	6	4	8	2
	0.50	10	3	10	2
25/75	0.75	12	30	10	3
	1.00	16	111	11	21
	0.25	7	4	8	2
	0.50	11	4	10	2
50/50	0.75	14	25	7	2
	1.00	40	68	10	12
ANOVA	F	32 ^{***}	356 ^{***}	3.3 ^{**}	37 ^{***}
	1.s.d.	5	9	2	7

*** significant at 0.005 level

** significant at 0.01 level

TABLE 5.6

Ammonium and nitrate nitrogen concentrations in Ilanot soil
Fertilizers applied before planting

N ratio	N appl.	mg	N	kg	soil,	at
NH ₄ /NO ₃		Flowering		Ripeness		
appl.	g/pot	NH ₄	NO ₃	NH ₄	NO ₃	
-	-	8	4	9	3	
	0.25	6	3	8	4	
	0.50	5	7	10	3	
-/100	0.75	6	97	8	6	
	1.00	10	178	10	79	
	0.25	5	2	9	6	
	0.50	6	2	12	4	
25/75	0.75	13	69	12	10	
	1.00	30	155	18	47	
	0.25	6	3	12	5	
	0.50	7	3	12	3	
50/50	0.75	18	28	12	3	
	1.00	67	68	19	13	
ANOVA	F	66***	186***	5***	97***	
	l.s.d.	6	15	4	6	

*** significant at 0.005 level.

TABLE 5.7

Ammonium and nitrate nitrogen concentrations in Ilanot soil
at ripeness of wheat
Fertilizer applied portionally in irrigation water

N ratio	N appl.	mg N per kg soil	
NH_4/NO_3			
appl.	g/pot	NH_4	NO_3
	0.25	11	5
	0.50	11	4
-/100	0.75	9	3
	1.00	13	41
	0.25	14	5
	0.50	10	5
25/75	0.75	10	4
	1.00	8	28
	0.25	10	5
	0.50	8	4
50/50	0.75	8	4
	1.00	8	11
ANOVA	F	5***	97***
	l.s.d.	4	6

*** significant at 0.005 level

Reduced nitrogen concentrations were determined in wheat plants cut at flowering and in straw and grain of mature plants. In Beeri soil and in treatments receiving 0.75 and 1.0gN/pot, the nitrogen concentration increases in general with increasing proportion of ammonium in the nitrogen mixture. (Table 5.8 and figure 5.4). The 50/50 ammonium to nitrate ratio generates statistically significant higher nitrogen concentrations in plants than found in the only nitrate treatment. Differences in nitrogen concentration between treatments receiving 0.25 and 0.50 gN/pot are small, probably due to nitrogen deficiency stress of plants growing in these rates of application. Soil analysis data (table 5.5) indicate mineral nitrogen exhaustion at flowering in these treatments. Calculated reduced nitrogen

accumulation by the vegetative parts of plants increases also with increasing ratio of ammonium in the nitrogen mixture. However, reduced nitrogen accumulation by grain is higher in the 25/75 mixture than in the two others. Accumulation of reduced N at maturity by both straw and grain at the 1.0g N/pot application is significantly lower in the only nitrate treatment than in the ammonium and nitrate mixed treatments.

TABLE 5.8
Reduced N concentration in wheat and N accumulation (uptake)
Beerli soil

N ratio NH_4/NO_3 appl.	N appl. g/pot	N in plants, % by			N uptake, g/pot, by		
		Flowering	R i p e n e s s		Flowering	R i p e n e s s	
			Straw	Grain		Straw	Grain
-	-	0.70	0.57	1.22	0.04	0.05	0.02
	0.25	1.25	0.25	1.01	0.16	0.04	0.12
	0.50	2.25	0.33	1.57	0.36	0.06	0.26
-/100	0.75	2.83	0.69	1.77	0.45	0.14	0.27
	1.00	2.77	1.01	1.98	0.44	0.21	0.31
	0.25	1.33	0.43	1.09	0.17	0.07	0.12
	0.50	2.33	0.60	1.50	0.36	0.12	0.22
25/75	0.75	2.80	0.99	1.76	0.49	0.23	0.24
	1.00	2.92	1.48	2.36	0.52	0.35	0.35
	0.25	1.30	0.51	1.15	0.16	0.09	0.10
	0.50	2.58	0.83	1.63	0.40	0.17	0.21
50/50	0.75	3.27	1.27	2.00	0.54	0.34	0.27
	1.00	3.48	1.50	2.43	0.57	0.34	0.28
ANOVA	F	110***	24***	57***	149***	27***	50***
	l.s.d.	0.25	0.26	0.18	0.04	0.07	0.04

*** significant at 0.005 level

In the parallel experiment in Ilanot soil, where fertilizers were applied before planting, very similar results were obtained. At the flowering stage and at the 1.0g N/pot application level (table 5.9) reduced

5.14

Figure 5.4

N uptake by wheat plants
Beerli soil

nitrogen concentration in plants increases with the ammonium proportion in the applied nitrogen mixture and the 50/50 mixture generated significantly higher nitrogen concentrations in plants than the other two treatments. The same is observed for reduced nitrogen accumulation (table 5.9 and figure 5.5). Concentration of reduced nitrogen and its accumulation in straw at maturity shows the same results (table 5.9). Concentration of reduced nitrogen in grain at the 1.0g N/pot level of application increases also with increasing proportion of ammonium in the nitrogen mixture, but this is not reflected in reduced nitrogen accumulation in the grain (table 5.9). However, the calculated reduced nitrogen accumulation by the whole plant (grain and straw) at maturity shows that the plants receiving only nitrate accumulated significantly less than those supplied with ammonium and nitrogen mixtures (figure 5.5).

TABLE 5.9
N concentration in wheat and N accumulation (uptake)
Ilanot soil
Fertilizers applied before planting

N ratio NH_4/NO_3	N appl. g/pot	N in plants, % by			N uptake, g/pot, by		
		Flowering	R i p e n e s s		Flowering	R i p e n e s s	
appl.			Straw	Grain		Straw	Grain
-	-	0.34	0.47	2.43	0.05	0.03	0.05
	0.25	1.32	0.24	1.24	0.19	0.04	0.15
	0.50	2.33	0.39	1.66	0.40	0.07	0.27
-/100	0.75	2.93	0.61	1.83	0.49	0.12	0.32
	1.00	2.62	0.62	2.33	0.43	0.11	0.38
	0.25	1.27	0.28	1.36	0.19	0.04	0.17
	0.50	2.09	0.44	1.44	0.38	0.08	0.23
25/75	0.75	2.69	0.68	2.07	0.49	0.14	0.33
	1.00	2.76	0.89	2.52	0.49	0.17	0.38
	0.25	1.36	0.32	1.25	0.20	0.05	0.15
	0.50	2.20	0.53	1.57	0.36	0.10	0.25
50/50	0.75	2.98	0.82	2.19	0.51	0.16	0.35
	1.00	3.37	1.35	2.77	0.55	0.28	0.33
ANOVA	F	57***	28***	17***	95***	30***	20***
	l.s.d.	0.35	0.15	0.46	0.05	0.03	0.08

*** significant at 0.005 level

Figure 5.5

N uptake by wheat plants

Ilanot soil

In the series of treatments in Ilanot soil with portional nitrogen fertilizer application, reduced nitrogen concentrations and accumulation in grain increased, with some exceptions, at all four levels of application with increasing proportion of ammonium in the nitrogen mixture (table 5.10). N concentration and accumulation in straw is higher in the 50/50 mixture than in other treatments at the 1.0gN/pot level of application (table 5.10). Reduced nitrogen accumulation by the whole plant (straw and grain) was significantly lower at the 0.75 and 1.0gN/pot levels of application in the nitrate-only treatment than in the ammonium and nitrate mixtures. These results indicate that gradual application of ammonium and nitrate mixtures without a nitrification inhibitor, in a soil like one used, may be an effective means for maintaining both ions in the soil solution with growing plants. It should be noted that in another work, the same loamy sand was found to have a slow rate of nitrification.

TABLE 5.10

N concentration in wheat and N accumulation (uptake) at maturity
 Ilanot soil
 Fertilizers applied portionally in irrigation water

N ratio NH_4/NO_3 appl.	N appl. g/pot	N in plants, %		N uptake, g/pot	
		Straw	Grain	Straw	Grain
-/100	0.25	0.26	1.01	0.04	0.13
	0.50	0.54	1.39	0.10	0.21
	0.75	0.67	1.78	0.14	0.28
	1.00	0.87	2.27	0.17	0.24
25/75	0.25	0.26	0.97	0.04	0.13
	0.50	0.43	1.63	0.08	0.27
	0.75	0.69	3.30	0.14	0.53
	1.00	0.82	2.50	0.17	0.40
50/50	0.25	0.28	1.70	0.04	0.21
	0.50	0.41	2.40	0.07	0.40
	0.75	0.49	2.90	0.10	0.48
	1.00	1.00	3.43	0.21	0.47
ANOVA	F	28***	17***	30***	20***
	l.s.d.	0.15	0.46	0.03	0.08

*** significant at 0.005 level

P concentrations in wheat were measured and P accumulation by the plants calculated. Results for the Beeri soil are given in table 5.11 and free hand curves relating P concentration to N applied in figure 5.6. At the flowering stage, level or form of N applied did not influence appreciably P concentration in plants nor P uptake. At the mature stage P concentration in straw was significantly lower in treatments receiving only nitrate than in those receiving ammonium and nitrate mixtures. In the grain this is true only for the 1.0gN/pot application. Differences between treatments in P accumulation are small, but in the straw a lower P accumulation is found in the only nitrate treatments than in the ammonium and nitrate mixtures.

TABLE 5.11
P concentration in wheat and P accumulation (uptake)
Beerli soil

N ratio NH_4/NO_3	N appl. g/pot	P in plants, % by			P uptake, g/pot by		
		Flowering	R i p e n e s s		Flowering	R i p e n e s s	
			Straw	Grain		Straw	Grain
-	-	0.40	0.11	0.36	0.02	0.01	0.01
	0.25	0.41	0.16	0.29	0.05	0.03	0.03
	0.50	0.43	0.12	0.33	0.07	0.02	0.05
-/100	0.75	0.45	0.14	0.33	0.07	0.03	0.05
	1.00	0.40	0.14	0.33	0.06	0.03	0.05
	0.25	0.47	0.21	0.31	0.06	0.04	0.03
	0.50	0.45	0.18	0.36	0.07	0.04	0.05
25/75	0.75	0.40	0.19	0.32	0.07	0.04	0.04
	1.00	0.45	0.17	0.42	0.08	0.04	0.06
	0.25	0.43	0.23	0.34	0.05	0.04	0.03
	0.50	0.44	0.20	0.39	0.07	0.04	0.05
50/50	0.75	0.40	0.20	0.37	0.07	0.05	0.05
	1.00	0.42	0.17	0.38	0.07	0.04	0.04
ANOVA	F	N.S.	10***	3.6***	22***	11***	22***
l.s.d.		0.06	0.03	0.05	0.01	0.01	0.01

*** significant at 0.005 level

Data for P concentration in wheat and accumulation for Ilanot soil receiving nitrogen fertilizers before planting are given in table 5.12. P concentration in the ripe vegetative parts of wheat at the 0.75 and 1.0g N/pot application were significantly lower when nitrate-only was applied than when ammonium and nitrate mixtures were applied. Other data do not show meaningful differences.

5.20

Figure 5.6

P concentration in mature wheat (dry matter)
Beeri soil.

TABLE 5.12

P concentration in wheat and P accumulation (uptake)

Ilanot soil

Fertilizers applied before planting

N ratio NH_4/NO_3	N appl. g/pot	P in plants, % by			P uptake g/pot by		
		Flowering	R i p e n e s s		Flowering	R i p e n e s s	
appl.	g/pot		Straw	Grain		Straw	Grain
-	-	1.11	0.50	0.48	0.05	0.03	0.01
	0.25	0.75	0.42	0.45	0.11	0.06	0.06
	0.50	0.83	0.44	0.47	0.14	0.08	0.08
-/100	0.75	0.79	0.42	0.50	0.13	0.09	0.09
	1.00	0.73	0.43	0.57	0.12	0.08	0.09
	0.25	0.73	0.51	0.39	0.11	0.08	0.05
	0.50	0.81	0.50	0.46	0.15	0.09	0.07
25/75	0.75	0.78	0.53	0.59	0.14	0.11	0.09
	1.00	0.72	0.59	0.60	0.15	0.12	0.09
	0.25	0.84	0.49	0.40	0.12	0.08	0.05
	0.50	0.67	0.57	0.43	0.14	0.10	0.07
50/50	0.75	0.90	0.61	0.50	0.15	0.12	0.08
	1.00	0.96	0.65	0.54	0.16	0.13	0.06
ANOVA	F	3.1***	6.9***	5.0***	28***	12***	15***
	l.s.d.	0.20	0.10	0.09	0.02	0.02	0.01

*** significant at 0.005 level

P percentages in vegetative parts of plants in Ilanot soil that received the ammonium and nitrate mixture at the 1.0g N/pot level in portions during the growing period are higher than those receiving nitrate-only (table 5.13). In grain the 50/50 mixture at the same level of application induced higher P concentrations than the other two nitrogen treatments.

Cations concentration (calcium, magnesium and potassium) in wheat plants was measured and calculated in m.eq. per kg dry plant material.

Cations accumulation was calculated in m.eq. per pot. Sodium was not determined, because of its very low concentration found in previous experiments.

TABLE 5.13
P concentration in wheat and P accumulation (uptake) at maturity
Ilanot soil
Fertilizers applied portionally in irrigation water

N ratio NH_4/NO_3	N appl. g/pot	P in plants, %		P uptake, g/pot	
		Straw	Grain	Straw	Grain
-/100	0.25	0.33	0.39	0.05	0.05
	0.50	0.34	0.44	0.06	0.06
	0.75	0.43	0.45	0.09	0.07
	1.00	0.34	0.44	0.07	0.07
25/75	0.25	0.32	0.35	0.05	0.05
	0.50	0.37	0.45	0.07	0.07
	0.75	0.38	0.53	0.08	0.09
	1.00	0.47	0.46	0.10	0.08
50/50	0.25	0.32	0.47	0.05	0.06
	0.50	0.33	0.53	0.06	0.09
	0.75	0.38	0.56	0.08	0.09
	1.00	0.45	0.61	0.10	0.08
ANOVA	F	6.9***	5.0***	12***	15***
	l.s.d.	0.10	0.09	0.02	0.01

*** significant at 0.005 level

Calcium and magnesium concentrations and accumulation by wheat plants at flowering stage in Beeri soil increase with increasing levels of nitrogen application, except at the highest level of nitrate only application (table 5.14). Potassium concentration in plants varies somewhat between treatments. Concentration and accumulation of all three cations is not related to nitrogen forms applied. Cations concentrations

TABLE 5.14

Cations concentration and accumulation in wheat plants
at flowering stage. Beeri soil.

N ratio NH_4/NO_3 appl.	N appl. g/pot	Ca		Mg		K		Cations
		m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	uptake m.eq/pot
-	-	114	0.6	168	0.9	434	2.3	3.8
-/100	0.25	165	2.1	208	2.7	402	5.3	10.1
	0.50	281	4.6	269	4.3	405	6.5	15.3
	0.75	352	5.6	293	4.7	374	5.9	16.2
	1.00	273	4.4	300	4.8	347	5.5	14.8
25/75	0.25	229	2.9	213	2.7	351	5.4	11.3
	0.50	245	3.8	282	4.4	397	6.2	14.3
	0.75	291	5.0	324	5.6	376	6.5	17.2
	1.00	311	5.5	301	5.3	332	5.9	16.6
50/50	0.25	179	2.2	212	2.7	430	5.3	10.1
	0.50	212	3.2	266	4.2	414	6.4	13.8
	0.75	236	3.8	304	5.0	404	6.6	15.4
	1.00	250	4.1	298	4.9	393	6.4	15.4
ANOVA	F		6.4***		40***		17***	20***
	l.s.d.		1.7		0.6		0.8	2.4

*** significant at 0.005 level.

and uptake in straw of mature plants (table 5.15) follow approximately the same trend as above, although no clear conclusions can be drawn.

Calcium concentration in wheat grain grown on Beeri soil (table 5.16) increases somewhat with increasing levels of nitrogen application, but nitrogen sources do not induce appreciable differences in calcium concentration. Magnesium concentration in grain varies very little between treatments. Potassium concentrations at the 1.0g N/pot level of nitrogen application decrease with increased ammonium ratio in the ammonium-nitrate mixture applied.

Table 5.17 gives cation concentrations and uptake in wheat plants at flowering in Ilanot soil. Calcium concentration increases in most treatments with increasing levels of nitrogen application. The same tendency is seen for magnesium, while there is no clear trend for potassium concentrations.

Table 5.18 shows similar trends in straw of mature plants. The results for grain (table 5.19) do not show clear trends.

TABLE 5.15

Cations concentration and accumulation in straw of
mature wheat plants. Beerli soil.

N ratio NH_4/NO_3 appl.	N appl. g/pot	Ca		Mg		K		Cation
		m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	uptake m.eq/pot
-	-	161	1.2	185	1.3	305	2.2	4.7
-/100	0.25	198	3.2	213	3.2	275	4.5	10.9
	0.50	285	5.4	233	4.0	249	4.7	14.1
	0.75	313	6.5	292	4.9	291	5.5	17.5
	1.00	457	8.7	212	5.9	355	5.4	22.9
25/75	0.25	200	3.4	251	3.6	264	4.5	11.5
	0.50	263	5.2	292	5.0	255	5.5	15.7
	0.75	338	7.8	313	6.9	256	6.1	25.5
	1.00	427	9.9	220	7.3	328	7.6	27.4
50/50	0.25	163	2.8	253	3.7	314	5.3	14.7
	0.50	253	5.2	302	5.2	301	6.2	16.6
	0.75	312	7.4	228	7.2	290	6.9	21.4
	1.00	342	7.8	270	5.2	263	6.0	19.1
ANOVA F			51 ***		38 ***		10 ***	14 ***
	l.s.d.		1.1		0.8		1.2	4.9

*** significant at 0.005 level.

TABLE 5.16

Cations concentration and accumulation in grain of
mature wheat plants. Beerli soil.

N ratio NH ₄ /NO ₃ appl.	N appl. g/pot	Ca		Mg		K		Cation
		m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	uptake m.eq/pot
-	-	17	0.0	124	0.2	111	0.2	0.4
	0.25	17	0.2	96	1.1	103	1.2	2.5
	0.50	21	0.3	100	1.7	127	2.1	4.2
-/100	0.75	21	0.3	101	1.5	119	1.8	3.6
	1.00	26	0.4	104	1.8	176	2.8	5.1
	0.25	20	0.2	104	1.1	109	1.2	2.5
	0.50	23	0.3	106	1.5	129	1.9	3.8
25/75	0.75	25	0.3	103	1.4	122	1.7	3.1
	1.00	25	0.4	143	2.1	140	2.1	4.6
	0.25	20	0.2	117	1.0	120	1.1	2.3
	0.50	23	0.3	121	1.6	131	1.7	3.6
50/50	0.75	23	0.3	119	1.6	124	1.7	3.6
	1.00	24	0.3	120	1.4	127	1.4	3.1
ANOVA	F		8.8***		22***		39***	27***
	l.s.d.		0.1		0.3		0.3	0.7

*** significant at 0.005 level.

TABLE 5.17

Cations concentration and accumulation in wheat plants
at flowering stage. Ilanot soil.

N ratio NH_4/NO_3 appl.	N appl. g/pot	Ca		Mg		K		Cation
		m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	uptake m.eq/pot
	-			126	0.6	747	3.4	4.1
-/100	0.25	244	3.6	204	3.0	463	6.7	13.3
	0.50	391	6.4	264	4.3	453	7.4	18.2
	0.75	420	5.7	271	4.5	409	6.8	17.0
	1.00	424	7.0	266	4.4	372	6.1	17.5
25/75	0.25	180	2.7	186	2.8	443	6.7	12.2
	0.50	350	6.3	243	4.4	385	7.0	17.7
	0.75	320	5.9	276	5.1	454	8.3	19.3
	1.00	450	8.0	263	4.7	417	7.4	20.2
50/50	0.25	238	3.5	197	2.9	520	7.5	13.9
	0.50	300	4.9	229	3.8	461	7.6	16.4
	0.75	335	5.7	271	4.6	468	8.0	18.4
	1.00	263	4.3	258	4.2	478	7.7	16.2
ANOVA	F		20***		59***		10***	22***
	l.s.d.		1.6		0.4		1.1	2.7

*** significant at 0.005 level.

5.20
TABLE 5.18

Cations concentration and accumulation in straw of mature
wheat plants. Ilanot soil.

N ratio NH ₄ /NO ₃ appl.	N appl g/pot	Ca		Mg		K		Cation uptake m.eq/pot
		m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	m.eq/kg	uptake m.eq/pot	
	-	110	0.6	154	0.9	498	2.8	4.3
-----Preplanting fertilizer application-----								
-/100	0.25	166	2.5	166	2.5	343	5.3	10.4
	0.50	251	4.7	175	3.3	305	5.7	13.7
	0.75	295	5.9	192	3.9	265	5.4	15.1
	1.00	366	7.8	222	4.1	261	4.7	16.5
25/75	0.25	229	2.4	180	2.9	394	6.3	11.5
	0.50	232	4.4	192	3.7	320	6.1	14.2
	0.75	225	4.6	222	3.9	307	6.3	14.9
	1.00	429	10.6	230	4.7	291	6.0	21.3
25/75	0.25	149	2.5	173	2.8	352	5.7	11.0
	0.50	227	4.1	195	3.5	383	6.8	14.3
	0.75	249	4.8	197	3.8	369	7.0	15.6
	1.00	309	6.4	195	4.0	290	6.0	16.4
-----Portional fertilizer application-----								
-/100	0.25	120	1.5	150	2.4	285	4.5	8.4
	0.50	492	8.0	182	3.5	315	6.1	17.6
	0.75	415	8.7	243	5.1	266	5.5	19.3
	1.00	527	10.6	226	4.5	263	5.3	20.4
25/75	0.25	199	3.2	182	2.9	353	5.7	11.8
	0.50	321	6.1	230	4.4	324	7.5	18.0
	0.75	342	6.8	217	4.3	267	5.3	16.4
	1.00	374	10.4	253	5.2	299	6.1	21.7
50/50	0.25	183	2.9	188	2.9	340	5.3	11.1
	0.50	295	5.3	199	3.6	313	5.6	14.5
	0.75	343	7.0	186	3.8	250	5.1	15.9
	1.00	360	7.9	232	5.1	277	6.1	19.1
ANOVA F			7.2***		7.1***		3.4***	12.0***
l.s.d.			3.1		1.0		1.4	3.4

*** significant at 0.005 level.

TABLE 5.19

Cations concentration and accumulation in grain of
mature wheat plants. Ilanot soil.

N ratio NH_4/NO_3 appl.	N appl. g/pot	Ca		Mg		K		Cation
		m.eq/kg	uptake	m.eq/kg	uptake	m.eq/kg	uptake	uptake
		m.eq/pot		m.eq/pot		m.eq/pot		m.eq/pot
-	-	15	0.1	142	0.3	304	0.6	1.0
-----Preplanting fertilizer application-----								
	0.25	50	0.6	155	1.9	141	1.7	4.3
	0.50	31	0.5	128	2.1	130	2.1	4.8
-/100	0.75	46	0.8	143	2.5	135	2.4	5.7
	1.00	42	0.6	161	2.5	153	2.4	5.5
	0.25	51	0.6	135	1.7	136	1.7	4.0
	0.50	20	0.3	113	1.8	157	2.5	4.7
25/75	0.75	22	0.3	145	2.3	145	2.3	4.9
	1.00	48	0.7	156	2.3	157	2.4	5.4
	0.25	15	0.2	135	1.6	130	1.5	3.3
	0.50	20	0.3	128	2.0	125	2.0	4.3
50/50	0.75	21	0.4	134	2.2	128	2.1	4.7
	1.00	32	0.4	181	2.1	150	1.7	4.2
-----Portional fertilizer application-----								
	0.25	18	0.2	134	1.8	121	1.6	3.6
	0.50	15	0.2	127	1.9	135	2.0	4.1
-/100	0.75	13	0.2	138	2.1	122	1.9	4.2
	1.00	27	0.4	128	1.9	155	2.3	4.6
	0.25	6	0.1	108	1.5	103	1.4	3.0
	0.50	30	0.5	140	2.3	153	2.5	5.3
25/75	0.75	16	0.3	164	2.6	228	9.2	12.1
	1.00	30	0.5	156	2.5	203	3.3	6.3
	0.25	50	0.5	163	2.1	266	4.5	7.1
	0.50	38	0.6	154	2.6	295	4.9	8.1
50/50	0.75	58	0.9	210	3.5	308	5.1	9.5
	1.00	36	0.5	202	2.7	269	3.6	6.8
ANOVA F			3.0***		9.0***		40.0***	5.6***
1.s.d.			0.3		0.5		0.7	1.6

*** significant at 0.005 level.

SUMMARY

A second greenhouse experiment was performed based on results obtained in the experiment described in chapter 4.

Wheat was grown to maturity on a loamy sand, noncalcareous neutral soil and on a silt loam, basic, calcareous soil. N was applied at rates of 0.0, 0.25, 0.50, 0.75 and 1.00 gN per pot. Ammonium to nitrate nitrogen ratios in applied fertilizers were 0/100, 25/75, 50/50. Fertilizers were applied and mixed into the soil prior to planting and in addition in one soil N was applied in 15 portions with irrigation water.

The 25/75 ammonium to nitrate mixture at the 1.0g N/pot rate of application was a more effective source of nitrogen for vegetative growth than nitrate-only or the 50/50 mixture, but it was not more effective for grain production. This was found on both soils with preplanting fertilizer application. When N fertilizers were applied in portions over the growing period the 50/50 mixture produced more vegetative yield than the 25/75 mixture or nitrate-only. The 25/75 mixture produced slightly more grain than the other treatments.

Reduced nitrogen concentrations in vegetative parts and in grain increased in general with increasing proportion of ammonium in the ammonium and nitrate mixtures, in both soils and in the two fertilizer application methods. This was mainly found at the 1.0g N/pot rate of application. At the lower rates of application the plants may have suffered of nitrogen deficiency stress.

Phosphorus concentrations in wheat are in general proportional to ammonium ratio in the mixture. They are lower in the nitrate-only treatments than in the ammonium and nitrate mixtures.

Variation in cations (Ca, Mg, K) concentrations is not related to form of nitrogen applied, except in one treatment where potassium concentration decreases with increasing proportion of ammonium in the fertilizer mixture.

Results obtained for yield and nitrogen concentrations in wheat in this experiment confirm those obtained in the first experiment. However,

cation concentrations found in this experiment are not in agreement with those in the previous one.

Chapter 6. Seasonal Patterns of Nitrogen Uptake from Nutrient Solutions
Containing Nitrate, or Mixtures of Nitrate and Ammonium by Durum
Spring Wheat.

INTRODUCTION

Nitrogen is a major component of numerous essential organic compounds such as amino acids, proteins and nucleic acids. The two most important inorganic N sources that are utilized by higher plants to create these organic forms are nitrate and ammonium.

Plant roots absorb nitrate actively against an electrochemical gradient (Mengel and Kirkby, 1978; Jackson et al., 1986). The driving force for nitrate uptake has been proposed to consist of a transmembrane acidity gradient in which nitrate influx into the root is either across a hydroxyl:nitrate antiporter or by cotransport of hydrogen and nitrate ions inward across a symporter (Jackson et al., 1986). To sustain the driving force for nitrate uptake, requires generation of cytoplasmic alkalinity. Generation of the needed alkalinity can be accomplished by three mechanisms: (1) an electrogenic release of protons to the ambient solution via a plasmalemma ATPase, (2) decarboxylation of organic acid anions, and (3) generation of hydroxyl ions during nitrate reduction (Jackson et al., 1986).

While the exact mechanism of ammonium absorption is not clearly defined, it is evident that the absorption of ammonium by plant roots is rapid, and highly controlled by genetic and environmental factors (Reisenauer, 1978). Because of the difference in charge of the two ions and the necessity of maintaining electrical neutrality, ammonium absorption causes the external medium to become acidic, while the absorption of nitrate results in an alkaline medium (Hageman, 1984). The change in acidity or alkalinity of the

external medium as a function of form of N may have an impact on subsequent uptake as total absorption of ammonium increases with pH, while nitrate absorption is favored by increased acidity (Rao and Rains, 1976; Weissman, 1950).

Along with root zone pH, the carbohydrate status of plants can also influence ion uptake. Adequate carbohydrate levels in the plant are required to provide the carbon skeletons and energy necessary for ammonium and nitrate assimilation (Kirkby and Hughes, 1970; Michael et al., 1970). The process of ammonium metabolism involves conversion by a reductive amination of alpha-ketoglutarate to glutamate and by enzymatic synthesis to glutamine and asparagine (Mifflin and Lea, 1976). The metabolism of ammonium decreases the pool of carboxylates and increases the level of amides in the roots (Reisenauer, 1978; Ganmore-Neumann and Kafkafi, 1983). Nitrate is metabolized partly in the roots and partly in the leaves; and therefore, requires lower consumption of root carbohydrates (Ganmore-Neumann and Kafkafi, 1983).

In contrast to nitrate, which can be stored in high levels in plant tissue and assimilated as required, ammonium can be toxic to plants and therefore must be assimilated immediately in the roots. The toxicity results mainly from free ammonia (Mengel and Kirkby, 1978). Although species differ in their tolerance to ammonium, general symptoms of ammonium toxicity include a restriction of plant growth, chlorosis of leaves, marginal necrosis, necrotic spots, stem lesions and plant death (Maynard and Barker, 1969). Roots exposed to toxic levels of ammonium are often short, thick, less branched, and darkly colored (Maynard and Barker, 1969; Cox and Reisenauer, 1973; Warncke and Barber, 1973). Kirkby and Hughes (1970) suggested that the ammonia toxicity may be due to an uncoupling of phosphorylation, thus decreasing available energy.

Although most crop species can utilize either nitrate or ammonium, numerous researchers, utilizing mixtures of these ions have shown that ammonium often has an inhibitory effect on nitrate uptake. While the precise manner in which ammonium inhibits nitrate absorption is not known, several studies have addressed this issue using various crop species.

Evidence of both direct and indirect mechanisms of ammonium induced inhibition of nitrate uptake have been suggested. As a direct action, ammonium may interact with nitrate transporters at either the external or internal surface of the plasmalemma (Jackson et al., 1986). For wheat (Triticum aestivum L.), Minotti et al. (1969) have suggested that ammonium and the high acidity resulting from its uptake caused an alternation in membrane permeability which restricted the capacity for nitrate absorption. This pH effect on membranes caused by ammonium absorption has also been shown to stimulate an efflux of nitrate in arabidopsis mutants (Doddema and Telkamp, 1979).

Some researchers have suggested that ammonium, or assimilation products of ammonium, (e.g., amino acids) affect nitrate uptake indirectly by inhibiting the reduction of nitrate, possibly by inhibiting the enzyme nitrate reductase (Lycklama, 1963). MacKown et al. (1982), working with maize (Zea mays L.) seedlings, observed a restriction of both net nitrate uptake and reduction. They found that the inhibition of reduction (48%) was greater than that of influx (20%), which indicated that ammonium, or a product of its assimilation, exerted differential effects on the nitrate transport and reduction processes. Nevertheless, uptake and reduction of nitrate appear to be closely related. Uptake regulates the availability of substrate while the process of reduction generates OH^- ions which contribute to the driving force for nitrate uptake (Rufy et al., 1982).

A review of the literature reveals contradictory findings with regard to inhibition of nitrate reduction in the presence of ammonium. Some researchers have observed a decrease in nitrate reduction that was primarily due to a limitation in availability of substrate for the reduction process (MacKown et al., 1982; Lycklama, 1963; Rufty et al., 1982). In contrast, other studies have indicated that ammonium has an indirect effect on nitrate uptake through an inhibition of nitrate reductase activity, (NRA) (Breteler and Smit, 1974; Frith, 1972). Frith (1972) reported that roots of apple (Malus sylvestris Mill.) seedlings grown in nutrient solution containing ammonium plus nitrate (equal amounts of N for each form) had an average NRA 20% less than that of plants grown in solutions containing solely nitrate. For leaves of young wheat plants grown on a mixture of nitrate and ammonium, Breteler and Smit (1974) found that along with the inhibition of nitrate uptake, ammonium metabolism predominated over nitrate assimilation and resulted in lower NRA, a higher free amino acid level, and a lower carboxylate level than in plants grown in nitrate. A similar decrease in NRA by ammonium was found by Smith and Thompson (1971) for excised barley (Hordeum vulgare L.) roots, and by Joy (1969) for Lemna minor.

The contradictions of conclusions among these studies may be due to different plant species and cultural procedures. For example, the ionic composition or acidity of the nutrient solution can alter the effect of ambient ammonium on NRA (MacKown et al., 1982).

In contrast, other studies which investigated a range of species have shown an inhibition of nitrate absorption without a decrease in nitrate reduction (Rao and Rains, 1976; Jackson et al., 1976; Bennett et al., 1964; Minotti et al., 1969). The uptake of ions by intact plants is dependent in a complex way on the plant growth rate and on the ionic and carbohydrate status

of the root (Jackson et al., 1976). In cases where nitrate uptake was inhibited without a corresponding decrease in nitrate reduction, it is possible that carbohydrate translocation to the root system may be insufficient for optimal nitrate uptake, but adequate for effective reduction and translocation. As a consequence of active ammonium assimilation, available carbon substrates for nitrate assimilation may be decreased. Both Minotti et al. (1969) and Rao and Rains (1976) found that beyond the saturation value for ammonium uptake (0.2 and 0.1 mM, respectively), there was no further restriction on nitrate absorption. These data can be interpreted to imply direct effects of ammonium on the nitrate uptake system.

Other research has shown that inclusion of K in treatment solutions lessens the inhibitory effects of ammonium on nitrate uptake. Experiments with wheat (Jackson et al., 1976) and maize (Rufty et al., 1982) indicate that K has a moderating influence on the inhibitory effects of ammonium on nitrate uptake, and while ambient ammonium decreased K uptake, K did not alter ammonium uptake or assimilation (Rufty et al., 1982).

Energy considerations may also be of importance in regard to the mechanism of inhibition. The first step in ammonium assimilation requires ATP, and therefore may compete for this substrate with plasmalemma ATPases, which contribute to the driving force of nitrate uptake (Jackson et al., 1986). Another contributing factor in the mechanism of inhibition may be related to the pH of the rooting zone. For wheat seedlings, Weissman (1950) observed that the rate of ammonium absorption was stimulated by increasing the pH of the rooting zone from 4.3 to 6.3, while nitrate absorption in the presence of ammonium exhibited a decrease over a similar pH range. At neutral to acidic pH values, many plant species tolerate high levels of ammonium because the higher H^+ concentration depresses the formation of $NH_3(aq)$ concentration.

Various crop species can therefore grow and develop normally at ammonium levels of up to several mM provided that the pH is in the region of 4 to 6. Crop species that have been shown to tolerate relatively high levels of ammonium include wheat (Blondel and Blanc, 1973; Breteler and Smit 1974; weissman 1950), rice (Oryza sativa L.) (Dijkshoorn and Ismunadji, 1972), and sugar beet (Beta vulgaris L.) (Breteler, 1973).

Despite the apparent inhibition of nitrate uptake by ammonium, various plant species grown in solution culture have been shown to absorb more total N when they were provided with a combination of nitrate and ammonium than when supplied with either form separately (Cox and Reisenauer, 1973; Gashaw and Mugwira, 1981; Schrader et al., 1972; Ganmore-Neumann and Kafkafi, 1983; Weissman, 1950). Cox and Reisenauer (1973) observed a 64% increase in the rate of N uptake by young wheat plants when 30% of the total N was supplied as $\text{NH}_4^+\text{-N}$, as compared to an all nitrate treatment. Gashaw and Mudwira (1981) found that wheat absorbed 30% more total N when grown on a 50/50 mixture of $\text{NO}_3^-\text{-N}/\text{NH}_4^+\text{-N}$ than when grown solely on nitrate. Under similar conditions they found a 43% increase in absorption of total N with triticale (Triticosecale) and a 46% increase with rye (Secale cereale L.). With maize grown in a 50/50 mixture of $\text{NO}_3^-\text{-N}/\text{NH}_4^+\text{-N}$, Schrader et al. (1972) found a 37% increase in total N absorption over nitrate-grown plants. For wheat seedlings, regardless of pH (within the range 4.3 to 6.3), Weissman (1950) observed an approximate 30% increase in total N absorbed as compared to plants grown in all nitrate. Although the presence of ammonium inhibited nitrate uptake in all the above mentioned studies, plants grown with N mixtures still accumulated more total N than did nitrate-grown plants. This result occurred because ammonium uptake more than compensated for the decreased nitrate absorption.

A specific explanation for the superiority of N mixtures over nitrate

alone is not clear. It has, however, often been ascribed to a lower energy requirement for utilizing ammonium rather than nitrate in protein synthesis (Cox and Reisenauer, 1973; Middleton and Smith, 1979). Another explanation may be the inability of the nitrate assimilation system to supply the plant with maximum useable levels of reduced-N (Reisenauer, et al., 1982). Support for this concept comes from the observation that quantities of reduced-N in xylem-sap are generally higher in plants fed with mixtures of nitrate and ammonium than with nitrate alone (Pate, 1973).

As previously discussed, plants supplied with ammonium frequently have a higher concentration of amides and total soluble organic N than nitrate-grown plants. Storage of excess N as amides could be one mechanism by which plants can tolerate high levels of ammonium. Because this mechanism can only function when carbohydrate supply is adequate, it may provide an explanation as to why some plants can thrive on ammonium while others show symptoms of ammonium toxicity (Kirkby and Hughes, 1970). In this view, Barker and Bradfield (1963) suggested that the tolerance of maize to ammonium results from high carbon fixation rates and the large capacity of the plant to detoxify and store ammonium in the soluble N pool.

There is some evidence that young plants absorb ammonium more rapidly than nitrate, however, as the plant ages, the reverse situation often occurs and more nitrate than ammonium is absorbed (Hageman, 1984; Hewitt, 1970). Spratt and Gasser (1970) suggest that young plants preferentially absorb ammonium because they lack a completely functional nitrate reductase system. Stahl and Shive (1933) found a preferential uptake of ammonium during early growth of oat (Avena sativa) plants, while Naftel (1931), observed a similar trend in maize, cotton (Gossypium hirsutum L.) and wheat. For sugar-beet, Michael et al. (1970) found that the ratio of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ uptake decreased as a function

of plant age from 1.1 at 5 weeks to 0.9 at 15 weeks. For strawberry plants (Fragaria ananasa Duch.), Ganmore-Neumann and Kafkafi (1985) found that a preference for nitrate uptake occurred during flowering and fruiting, while ammonium was preferred during vegetative growth. This difference in predominant N form absorbed may be associated with carbohydrate status of the roots.

For soil grown wheat, the greatest uptake of N occurs at heading (Boatwright and Haas, 1961), and little N is absorbed between heading and maturity. For maize, N accumulation is greatest up to tasseling, with a significant decrease in uptake after silking (Andre et al., 1978; Hanway, 1962; Sayre, 1948; Thermon and Noogle, 1973). Soybean (Glycine max L.) plants grown in nutrient solution absorbed most of their total N from full bloom through pod filling stages (Harper, 1971; Lathwell and Evans, 1951). McElhannon and Mills (1978), also observed that the absorption of N by lima bean (Phaseolus lunatus L.) varied with growth stage. They found that maximum absorption of nitrate and ammonium occurred at flower initiation and at pod filling, while absorption of ammonium, but not nitrate, was also high at pod initiation.

The above literature review indicates that the amount of total N accumulated is influenced by the form of N supplied, plant species and the stage of plant development. Therefore, the current study was undertaken to investigate the interaction between nitrate and ammonium absorption throughout the life cycle of spring wheat.

MATERIALS AND METHODS

PLANT CULTURE AND TREATMENTS

Durum spring wheat seeds (Triticum durum cv. Inbar) were germinated and

grown to maturity in hydroponic culture in the greenhouse. Hydroponic culture vessels were 7 liter polyethylene pots painted black and covered with aluminum foil. Natural lighting was supplemented with metal halide lamps (approximately $300 \text{ } \mu\text{mol}/\text{m}^2/\text{s}$, between 400 and 700 nm wavelength) to provide 14 h of illumination. Greenhouse temperatures were maintained at 24°C during the light cycle and 18°C during the dark cycle. Under these conditions, color and morphological development of plants were similar to field grown plants.

Treatments consisted of three ratios of NO_3^- -N to NH_4^+ -N (100/0, 75/25, and 50/50) arranged in a randomized complete block design with five replications. The concentration of nutrients of full strength solution for each of the treatment solutions are shown in Table 6-1. In these solutions, S, K, Ca, and Mg are all allowed to vary (largest fluctuation in S) in order to achieve the best possible balance between the ion concentrations. This problem results from the difference in charges of the nitrate and ammonium ions, and from limitations in the number of mineral salt combinations that are suitable for preparing nutrient solutions. Iron was added daily as ferrous sulfate to maintain the Fe level at $2 \text{ } \mu\text{g}/\text{L}$ (1.5 ml/pot of 0.03 M FeSO_4) (Hageman et al., 1961). A full complement of micronutrients (Cu, Zn, B, Mn, and Mo) was included at concentrations according to Hoagland and Arnon (1950), except for Mn which was four times higher.

For aeration and pH control of the solution a recirculating ion exchange column was added to each culture vessel. This column was loaded with 75 ml of the appropriate (approximately 3:1) mixture of H^+ and Ca^{2+} forms of Amberlite IRC 50 cation exchange resin to maintain a pH of 5.5 ± 0.5 (Harper and Nicholas, 1976). When the solution pH deviated more than 0.5 unit the exchange column was replaced.

Table 6-1. The concentration of ions in the three treatment solutions used to evaluate the effect of mixtures of nitrate and ammonium versus nitrate alone on the cumulative uptake of N for spring wheat.

Element	$\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$		
	100/0	75/25	50/50
	----- mg liter ⁻¹ -----		
N (NO_3^-)	125	94	62
N (NH_4^+)	0	31	62
P	30	30	30
K	160	140	120
Mg	48	44	40
Ca	120	100	80
S	67	94	152

GERMINATION

Six seeds per pot were germinated directly into one-quarter strength treatment solution by the use of a "collar-seed-wick" assemblage inserted into holes (1.5 cm) on the perimeter of the culture vessel lid. The collar-wick system involved enclosing the seed within a collar (a 3 cm section of plastic tubing cut lengthwise and filled with vermiculite) which was connected to a wick (an 8 cm section of cotton string inserted in the overlap of the collar). The wick served to wet the vermiculite which supplied the imbibing seed with water. The moisture level of the seed was controlled by adjusting the solution level in the culture vessel. This technique allowed for undisturbed growth of the newly emergent radicle and resulted in excellent seedling uniformity. Emergence was defined as the date when 75% of all coleoptiles were visible. Date of emergence was not affected by N treatment (data not shown). At 5 days after emergence (DAE) the majority of radicles had penetrated the solution surface and the wicks between the collar and the solution were removed. The solution level was adjusted so that roots were sufficiently submerged to absorb water, while maintaining the proper moisture status of the seed crown. Two days later the plants were thinned to four uniform plants per pot, giving a stand density of 125 plants/m², and measurements commenced for NO₃⁻-N and NH₄⁺-N uptake.

ION UPTAKE

The daily rates and cumulative uptake of NO₃⁻-N and NH₄⁺-N were determined over the entire growing season by monitoring the loss of these ions from solution. For maintenance of a constant solution volume, pots were equipped with side-arm manometers and were regularly filled with dionized water. To determine the amount of NH₄⁺-N remaining in the solution, an appropriate

aliquot was taken from each pot and assayed using a colorimetric procedure (Cataldo et al., 1974). $\text{NO}_3\text{-N}$ was determined using absorbance at 210 nm (Cawse, 1967). Solutions were renewed (a total of 40 times throughout the experiment) when either of the N forms, in any of the treatment solutions, dropped below half their initial concentration. As the N uptake rates increased, solution ion concentrations were gradually increased, in a stepwise manner, to those listed in Table 6-1. Following germination on 25% of full strength solution, the nutrient ion concentration was increased according to the following schedule: 50% at 26 DAE, 60% at 35 DAE, 70% at 37 DAE, 85% at 44 DAE, and 100% at 53 DAE. Cumulative uptake was calculated by summation of net loss from the solution between time intervals.

RESULTS

CUMULATIVE UPTAKE OF $\text{NO}_3\text{-N}$ AND $\text{NH}_4\text{-N}$

For plants receiving either nitrate/ammonium mixture, similar trends in the uptake of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were observed throughout the experimental period. During the first 38 days of growth, plants grown on either N mixture accumulated 2.2-fold more total N than those grown solely on nitrate (Fig. 6-1). Compared to plants grown with only nitrate, total-N uptake was higher by 16 and 22 DAE for plants receiving the 50/50 and 75/25 N treatments, respectively. By 38 DAE plants receiving either N mixture had absorbed 0.29 grams of total N/plant, while plants grown solely on nitrate had absorbed 0.14 grams total N/plant.

During the early vegetative growth period (up to 32 DAE), the amount of $\text{NO}_3\text{-N}$ absorbed was similar for all treatments (Fig. 6-1). In contrast, between the period 30 to 70 DAE, plants grown on the 75/25 N mixture absorbed approximately 34% more $\text{NO}_3\text{-N}$ than nitrate grown plants, after which $\text{NO}_3\text{-N}$

Figure 6-1. Effect of two nitrate/ammonium mixtures (75/25, A and 50/50, B) versus nitrate alone on the cumulative uptake of total N (both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) over 37 days of growth. Values represent the mean of 5 replications with one standard error of the mean above and below the actual data point. In all instances where error lines overlap, only one standard error, either above or below, is presented. For points with no error bars, the standard error fell within the data point symbol. For ease of comparison, $\text{NO}_3^-\text{-N}$ uptake of the 100/0 treatment was plotted twice. Also, for graphical clarity, the uptake of $\text{NH}_4^+\text{-N}$ is not presented but can be depicted by the difference between total N uptake and $\text{NO}_3^-\text{-N}$ uptake. Days after emergence when tillering occurred is indicated.

absorption was similar for both treatments (Fig. 6-2). Between the period 30 to 65 DAE, plants grown with the 50/50 N mixture also absorbed more NO_3^- -N (17%) than nitrate-grown plants, with similar absorption rates for both treatments following 65 DAE. Despite the differences in NO_3^- -N uptake between plants receiving the N mixtures and plants grown on only nitrate, cumulative NO_3^- -N absorption was similar for all three treatments by physiological maturity (PM) (133 DAE).

Throughout the life cycle, plants grown on either N mixture absorbed more total N (approximately 2-fold) than did plants grown only with nitrate (Fig. 6-2). By 133 DAE, cumulative N uptake of plants receiving either N mixture was 3.3 grams N/plant while those receiving only nitrate had absorbed 1.6 grams N/plant.

Although not graphically presented, NH_4^+ -N uptake is represented by the difference total-N uptake and NO_3^- -N uptake. Cumulative NH_4^+ -N uptake over the course of the experiment was similar for the two N mixtures, and the amount absorbed was similar to NO_3^- -N absorption in nitrate-grown plants.

RATES OF N UPTAKE DURING DEVELOPMENT

The daily rate of N uptake was calculated as mg N absorbed per plant per day at intervals during the life cycle (depicted as the slope of the line between the various time intervals of the cumulative uptake plot). In general, rates of uptake of total N for all treatments, increased between 10 and 48 DAE, and reached a maximum daily rate during reproductive development (48-68 DAE) (Table 6-2). By the final stages of grain development (109-121 DAE), daily rates of total-N uptake were 38% of maximum uptake rate for plants receiving the 100/0 treatment and about 55% of the maximum rate for plants grown on either N mixture. Averaged over the life cycle, absorption rates of

Figure 6-2. Effect of two nitrate/ammonium mixtures (75/25, A and 50/50, B) versus nitrate alone on the cumulative uptake of total N (both NH_4^+ -N and NO_3^- -N) and NO_3^- -N over the entire experimental period. Days after emergence when flowering occurred is indicated. Other details as in Figure 6-1.

Table 6-2. The rates of N (NO_3^- -N, NH_4^+ -N and total-N) uptake during various time intervals (ca. 20 days) throughout the life cycle of wheat grown on nutrient media containing varying ratios of nitrate to ammonium.

Interval	NO_3^- -N			NH_4^+ -N		total-N		
	100/0	75/25	50/50	75/25	50/50	100/0	75/25	50/50
DAE	----- mg/plant/day -----							
10-28	3	4	3	2	2	3	6	6
28-48	15	23	20	9	12	15	32	31
48-68	21	27	29	14	20	21	41	48
68-89	20	21	20	19	20	20	40	40
89-109	17	16	15	21	23	17	37	37
105-121	8	10	12	13	15	8	22	27

total N for plants grown on N mixtures exceeded uptake rates of nitrate grown plants by 100 and 130% (75/25 and 50/50, respectively) (Table 6-2).

Rates of NO_3^- -N uptake followed a trend similar to total-N uptake. For all three treatments, rates of NO_3^- -N uptake increased during vegetative growth (10-48 DAE), and reached a maximum daily rate during reproductive development (48-68 DAE) (Table 6-2). Following this peak, the rates of nitrate uptake for all three treatments declined and by the final stages of grain development (109-121 DAE) were approximately 38% of maximum NO_3^- -N uptake rates. During vegetative and reproductive development (28-68 DAE) NO_3^- -N uptake rates of plants grown on N mixtures were higher than NO_3^- -N uptake rates of plants grown only on nitrate, while for the remainder of the experiment, NO_3^- -N uptake rates were similar for all three treatments.

Plants grown in both N mixtures absorbed NH_4^+ -N at similar rates throughout the experimental period (Table 6-2). However, in contrast to the uptake of total N and NO_3^- -N, the uptake rate of NH_4^+ -N increased throughout vegetative and reproductive development and reached a maximum rate during the grain fill period (89-121 DAE). During the final stages of grain development (109-121 DAE), daily rates of NH_4^+ -N uptake were approximately 64% of the maximum NH_4^+ -N uptake rate for both N mixtures.

PREDOMINANT N FORM ABSORBED VERSUS STAGE OF GROWTH

For plants receiving N mixtures, the predominant N form absorbed was estimated by calculating the uptake ratio of NH_4^+ -N/ NO_3^- -N at various time intervals throughout plant development (Table 6-3). During the vegetative and reproductive stage (up to 68 DAE), plants grown on mixtures of nitrate and ammonium absorbed more NO_3^- -N than NH_4^+ -N. For plants grown on the 75/25 N mixture, approximately 68% of the total N absorbed (28-68 DAE) was NO_3^- -N, and

Table 6-3. The uptake ratio of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ during various time intervals (ca. 20 days) throughout the life cycle of wheat grown on nutrient media containing mixtures of nitrate and ammonium.

Interval	$\text{NO}_3^-\text{-N}/\text{NH}_4^+\text{-N}$	
	75/25	50/50
DAE	----- $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ -----	
28-48	0.4	0.6
48-68	0.5	0.6
68-89	1.0	1.0
89-109	1.3	1.6
109-121	1.2	1.3

for plants receiving the 50/50 N mixture NO_3^- -N represented 60% of the total N absorbed.

For either N mixture, equal amounts of NO_3^- -N and NH_4^+ -N were removed from the media during early grain development (68-89 DAE), and greater amounts of NH_4^+ -N than NO_3^- -N were absorbed during mid and late grain fill (89-121) (Table 6-3). From 89 to 121 DAE, NH_4^+ -N uptake accounted for 55% of the total N absorbed by plants grown on the 75/25 N mixture and approximately 59% of the total N absorbed by plants receiving the 50/50 mixture.

Rates of N uptake were calculated at physiological maturity as total cumulative uptake (mg N) per gram root and per gram vegetative shoot mass (Tables 6-4, 6-5) (For root and shoot mass data, see Chapter 3). On a per root mass basis, plants grown on N mixtures accumulated less NO_3^- -N (20%) than plants grown solely on nitrate (502 mg NO_3^- -N/g root for nitrate grown plants and 414 mg NO_3^- -N/g root for either N mixture). However, with the additional NH_4^+ -N absorption, plants grown on mixed N nutrition accumulated more total N per root mass (43% for 75/25 and 56% for 50/50) than nitrate-grown plants (Table 6-4).

Similar to uptake rates calculated on a per root mass basis, when expressed on a per vegetative shoot mass basis, plants grown on either N mixture accumulated less NO_3^- -N than nitrate grown plants. In contrast to the 20% decrease per root mass, when expressed on a shoot mass basis, total NO_3^- -N uptake was reduced by 69% (Table 6-5). Contrary to accumulation of total N per root mass, all three treatments accumulated similar amounts (approximately 103 mg) of total N per gram of vegetative shoot mass.

TIMING OF N ACQUISITION

At physiological maturity (133 DAE), nitrate-grown plants had adsorbed

Table 6-4. The total accumulation of N (NO_3^- -N, NH_4^+ -N and total-N) expressed on a per root mass basis for wheat grown on nutrient data containing varying ratios of nitrate to ammonium.

NO_3^- -N/ NH_4^+ -N	NO_3^- -N	NH_4^+ -N	total-N
	----- mg N/g root -----		
100/0	502	-	502
75/25	415	304	719
50/50	413	370	783

Table 6-5. The total accumulation of N (NO_3^- -N, NH_4^+ -N and total-N) expressed on a per vegetative shoot mass basis for wheat grown on nutrient media containing varying ratios of nitrate to ammonium.

NO_3^- -N/ NH_4^+ -N	NO_3^- -N	NH_4^+ -N	total-N
----- mg N/g vegetative mass -----			
100/0	98	-	98
75/25	58	43	101
50/50	58	52	109

1605 mg total N and plants grown on N mixtures had absorbed 3237 mg and 3445 mg total N (75/25 and 50/50, respectively) (Fig. 6-2). Of the total N absorbed by plants receiving either N mixture, 45% was NH_4^+ -N and 55% was NO_3^- -N. By flowering (50 DAE), plants in all three treatments had absorbed 35% of the N accumulated by maturity (Table 6-6). In contrast to total N absorption, plants receiving N mixtures had absorbed 41% of their final NO_3^- -N absorption and 27% of their final NH_4^+ -N by flowering.

DISCUSSION

The greater uptake of total N by plants grown in N mixtures appears to be associated with NH_4^+ -N uptake as plants in all treatments absorbed similar amounts of NO_3^- -N until 28 DAE for the 75/25 mixture and 32 DAE for the 50/50 mixture (Fig. 6-1). Apparently, these young wheat plants were absorbing NO_3^- -N at their maximal rates and the absorption of NH_4^+ -N was in addition to and independent of, the uptake of NO_3^- -N. The data indicate that these young wheat plants were unable to take up and/or assimilate enough N for full genetic expression when nitrate, as the sole source of N, was maintained between 5 and 20 mM in the nutrient solution.

Throughout the experimental period, plants grown on either N mixture absorbed approximately 2-fold more total N than nitrate grown plants (Fig. 6-2). Compared to other studies in the literature reporting an increase in total N uptake with mixtures of nitrate and ammonium (Cox and Reisenauer, 1973; Gashaw and Mugwira, 1981; Schrader et al., 1972), the plants in this experiment absorbed up to 70% more total N. Apparently, for this species (Triticum durum) the presence of ammonium in the rooting zone did not inhibit net nitrate utilization. These data are in contrast to numerous other studies involving mixed N nutrition which have shown an inhibition of nitrate

Table 6-6. The percentage of the final cumulative N (NO_3^- -N, NH_4^+ -N and total-N) that was absorbed by flowering (50 DAE) for wheat plants grown on nutrient media containing varying ratios of nitrate to ammonium.

NO_3^- -N/ NH_4^+ -N	NO_3^- -N	NH_4^+ -N	total-N
	----- % of total* -----		
100/0	-	-	34
75/25	42	26	35
50/50	40	29	35

*Represents percentage of total cumulative uptake for a given N form.

utilization in the presence of ammonium (Minotti et al., 1969; MacKown et al., 1982; Rufty et al., 1982; Breteler and Smit, 1974; Frith, 1972; Rao and Rains, 1976).

The physiological explanation for lack of inhibition is not clear, but a review of the literature reveals several possibilities. Potassium has been shown to mediate inhibitory effects of ammonium on nitrate uptake without altering uptake or assimilation of ammonium (Rufty et al., 1982). The inclusion of adequate levels of K in our nutrient solutions (Table 6-1) may have contributed to the lack of inhibitory effects. Rigorous control of nutrient solution pH in the range 5.0 to 6.0 may have also contributed to the lack of an inhibition of nitrate uptake as plants can often tolerate levels of ammonium up to several mM if the pH is maintained between 4.0 to 6.0. A final possibility is that this particular durum species exhibits an allocation of carbohydrates between shoot and root which circumvents the inhibitory effects of ammonium by providing the root with adequate energy to simultaneously assimilate both N forms. Although speculative, future tests of these concepts may elucidate mechanisms involved in the lack of (or presence of) ammonium induced-inhibition of nitrate uptake.

The similar results for the two N mixtures (75/25 and 50/50) (Fig. 6-2) indicates that the enhanced total N uptake from N mixtures is not a function of the level of NH_4^+ -N present in the rooting zone. For either N mixture, plants absorbed approximately 45% of their total N as NH_4^+ -N (Fig. 6-2).

Peak absorption rate of total N for plants grown in N mixtures and with nitrate alone occurred during reproductive development (Table 6-2). For all treatments, the maximum absorption rate of nitrate also occurred during reproductive development. In contrast, ammonium absorption rate increased throughout reproductive development and reached a maximum during the grain

filling period. These data indicate that when N is available, uptake, especially of $\text{NH}_4^+\text{-N}$ is maintained at relatively high rates throughout grain development. In this study, plants absorbed 65% of their total N, 73% of total $\text{NH}_4^+\text{-N}$ and 59% of total $\text{NO}_3^-\text{-N}$, during the post-anthesis period (Table 6-6). In contrast, for nine field-grown wheat cultivars, Van Sanford and MacKown (1987) found that an average of 82% of the total plant N accumulated by maturity was present at anthesis.

The ratios of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ absorbed during various time intervals of the experiment (Table 6-3) indicate that the stage of plant development can influence the relative proportion of uptake between the two N forms. These data indicate a preference for nitrate utilization by vegetative plants and a preference for ammonium utilization during reproductive growth. The data are in agreement with those of Andre et al. (1978), who found a preference for nitrate use during vegetative growth and a change in preference during the flowering period, for maize. Other researchers, working with various crop species, have also observed that the uptake ratio of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ changes with plant development (Hageman, 1984; Hewitt, 1970; Spratt and Gasser, 1970; Michael et al., 1970; Ganmore-Neuman and Kafkafi, 1985). However, in contrast to the current study, most of the previous studies report the predominant N form absorbed by young plants was ammonium. It has been suggested that the carbohydrate status of the plant influences which form is preferentially absorbed (Michael et al., 1970), thus the lower ammonium uptake by older plants could be due to a decreased carbohydrate level in the roots.

When expressed on a per vegetative shoot mass basis (Table 6-5), nitrate uptake was lower for plants receiving N mixtures than those receiving solely nitrate. In contrast to $\text{NO}_3^-\text{-N}$, all plants accumulated similar amounts of total N per gram of vegetative shoot mass. Because plants grown on N mixtures

produced twice as much vegetation (see Chapter 3) and absorbed twice as much total N (Fig. 6-2), these data indicate a strong association between total N accumulation and vegetative growth. Total N accumulation, expressed on a per root mass basis (Table 6-4), was higher for plants grown with mixed N nutrition compared to nitrate grown plants. These data indicate that roots are more efficient at N absorption when both forms are available compared to when only nitrate is present.

Overall, these data indicate that inhibition of nitrate uptake in the presence of ammonium is not obligatory. For the wheat cultivar used in this study, lack of this inhibition could be the reason for the substantial increase in absorption of total N. Apparently, under these experimental conditions, wheat plants cannot absorb maximal N when supplied only with nitrate. Increased N absorption by the durum wheat was achieved through the use of mixed N nutrition.

CONCLUSIONS

Wheat (Triticum durum cv. Inbar) was grown to maturity in hydroponic culture under greenhouse conditions during the spring of 1986 to investigate the amount and patterns of absorption of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. Three N treatments were used by supply equal amounts of N in the following ratios 100/0, 75/25 and 50/50 of $\text{NO}_3^-\text{-N}/\text{NH}_4^+\text{-N}$. Rates and cumulative uptake of $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ were determined throughout the life cycle by monitoring the loss of these ions from the solutions. The following conclusions were drawn:

1. Plants grown on both mixture of nitrate and ammonium absorbed more total N (about 2-fold) than plants grown on nitrate alone.

2. Plants grown on the 50/50 mixture absorbed slightly more total N (6%) and slightly less NO_3^- -N (3%) than plants grown on the 75/25 N mix. This data indicates that, at these concentrations, the presence of NH_4^+ -N was more important than the concentration on N uptake.
3. During the first 30 days after emergence, plants of all N treatments absorbed similar amounts of NO_3^- -N, indicating that ammonium absorption was not inhibiting nitrate absorption.
4. When N mixtures were supplied, the patterns of uptake of NO_3^- -N and NH_4^+ -N varied with plant development. During the 68 days after emergence, NO_3^- -N uptake exceeded NH_4^+ -N uptake (about 25%), for the next 21 days the rates of uptake were similar, while during the last 32 days, NH_4^+ -N uptake exceeded NO_3^- -N uptake (about 35%).

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General Discussion and Conclusions

This project was undertaken to clarify the role of ammonium per se in production of crop plants. The need for such studies exists because when ammonium salts are applied to soil under conditions favorable for crop growth, ammonium is rapidly converted to nitrate the soil organisms. It is generally assumed that nitrate is the primary form of N available to most crop plants.

Although nitrification inhibitors have been developed and used, there is disagreement among researchers over the effectiveness (duration, especially in diverse soils) of the inhibitors. In central Illinois soils, samples taken in the fertilizer application zone show that the applied ammonium (with or without nitrification inhibitors) decreases with time and is usually depleted by mid-June. In these studies, the conversion of ammonium to nitrate was not markedly affected by the nitrification inhibitor. The duration of the effectiveness of the inhibitor and the presence of ammonium (with or without the inhibitor) was a function of the soil type and the environmental conditions.

Many workers have noted yield increases in maize when the ammonium fertilizer salts have been treated with nitrification inhibitors. However, the major point of disagreement among investigators lies in whether the increases in yields can be attributed directly or indirectly to ammonium. The indirect effect arises from the ability of the nitrification inhibitor to maintain ammonium only during the early phase of plant growth. During this period, the ammonium was bound to the soil and not leached by the normally heavy spring rains. Consequently more N was made available to the plant as nitrate latter stages of plant development.

As noted in the introductory sections of this report, there is a substantial body of literature that indicates that mixtures of ammonium and nitrate produce more vegetative growth and in a few instances more grain yield than either form alone as the sole source of N. However, in some of these studies, the evidence that the increased yields were due to ammonium per se was not compelling. For example, when barnyard manure has been used to obtain greater yields than obtained with inorganic fertilizers, it is not known that ammonium per se caused the increase in yields. The yield increase could have been due to (a) better soil structure, aeration and water holding potential; (b) availability to the plant of organic compounds including amino acids; (c) improved mycorrhiza activity, etc.

Hydroponic cultures provides an approach that insures the presence of ammonium in the rooting medium throughout the life cycle of the plant, the major advantage of nutrient solution over soil pot culture is that the solution cultures minimizes nitrification. Further the ratio of $\text{NO}_3^- \text{--N} : \text{NH}_4^+ \text{--N}$ can be monitored and modified daily if needed. Numerous hydroponic cultures have been provided evidence that plants grown of nitrate/ammonium mixtures produce more vegetative mass than plants grown solely on nitrate or ammonium. However, in only a limited number of experiments have the plants been grown to maturity. For rice it was reported that mixed N nutrition produced more grain than either nitrate or ammonium nutrition. One of the problems with hydroponic cultures is the maintenance of the solution pH. However this problem can and has been solved. Another problem is that it is difficult to extrapolate the results obtained with hydroponic cultures to field production conditions.

Under field conditions, it is most laborous to sample in sufficient detail to establish the ratios of $\text{NO}_3^- \text{--} \text{N} : \text{NH}_4^+ \text{--} \text{N}$ throughout the entire rooting zone and for the life cycle of the plant. While these data can be collected these values do not establish that the plant is absorbing these ions in proportion to the measured ratios. This last statement indicates the desirability of utilizing the plant as a sampling tool. It is known that plants grown on ammonium have higher concentrations of reduced N, amides and lower concentrations of organic acids (especially malate), nitrate, calcium, magnesium and sometimes K^+ than plants grown on nitrate. Because the changes in these metabolites is the direct result of changes in uptake of ions and the subsequent metabolism of nitrate and ammonium, analysis of the plant for these constituents should reflect the availability (absorption by) of ammonium the plant.

Chapter 1. Nitrification Inhibition. The objective was to define conditions that would maintain predetermined ratios of ammonium and nitrate in soils kept under laboratory or greenhouse conditions.

The conclusions were: (a) Ammonium could be maintained in soil for several (6) weeks by treating ammonium sulfate with the nitrification inhibitor, dicyandiamide (DCD) (10:1 w/w) prior to its incorporation in the soil sample; and (b) Predetermined ratios of $\text{NO}_3^- \text{--} \text{N} : \text{NH}_4^+ \text{--} \text{N}$ could be maintained reasonably well over a six week period for two soil types when the soil was fortified with various ratios of calcium nitrate and DCD treated ammonium sulfate.

Chapter 2. Part A. Effect of $\text{NO}_3^- \text{--} \text{N} : \text{NH}_4^+ \text{--} \text{N}$ Ratios in the Nutrient Medium on Metabolite Concentrations of Maize Plants.

The objective was to show that certain plant constituents reflect the form of N being metabolized which permits the plant to be used as a sampling tool to determine the relative amounts of nitrate and ammonium available (absorbed

by) to the plant from nutrient solutions.

The conclusion reached when plants were grown for 30 days in nutrient solutions having $\text{NO}_3^- \text{--} \text{N} : \text{NH}_4^+ \text{--} \text{N}$ ratios of 100/0, 75/25, 50/50, 25/75 and 0/100 and plant parts or whole shoot assayed for malate, reduced N and $\text{NO}_3^- \text{--} \text{N}$ were:

(a) Concentrations of each of the three constituents showed proportional changes (significant linear regression) in response to increased levels of ammonium in the media; and (b) Although the data obtained in these experiments was limited, the results indicate that plants can serve as a sampling tool to determine the relative amounts of nitrate and ammonium absorbed.

The last conclusion is supported by the data of Table 4.7 from Chapter 4 on wheat and millet. The regression of reduced N against percentage of ammonium applied to the soil for the three harvest dates are as follows:

Wheat				
Total N Applied	Date Harvested	Plant Part	Regression equation	r
0.5	Dec 27	Shoot	$0.012X + 3.0$	.83
0.5	Jan 29	Shoot	$0.012X + 2.6$	.88
0.5	April 1	Straw	$0.0066X + 0.67$	.96
1.0	Dec 27	Shoot	$0.012X + 3.5$	.95
1.0	Jan 29	Shoot	$0.027X + 3.3$	.95
1.0	April 1	Straw	$0.030X + 1.4$	.98
Millet				
0.5	June 2	Shoot	$0.019X + 2.3$	.97
0.5	June 9	Shoot	$0.012X + 2.1$	.58
0.5	June 23	Shoot	$0.0069X + 0.92$	.91
1.0	June 2	Shoot	$0.021X + 2.6$	.98
1.0	June 9	Shoot	$0.022X + 1.9$	.93
1.0	June 23	Shoot	$0.018X + 1.1$	.98

Significance at 5% level = .88

It was not feasible to establish regression equations for the data from Tables 5.8 and 5.9 from Chapter 5 because there were only three levels of ammonium. However in only three of the eight possible tests were the reduced N concentrations in the plant approximately proportional to the increased level of ammonium in the soil media when the harvests was made at flowering. In five of the eight tests the concentration of reduced N were relatively low for the 75/25 ratio of $\text{NO}_3^- \text{--} \text{N} : \text{NH}_4^+ \text{--} \text{N}$. Regardless of the amount of total N applied per pot, the reduced N concentrations of plants treated with the 50:50 N mix were greater than for the all nitrate treatment.

When the reduced N concentration of the stover (harvest at maturity) was plotted against the percentages of ammonium in the media (either Beeri or Ilanot soil), the increases in N were approximately proportional to the increases in ammonium in the soil media for all eight tests.

When the reduced N concentration of leaves and stems (Table 2.2, Chapter 2) were plotted against the three levels of NH_4^+ in the nutrient cultures, again the lines were nearly linear.

In summary, these data show that certain constituents of the plant are affected by the uptake and metabolism of nitrate and ammonium. However the data is too limited to permit the conclusion that the plant is the ideal sampling tool for establishing the availability of nitrate and ammonium to the plant throughout its life cycle. For example, the nearly linear plots of the reduced N concentration in the straw at maturity versus the percent of ammonium in the media indicate only that the plant was exposed to various ratios of $\text{NO}_3^- \text{--} \text{N} : \text{NH}_4^+ \text{--} \text{N}$ at some stage in its life cycle. It does not specify when or the duration of the exposure. The reason for the failure to obtain linear plots (reduced N vs % NH_4^+) for the plant material harvested at the flowering stage is not clear. There are at least three possible explanations:

(a) The apparently aberrant values are correct and accurately reflect the ratios of NO_3^- -N: NH_4^+ -N absorbed; (b) The basic theory is wrong; or (c) Errors in the sampling and assay procedures.

Part B. Maize Yields.

The objective was to compare the effects of a mixture of nitrate and ammonium (50:50) versus all nitrate in hydroponic cultures on dry mass and grain yield, at maturity, yield components and partitioning of dry mass, N, P and K for two maize hybrids. The conclusions were: (a) Both the prolific hybrid (FS854) and the single eared hybrid produced more grain (14 and 8%, respectively) when grown on a mix of equal proportions than when grown on nitrate alone; (b) The increase in grain yield for both hybrids was the result of an increase in the number of kernels per plant; (c) Only when grown on mixed N did the single eared hybrid produce a full sized second ear (shank, cob and husks but few kernels); (d) For FS854, the increase in grain was associated with an increase in partitioning of dry matter from the vegetation to the grain and not an increase in total dry mass; (e) For B73xLH51, equal increases in grain and total dry mass were observed; and (f) The two hybrids different in ability to absorb nitrate, ammonium and other nutrients.

In summary, these data indicate that in general grain yields of maize can be increased by about 10% when ammonium is available to the plant throughout its life cycle. Prolific hybrids, appear to be more responsive to ammonium nutrition than single eared type. Because most prolific hybrids have poor stalk quality, it is suggested that a prolific hybrid with good stalk quality would be a most useful hybrid for experimentation with supplemental ammonium nutrition.

Chapter 3. Effect of Mixtures of Nitrate and Ammonium on Nitrogen Utilization and Productivity of Wheat:Hydroponic and Soil Cultures.

The objectives were to: (a) Compare the effects of varying NO_3^- -N: NH_4^+ -N ratios (100:0, 75:25 and 50:50) in hydroponic and soil cultures on the growth and yield of durum wheat (cv. Inbar); and (b) identify factors associated with differences in productivity resulting from either N treatment or culture method.

The conclusions were: (a) The hydroponically-grown plants produced more (40%) tillers, total dry mass (80%) and grain (80%) when grown on the N mixes than when grown on nitrate alone; (b) The increase in yield resulted from more tillers per plant as kernel weight remained relatively constant regardless of N treatment; (c) Plants cultured on mixed N accumulated more total reduced N (2-fold) and P (1.7-fold) than plants grown on nitrate alone. Consequently at maturity the concentrations of N were slightly higher while concentrations of P were similar; (d) At maturity the concentrations of reduced N in both leaves and stems increased progressively (nearly linear) with the increased amounts of ammonium (0 to 50%) in the nutrient solutions; (e) The wheat cv. Inbar was unable to absorb sufficient N for maximum plant development when the nutrient solution contained only nitrate (f) For soil grown plants, N mixtures caused no significant effects on growth, tillering, grain yield, reduced N and P contents relative to nitrate as the sole source of N; (g) The reason for the lack of plant response to the mixed N treatment is not clear. However these data support the conclusion that the plants grown in soil fortified with mixed N forms were not absorbing any more ammonium than plants grown on soil fertilized only with nitrate. (The effectiveness of the nitrification inhibition was verified by control pots without plants). (h) When the N source was mixed N, the concentrations of reduced N of the vegetative parts at maturity were higher for the hydroponic than for the soil cultured plants. When the N source was nitrate, the concentrations of reduced N in the

vegetative parts were similar regardless of rooting media; (i) Regardless of the culture method or treatment, the partitioning of the dry matter among the plant parts was similar. In contrast, the distribution of N and P among plant parts differed for soil- and hydroponically-grown plants and the distribution of P was altered for hydroponically grown plants; and (j) The hydroponic cultures show that mixtures of nitrate and ammonium markedly increased the productivity of the wheat cv. Inbar. Thus, there is a potential for increasing wheat production by supplying supplemental ammonium. The soil experiment shows that there are unknown factors that apparently prevented the absorption of ammonium from this support medium.

Chapter 4. The Effect of Various Mixtures of Ammonium and Nitrate on the Growth and Yield of Durum Wheat and the Growth of Millet: Soil Experiment.

The objectives were to: (a) Maintain the applied ratios of ammonium to nitrate in the soil by addition of dicyandiamide (DCD); and (b) Determine the effect of the amount, form and various ratios of ammonium to nitrate on plant growth, including yield, content of reduced N and cation uptake in wheat and millet.

Conclusions were: (a) Addition of DCD to various mixtures of ammonium and nitrate fertilizers maintained the desired and predetermined ratio of ammonium to nitrate in the soil during incubation and plant growth experiments; (b) For both wheat and millet, plants grown on all ammonium fertilizer produced lower yields of dry matter than plants grown on mixtures of the two forms of N; (c) Plants grown on all nitrate produced less (statistically insignificant) dry matter than when grown on mixed N; (d) Supplying nitrate alone resulted in lower accumulation of reduced N compounds in wheat or millet than when these

plants were grown on N mixtures or on all ammonium and (e) Controlling the ratio of ammonium to nitrate in the soil leads to differences (up to 300%) in total accumulation of Mg^{2+} and Ca^{2+} in the plant tops.

Chapter 5. The Effect of Total Amount, Form and Ratio of Forms of N on Growth and Grain Yield of Wheat grown on Two Soil Types.

The objectives were to compare the growth, grain yield, tillering, and cation accumulation at various stages of growth when wheat was grown on two soil types, total N was supplied at four levels and ratios of NH_4^+-N and NO_3^--N were 0:100, 25:75 and 50:50. The ammonium salts were treated with DCD prior to adding to soil. In one experiment the N treatments were supplied in 15 increments.

The conclusions are: (a) The mixture of $NH_4^+:NO_3^-$ of 25:75 at the 1 g N per pot was a more effective source of N for vegetative growth than nitrate alone or the 50:50 mixture, but it was not more effective for grain production. This finding was true for both soil types when N was applied prior to planting; (b) When N fertilizers were applied in increments during the experimental period at the 0.5, 0.75 and 1.0 g N/pot levels, the N mixes resulted in greater (up to 14%) grain yields than the nitrate only treatment; (c) When the N fertilizer was applied in portions over the growing period, the 50:50 mixture produced more vegetative yield than the other two treatments; (d) At the 1 g N/pot level, the reduced-N concentrations in vegetation or grain increased in general with increasing proportions of ammonium in the rooting zone. This finding was true for both soils and for both methods of fertilizer application; (e) Phosphorus concentrations in wheat are in general proportional to the ammonium ratio in the mixture. They are lower in the nitrate only treatment than in the N mixes; (f) Variation in cation (Ca^{2+} , Mg^{2+} , K^+) concentrations were not related to form of N applied except in one

treatment where K^+ concentrations decreased with increasing proportions of ammonium in the fertilizer mixture; and (g) Results obtained for yield and N concentrations in wheat are similar to those of the first experiment (see Chapter 4). However, divergent results were noted for the cation concentrations.

Chapter 6. Seasonal Patterns of Nitrogen Uptake from Nutrient Solutions Containing Nitrate on Mixtures of Nitrate and Ammonium by Durum Wheat.

The objective was to determine the patterns (rate and cumulative) uptake of N from nutrient solutions containing nitrate and mixtures of nitrate and ammonium (100:0, 75:25, 50:50) throughout the life cycle of the durum wheat cv. Inbar.

The conclusions were: (a) Plants grown on both mixtures of nitrate and ammonium absorbed more total N (about 2-fold) than plants grown on nitrate alone; (b) Plants grown on the 50:50 N mix, absorbed slightly more total N (6%) and slightly less NO_3^- (3%) than plants grown on the 75:25 N mix. This data indicates that, at these concentrations, the presence of ammonium was more important to N uptake than the concentrations; (c) During the first 30 days after emergence, plants of all N treatments absorbed similar amounts of nitrate indicating that ammonium absorption was not inhibiting nitrate absorption; and (d) When N mixtures were supplied, the patterns of uptake of nitrate and ammonium varied with plant development. During the 68 days after emergence, nitrate uptake exceeded ammonium uptake (about 25%), for the next 21 days the rates of uptake were similar while during the last 32 days, ammonium uptake exceeded nitrate uptake (about 35%).

Cooperation

Personnel

Haifa, Israel

Investigators: J. Hagin and A. Shaviv
Investigator:
(first year) Shelly Katz

Greenhouse: A. Moses and B. Kosta
Analytical
technician: Ronit Faran

Urbana, Illinois

Investigators: F. E. Below and R. H. Hageman
Investigator:
(first year) R. G. Hoeft

Graduate Student: Jill Heberer

Technicians: Lowell Gentry
JoEllen Pauss

From its inception this project has been characterized by excellent cooperation. This cooperation is illustrated by the following: (a) Shelly Katz on her visit to Urbana was a major force in formulating the broad outlines of the initial project proposal; (b) There has been a continuation of exchange of data and personnel (visits by Professors Hagin, Hageman and Below; (c) The use of common cultivars for experimental work at Haifa and Urbana; and (d) The project combines two disciplines, (Professor Hagin and coworkers providing the expertise in soil science and Professor Below et al. providing the expertise in plant physiology) which facilitated the acquisition of knowledge needed to permit the use of ammonium to increase grain yield of crop plants.

Benefits to Agriculture

The finding that the nitrification inhibitor (dicyandiamide) would maintain the desired and predetermined levels of ammonium in soils is beneficial to agriculture in that ammonium is more stable and is not leached from the soil. This minimizes environmental pollution.

The substantial (80%) increase in grain yields of the durum wheat (cv Inbar) grain in nutrient culture containing a mixture of nitrate and ammonium relative to a nitrate control, shows the benefits that may be derived when ammonium is freely available in the rooting zone. This work and a pending proposal provides an approach to understanding how to make ammonium freely available to soil grown plants. The current work also provides a way of identifying plants that are more responsive to ammonium. This information would permit a marked increase in crop yield and be of major importance to both Israel and the U.S.

Publications

Illinois

Below, F. E., and L. E. Gentry. 1987. Can ammonium nutrition increase productivity of corn? p. 28-37. In R. G. Hoelt (ed.) 1987 Illinois Fertilizer Conference Proceedings, Springfield, Il., 27-28 January. Illinois Coop Exten. Serv., Univ. of Illinois at Urbana-Champaign.

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Gentry, L. E., W. T. Wang, and F. E. Below. 1987. Influence of mixtures of nitrate and ammonium on nutrient uptake by wheat seedlings. Agron. Abstracts. (in press).

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Israel

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