

# A comparison between ammonium and nitrate nutrition of young sugar-beet plants grown in nutrient solutions at constant acidity. 1. Production of dry matter, ionic balance and chemical composition

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## Summary

In trials concerning ammonium and nitrate nutrition of sugar-beet plants changes in medium pH were eliminated by automatic titration and recording of the acidity or alkalinity released during growth. Yields of the plants on both N forms differed by only 12 % so that the effect of the N form on inorganic chemical composition, ionic balance, carboxylate content, soluble-sugar content and content of some nitrogenous compounds could be compared at similar yields

Cation excess (number of milliequivalents of inorganic cations minus those of inorganic anions; C-A) in seedlings grown on nitrate was over thrice that with ammonium.

The balance of ion uptake and the H<sup>+</sup> production curves showed that the acidity evolved is a good measure of the ammonium uptake. In nitrate nutrition the evolved alkalinity was small compared with the amount of nitrate absorbed by the plants and was no measure of nitrate uptake.

The diurnal changes in rate of release of H<sup>+</sup> and OH<sup>-</sup> by plants in either ammonium or nitrate nutrition were measured and related to plant composition, factors regulating ammonium and nitrate uptake, and the effect of light and darkness. There was a decrease in the rate of H<sup>+</sup> production during the dark period and a decrease in the rate OH<sup>-</sup> production during or just after the dark period.

## Introduction

Sugar-beet crops are usually dressed with nitrate, which gives higher yields than ammonium (Crowther & Garner, 1960, van Tuil, cited by Schuffelen et al., 1965). However, nitrate may be lost by leaching and denitrification, and heavy nitrogen dressings may cause high levels of nitrate and oxalate in foliage and root (Table 1).

Related species, such as spinach, mangold and fodder-beet, show similar contents when heavily dressed with nitrate (Prummel, 1966; Jurkowska, 1971; and others).

The nutritional value of crops is lowered by high nitrate and oxalate contents and with sugar-beets, a high oxalate content may be related to a low sugar content.

Sugar-beet plants grown on NH<sub>4</sub> contain virtually no NO<sub>3</sub> and less carboxylates than

Table 1. Maximum nitrate and oxalate content in various parts of the sugar-beet plant sampled on various dates in a field trial with 250 kg N/ha as Ca(NO<sub>3</sub>)<sub>2</sub> (van Egmond, 1973a, b).

Plant part	Oxalate		Nitrate	
	meq/kg DM	% of DM	meq/kg DM	% of DM
Leaf blades	5800	26	500	3
Petioles	2800	13	2000	12
Tops	600	3	330	2
Beets	500	2	370	2

plants grown on NO<sub>3</sub> (Houba et al., 1971). Other factors may also be influenced by the N form, such as free nitrogenous compounds, Na and K in the juice affecting industrial sugar processing (Loué, 1970).

To compare the real effect of NO<sub>3</sub> and NH<sub>4</sub> nutrition, changes in pH of the substrate caused by nitrification and excess anion or cation uptake were eliminated by using a nitrification inhibitor and equipment for control of pH.

The present experiments concern substitution of ammonium for nitrate. The major effect of this substitution, the change in the ionic balance (excess cation versus excess anion uptake) associated with H<sup>+</sup> or OH<sup>-</sup> release and the carboxylate accumulation by the plants, is considered in detail.

In addition, some aspects of nitrogen and carbohydrate metabolism are discussed.

### Material and methods

Diploid sugar-beet seeds were germinated in quartz sand moistened with demineralized water. The seedlings were transferred to well-aerated nutrient solutions with either NO<sub>3</sub> or NH<sub>4</sub> as the source of nitrogen. The composition of the solution is given in Table 2.

Besides N form, the solutions differed in Na and Cl concentrations. Sodium hydroxide was added to the NH<sub>4</sub> solution and HCl to the NO<sub>3</sub> solution during pH control by automatic titration.

Two 60-litre PVC boxes contained nutrient solution, which was circulated by an electric pump with a capacity of 12 litres/minute (Fig. 1). At intervals of 2 to 30 weeks the solutions were renewed and the boxes and pipe system thoroughly cleaned. The solution level in the boxes was kept constant by adding demineralized water daily. Nitrification was effectively inhibited by the N serve as there was no acidification of the nutrient solution when the plants were removed and nitrate in the plants was negligible

Table 2. Composition of the nutrient solutions (meq/litre).

	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
NO <sub>3</sub>	3	4	2	2	0	3	2	1	5
NH <sub>4</sub>	2	4	2	2	3	0	7	1	5

Trace elements: 0.5 ppm B, 0.5 ppm Mn, 0.4 ppm Fe, 0.05 ppm Zn, 0.02 ppm Cu, 0.01 ppm Mo, 0.01 ppm N-serve (2-chloro-6-trichloromethyl pyridine).

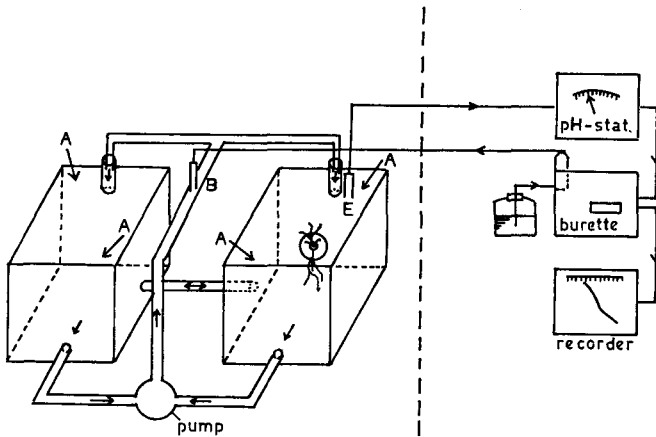


Fig. 1. Equipment for growing plants in nutrient solution at constant pH. For explanation, see text.

in ammonium nutrition. The N serve was added to both media and had no detectable effect on growth. The boxes were covered with perforated PVC lids in which the plants were accommodated by means of foam plastic.

The experiments were done in a growth cabinet kept at 20°C (night and day), a relative air humidity of 70–80 %, a daylength of 16 hours and a light intensity of 20 000 lx. The pH was regulated as outlined in Fig. 1. A glass and a reference (calomel) electrode (E) were pierced through the lid of the boxes close to the point where the nutrient solution was injected. At this point mixing was severe due to the free fall of the solution and aeration (arrows A). Any change in the pH value of the solution was measured by a pH-meter with pH-stat (Radiometer, Copenhagen), preset to pH 5.50 and operating an automatic burette with a capacity of 25 ml and filled with 0.100 N HCl (NO<sub>3</sub> nutrition), 0.100 N NaOH (NH<sub>4</sub> nutrition, young plants) or 1.00 N NaOH (NH<sub>4</sub> nutrition, older plants). When the pH value changed, a small volume (< 0.01 ml) of acid or base was injected in the central pipe (B) to readjust it.

Damage to the plants by local high or low pH values was prevented by effective dilution (liquid speed at point B: 0.7 m/s) and mixing. The injected volumes of acid or base were integrated and recorded continuously.

#### Analytical methods

Inorganic cations and anions in the plants were determined as follows (Slangen, 1971). A sample of dried and ground plant material was digested in concentrated sulphuric acid and hydrogen peroxide in the presence of salicylic acid. Na, K and Ca were determined flame-photometrically, H<sub>2</sub>PO<sub>4</sub> colorimetrically, Mg by atomic absorption spectrometry and total nitrogen colorimetrically with the indophenol-blue method. Another subsample was extracted with demineralized water and in the extract NO<sub>3</sub> was determined with a nitrate electrode, Cl coulometrically and SO<sub>4</sub> turbidimetrically.

Carboxylates were first converted into organic acids by decationization with a H<sup>+</sup> sulphonic acid resin and separated by gradient elution chromatography with n-butanol/chloroform on a silicagel column and continuous titration of the eluate. Oxalates in the

plant residue were determined potentiometrically after extraction with HCl (van Egmond, 1973b). Ammonium ions, glutamine and asparagine were determined after extraction of fresh plant material with cold 70 % ethanol in a cooled Bühler homogenizer. The amide groups were hydrolysed for 3 hours at 100°C at pH 6.5 (glutamine) and in 1 N H<sub>2</sub>SO<sub>4</sub> (asparagine and glutamine).

Ammonium ions in the extract and in the hydrolysates were determined by distillation in a Parnas-Wagner apparatus and automatic titration (Breteler et al., 1972). In the same alcoholic extract total free amino compounds were estimated according to Rosen (1957), with a ninhydrin-hydrindantin mixture and dimethyl sulphoxide instead of methyl cellosolve as solvent (Moore, 1968). Results were calculated as mmoles glutamine per kg dry matter. Water-soluble carbohydrates were estimated after extraction under reflux with boiling demineralized water and clarification with Carrez reagents. Total water-soluble carbohydrates were determined with a Technicon auto-analyser with anthrone reagent. Glucose, fructose and sucrose were determined in the extracts by enzymatic tests (Anon., 1971): glucose by hexokinase and glucose 6-P-dehydrogenase, fructose by P-glucose-isomerase and then as for glucose, and sucrose by  $\beta$ -fructosidase and then as for glucose.

## Results

The plants (10 per cultivation box) were grown under constant conditions for periods of about 6 weeks. Per box 5 plants were harvested, separated into leaves (tops) and roots with hypocotyledon (roots), weighed, dried at 70 °C for 24 h, weighed again, ground and analysed. A part of the plants was kept apart for analysis of fresh material. The roots were rinsed for 1 minute in 0.01 N HCl and then cleaned with demineralized water. During the experiments H<sup>+</sup> or OH<sup>-</sup> production was recorded continuously. The experiments were repeated several times under the same conditions.

Experiments with one N source were carried out after experiments with the other. All results are averages of samples of 2 cultivation boxes (Fig. 1) and data on inorganic composition are averaged duplicates.

## Results

### *Dry matter production*

The fresh and dry weights of tops and roots of sugar-beet plants with about 15 leaves grown with nitrate or ammonium for a period of about 6 weeks is given in Table 3.

Table 3. Fresh and dry weight (g) of tops and roots of 10 sugar-beet plants grown on different N sources. Age of the plants in days after transfer to the nutrient solution.

Age (days)	N source	Tops		Roots		Total	
		fresh	dry	fresh	dry	fresh	dry
38	NO <sub>3</sub>	424	42	195	11	619	53
42	NH <sub>4</sub>	486	47	240	10	726	57

The dry matter yield of the treatments was of similar magnitude. The small difference in age does not influence this conclusion. In 42 days at a relative growth rate of 5 % per day (own observations and van Egmond & Houba, 1970) the NO<sub>3</sub> plants would have yielded 64 g DM per 10 plants, which means 12 % more dry matter. With this small difference the results can be considered independent of difference in yield.

#### *Inorganic chemical composition*

Results are shown in Table 4. The total inorganic cation content (C) in the tops is higher and the total inorganic anion content (A) is lower with NO<sub>3</sub> nutrition than with NH<sub>4</sub> nutrition. In the roots A was much higher in NH<sub>4</sub> plants than in NO<sub>3</sub> plants, and although C is somewhat higher, the cationic composition of the roots was the same for both nitrogen sources.

The content of monovalent and divalent cations in the tops was influenced by the N form in different ways. There was not much difference in cationic composition of roots and in dry matter distribution between plant parts and the corrected dry matter differed by only 12 %. Therefore, it can be inferred that uptake of the monovalent cations sodium and potassium was mainly repressed by ammonium nutrition (see composition whole plant). This agrees with the findings of Epstein (1962), on competitive uptake among equally charged cations. De Jaegere et al. (1963) found a decrease mainly in monovalent cation content in the roots, and in Mg and Ca content in the stems of tomato plants transferred from nitrate to ammonium medium. Data on nutrient uptake by the plants are given in Table 5.

Table 4. Inorganic constituents and organic N in tops and roots and in whole sugar-beet plants after about 6 weeks growth on nitrate or ammonium medium. Contents in meq or mmol (N)/kg DM. NO<sub>3</sub>: 38 days, 53 g DM; NH<sub>4</sub>: 42 days, 57 g DM.

	NO <sub>3</sub>			NH <sub>4</sub>		
	tops	roots	whole plant	tops	roots	whole plant
K	2003	1550	1888	1264	1582	1315
Na	679	182	585	414	182	316
NH <sub>4</sub>	27	15	25	160	50	140
Mg	1136	341	962	1025	374	948
Ca	427	154	377	430	134	369
C	4272	2242	3837	3293	2322	3088
Cl	226	283	246	918	928	920
SO <sub>4</sub>	98	31	94	87	88	87
H <sub>2</sub> PO <sub>4</sub>	306	387	321	1296	708	1104
NO <sub>3</sub>	324	523	377	0	0	0
A	954	1224	1038	2301	1724	2111
N <sub>tot</sub>	3182	2935	3152	4057	2970	3704
N <sub>org</sub>	2831	2397	2749	3897	2920	3564
C-A	3318	1018	2799	992	598	977

C = sum of inorganic cations in meq/kg DM.

A = sum of inorganic anions in meq/kg DM.

N<sub>org</sub> = N<sub>tot</sub> - N<sub>nitrate</sub> - N<sub>ammonium</sub>

	NO <sub>3</sub>	NH <sub>4</sub>	NO <sub>3</sub> /NH <sub>4</sub>
K	121	75	1.61
Na	37	18	2.06
NH <sub>4</sub>	1	8	0.13
Mg	62	54	1.15
Ca	24	21	1.14
N <sub>tot</sub>		211	0.96
C	245	176	1.39
C <sub>a</sub>	244	379	0.64
Cl	16	52	0.31
SO <sub>4</sub>	6	5	1.20
H <sub>2</sub> PO <sub>4</sub>	21	63	0.33
NO <sub>3</sub>	24	0	
N <sub>tot</sub>	202		0.96
A	67	120	0.56
A <sub>a</sub>	253	132	1.92
C-A	178	56	3.18
C <sub>a</sub> -A <sub>a</sub>		247	
A <sub>a</sub> -C <sub>a</sub>	9		
N <sub>org</sub>	178	203	0.88
S <sub>org</sub> *	8	12	

Table 5. Inorganic cations, inorganic anions and nitrogen in 10 sugar-beet plants (meq or mmol (N)) after 42 days of growth on NO<sub>3</sub> or NH<sub>4</sub> media. The data for NO<sub>3</sub> plants of 38 days are recalculated for 42 days, assuming relative growth rate of 5 % per day, no change in shoot/root ratio, and no change in chemical composition between days 38 and 42. The content of most inorganic constituents drops by 1 % per day (Houba et al., 1971).

C, A = sum of inorganic cations and inorganic anions in the plants, respectively.  
C<sub>a</sub>, A<sub>a</sub> = sum of inorganic cations and inorganic anions absorbed by the plants, respectively.

\* S<sub>org</sub> = S<sub>tot</sub> - S<sub>SO<sub>4</sub></sub>, organic sulphur estimated as N<sub>org</sub> × 0.054 (Dijkshoorn, 1964, de Wit et al., 1963).

In both plant parts, the anions Cl and H<sub>2</sub>PO<sub>4</sub> were higher in concentration on ammonium than on nitrate partly because more chloride was available (Table 2). Of course the NH<sub>4</sub> and NO<sub>3</sub> content in the plants was influenced by the N source. Total nitrogen in the NH<sub>4</sub> plants was higher than in the NO<sub>3</sub> plants.

Higher N contents in NH<sub>4</sub> plants (Kirkby (1968) in white mustard plants and Coïc et al. (1962) in tomato plants) may come from a lower yield caused by ambient acidification. But higher nitrogen contents in nitrate nutrition were found by Weissman (1964) in sunflower and Houba et al. (1971) in sugar-beet plants. Such data are not strictly comparable because pH control was not always effective. Data on organic N may be more relevant.

Welte & Werner (1962) investigated growth and composition of several plant species at different NH<sub>4</sub>/NO<sub>3</sub> ratios in the soil, established by synthetic ion exchange resins. The effect of the nitrogen source on the nitrogen content depended on plant species and plant part. With increasing NH<sub>4</sub> supply, the Ca, Mg and K contents of the leaves and the stems decreased.

Organic nitrogen values in the NH<sub>4</sub> plants were 1000 meq/kg DM (tops) or 500 meq/kg (roots) higher than in the NO<sub>3</sub> plants. As observed by many authors, the (C-A) content of nitrate plants is much higher than that of ammonium plants. Free ammonium ions are included in the (C-A) data.

Omission of NH<sub>4</sub> from the calculation lowers (C-A) in the tops of the ammonium plants from 992 to 832, or by 16 %.

### Nutrient absorption

The amounts of nutrients absorbed from the solutions (DM x concentration) are given in Table 5.

The influence of the form of N on the amount of inorganic elements in the plants concerns mainly the anion content, especially chloride and phosphate. Higher P contents were also found by DeKock (1970) in tobacco leaves grown on  $\text{NH}_4$  or urea in place of  $\text{NO}_3$ . To a somewhat lesser extent the cation content too was influenced by the N source.

As mentioned before, replacing  $\text{NO}_3$  by  $\text{NH}_4$  affected monovalent and divalent cation uptake differently. Both total cation and anion absorption were distinctly affected by form of N.

The (C-A) in the  $\text{NO}_3$  plants was more than three times as high as in the  $\text{NH}_4$  plants, with 122 meq more organic anions in the nitrate plants.

### *Hydrogen and hydroxyl ion production and the ionic balance*

#### *Theory*

To keep the plant tissue electroneutral, differential cation-anion uptake is balanced by  $\text{OH}^-$  or  $\text{H}^+$  uptake or release. Whether electroneutrality is maintained by uptake or release of  $\text{H}^+$ ,  $\text{OH}^-$  or  $\text{HCO}_3^-$  (Dijkshoorn & Ismunadji, 1972) is not essential as far as the ionic balance is concerned. The following equations in ion equivalents per plant are valid for the 2 forms of N.

#### *$\text{NO}_3$ nutrition:*

uptake of anions = uptake of cations +  $\text{H}^+$  uptake

$$A_a = C_a + \text{H}^+_a$$

$$(\text{NO}_3 + \text{Cl} + \text{H}_2\text{PO}_4 + \text{SO}_4)_a = (\text{K} + \text{Na} + \text{Mg} + \text{Ca})_a + \text{H}^+_a \quad (1)$$

$$\text{efflux of } \text{OH}^- = \text{H}^+_a = A_a - C_a$$

#### *$\text{NH}_4$ nutrition:*

uptake of cations = uptake of anions +  $\text{OH}^-$  uptake

$$C_a = A_a + \text{OH}^-_a$$

$$(\text{NH}_4 + \text{K} + \text{Na} + \text{Mg} + \text{Ca})_a = (\text{Cl} + \text{H}_2\text{PO}_4 + \text{SO}_4)_a + \text{OH}^-_a \quad (2)$$

$$\text{efflux of } \text{H}^+ = \text{OH}^-_a = C_a - A_a$$

Most of the nitrogenous and sulphate ions are converted into organic N and S compounds and the balance of uptake in terms of plant composition is as follows:

#### *$\text{NO}_3$ nutrition:*

Eq. 1 yields:

$$\text{efflux } (\text{OH}^-) = (\text{N}_{\text{org}} + \text{NO}_3 + \text{NH}_4 + \text{Cl} + \text{H}_2\text{PO}_4 + \text{S}_{\text{org}} + \text{SO}_4) - (\text{K} + \text{Na} + \text{Mg} + \text{Ca}) = \text{N}_{\text{org}} + \text{S}_{\text{org}} - (\text{C-A}) + 2\text{NH}_4 \quad (3)$$

$\text{N}_{\text{org}}$ ,  $\text{S}_{\text{org}}$ , C, A and (C-A) are defined as in Tables 4 and 5, C and (C-A) inclusive  $\text{NH}_4$  ions,  $\text{H}_2\text{PO}_4$  stands for total P because organic and inorganic phosphates are mainly monovalent at the common tissue pH.

#### *$\text{NH}_4$ nutrition:*

Eq. 2 yields:

$$\text{efflux } (\text{H}^+) = (\text{N}_{\text{org}} + \text{NH}_4 + \text{K} + \text{Na} + \text{Mg} + \text{Ca}) - (\text{Cl} + \text{H}_2\text{PO}_4 + \text{S}_{\text{org}} + \text{SO}_4) = \text{N}_{\text{org}} - \text{S}_{\text{org}} + (\text{C-A}) \quad (4)$$

All diurnal  $\text{H}^+$  and  $\text{OH}^-$  production curves showed a decrease in rate in or shortly after the dark period and a constant rate in the light period.

Fig. 2 shows a model. To describe the influence of light and nutrients on the shape of the curves the following characteristics were applied:

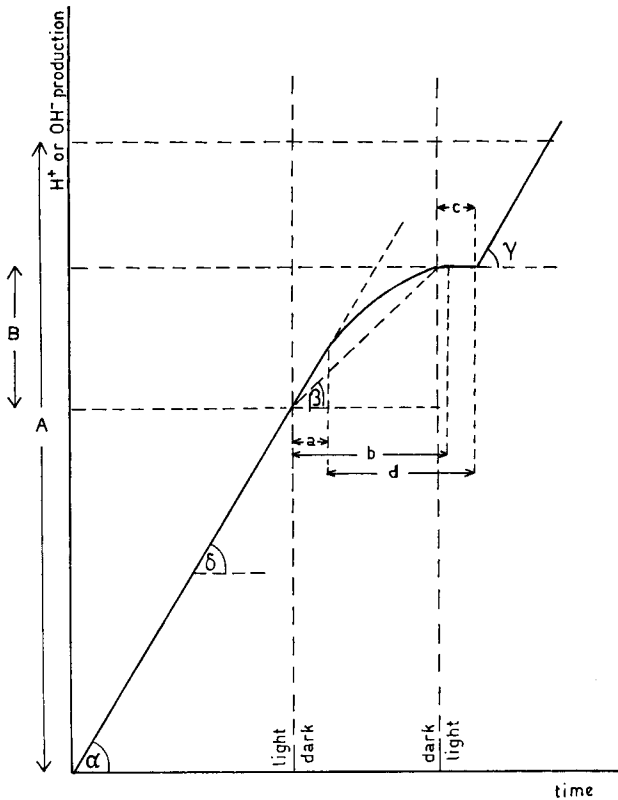


Fig. 2. Idealized shape of a daily  $H^+$  or  $OH^-$  production curve. For explanation, see text.

*In units of time (minutes of hours):*

a: time in which the original rate of  $H^+$  or  $OH^-$  production is maintained in the dark period;

b: time in which the  $H^+$  or  $OH^-$  production rate drops to zero after the beginning of the dark period;

c: time after the start of the light period required to restore a rate of  $H^+$  or  $OH^-$  production (almost) equal to the rate in the middle of the light period (see  $\delta$ );

d: shortest time interval between two (almost) equal rates of  $H^+$  or  $OH^-$  production. Duration of the periods fixed at 05h00–21h00 light, 21h00–05h00 dark.

*In units of  $H^+$  or  $OH^-$  production (meq):*

A: daily  $H^+$  or  $OH^-$  production, e.g. from 09h00 to 09h00;

B:  $H^+$  or  $OH^-$  produced in the dark period.

*In units of  $H^+$  or  $OH^-$  production rate (meq/h):*

$\alpha$ : rate of  $H^+$  or  $OH^-$  production at 09h00 or any other suitable time;

$\beta$ : mean rate of  $H^+$  or  $OH^-$  production in the dark period;

$\gamma$ : rate of  $H^+$  or  $OH^-$  production after restart of acidification or alkalization;

$\delta$ : rate at any time, e.g. if the curves are not straight; e.g.,  $\delta_{14}$  means  $\delta$  at 14h00.



Fig. 2 is based on results with ryegrass, corn, sugar-beet, spinach and wheat under controlled and natural climatological conditions on solutions with ammonium, nitrate or both N forms.

**Results**

Substitution of the data of Table 5 in Eq. 3 and 4 yields the following results in meq per 10 plants.

NO<sub>3</sub> nutrition: (3) efflux (OH<sup>-</sup>) = 178 + 8 - 178 + 2 = 10 meq OH<sup>-</sup>

NH<sub>4</sub> nutrition: (4) efflux (H<sup>+</sup>) = 203 - 12 + 56 = 247 meq H<sup>+</sup>

Hydrogen and hydroxyl ion production integrated over the test period is represented in Fig. 3.

The results show good agreement between the recorded H<sup>+</sup> production of 250 and C<sub>a</sub> - A<sub>a</sub> of 247 in NH<sub>4</sub> nutrition. For nitrate nutrition the recorded OH<sup>-</sup> production of 30 (35 days) was at least 3 times as high as the calculated value of 9 (42 days). Here the difference A<sub>a</sub> - C<sub>a</sub> is small compared with A<sub>a</sub> and C<sub>a</sub> and sampling or analytical errors are important. A 5% error in the determination of organic nitrogen or (C-A) corresponds with 9 meq OH<sup>-</sup> production. Moreover, the measurement of H<sup>+</sup> or OH<sup>-</sup> production was less accurate in the first week.

Fig. 4 shows a considerable daily variation in H<sup>+</sup> and OH<sup>-</sup> production rate. At the

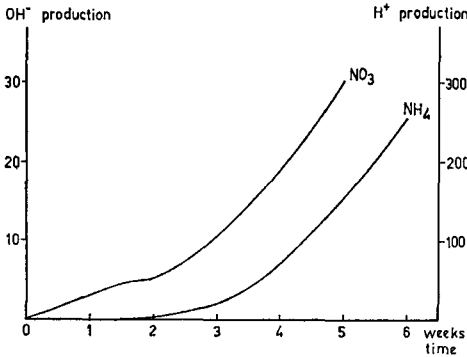


Fig. 3. Cumulative H<sup>+</sup> (NH<sub>4</sub> nutrition) and OH<sup>-</sup> (NO<sub>3</sub> nutrition) production expressed as meq per 10 plants in the course of time.

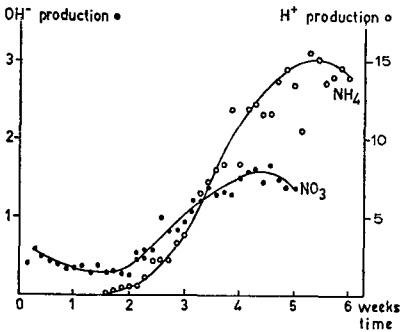


Fig. 4. The daily production rate of OH<sup>-</sup> (NO<sub>3</sub> nutrition) and H<sup>+</sup> (NH<sub>4</sub> nutrition) ions, expressed as meq per 10 plants per day (09h00-09h00).

end of the test the rate decreased with both nitrogen sources. The  $H^+$  or  $OH^-$  production lines are not always straight as drawn in Fig. 2 (see following figures). The decrease in rate is not always to zero rate, while this may be reached with the dark period as well. Decrease in  $H^+$  or  $OH^-$  production depends on plant species, stage of development, photoperiod etc. Of course more characteristics of the diurnal pattern of  $H^+$  or  $OH^-$  production could be derived from the curves, but then little is gained in view of the large variation in rate from day to day (see Fig. 4 where the daily production is plotted against time).

The diurnal pattern of  $OH^-$  production by plants grown on nitrate medium for 20, 30 or 42 days is given in Fig. 5, 6 and 7. With age  $a$  (time interval in which 21h00 production rate is maintained),  $b$  (time from 21h00 to zero production),  $A$  (daily pro-

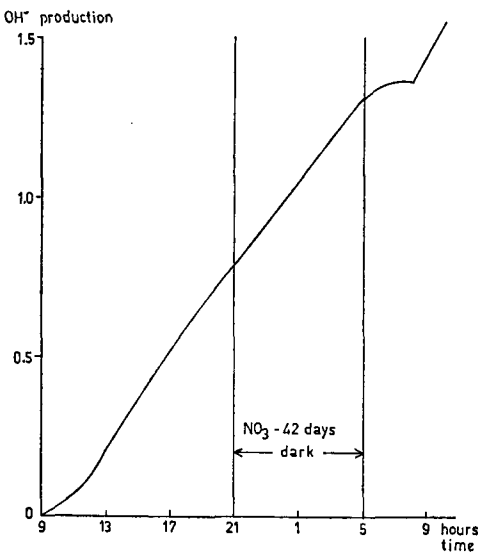
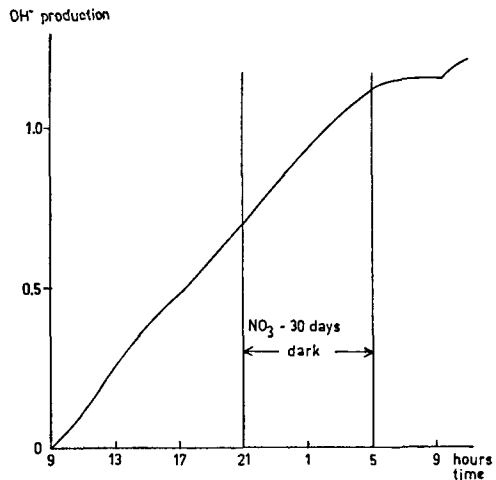
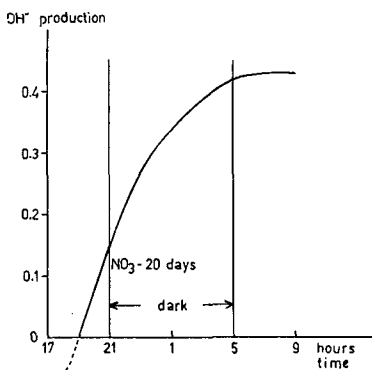


Fig. 5 (top left). Diurnal pattern of  $OH^-$  production of 10 sugar-beet plants (meq), 20 days after transfer to the nutrient solution. In Fig. 2,  $a = 2.3$  h,  $b = 8.3$  h,  $B = 0.28$  meq,  $\delta_{21} = 0.07$  meq/h,  $\beta = 0.03$  meq/h.

Fig. 6 (top right). Diurnal pattern of  $OH^-$  production of 10 sugar-beet plants (meq), 30 days after transfer to the nutrient solution.  $a = 5.3$  h,  $b = 9.5$  h,  $c = 4.2$  h,  $d = 7.2$  h,  $A = 1.15$  meq,  $B = 0.42$  meq,  $B/A = 37\%$ ,  $\alpha = 0.05$  meq/h,  $\beta = 0.05$  meq/h,  $\gamma = 0.04$  meq/h,  $\delta_{21} = 0.06$  meq/h.

Fig. 7 (left). Diurnal pattern of  $OH^-$  production of 10 sugar-beet plants (meq), 42 days after transfer of the nutrient solution.  $a = 7.3$  h,  $b = 9.7$  h,  $c = 2.9$  h,  $d = 4.0$  h,  $A = 1.54$  meq,  $B = 0.52$  meq,  $B/A = 34\%$ ,  $\alpha = 0.04$  meq/h,  $\beta = 0.07$  meq/h,  $\gamma = 0.09$  meq/h,  $\delta_{21} = 0.07$  meq/h.

duction), B (production in the dark),  $\beta$  (mean dark production rate),  $\gamma$  (regained rate) and  $\delta_{21}$  (production rate at the end of the light period) increase, while c (time from 05h00 to production restart) and d (time interval between two high production rates) decrease. The initial slope at 21h00 ( $\delta_{21}$ ) is maintained for at least several hours of darkness while the rate of  $\text{OH}^-$  production does not fall to zero until early in the subsequent light period. In general, the older the plants were, the longer the  $\text{OH}^-$  production rate concluding the light period ( $\delta_{21}$ ) was maintained in darkness and the longer the plants took to completely stop  $\text{OH}^-$  production.

Young plants had longer periods with zero production rates than older plants.

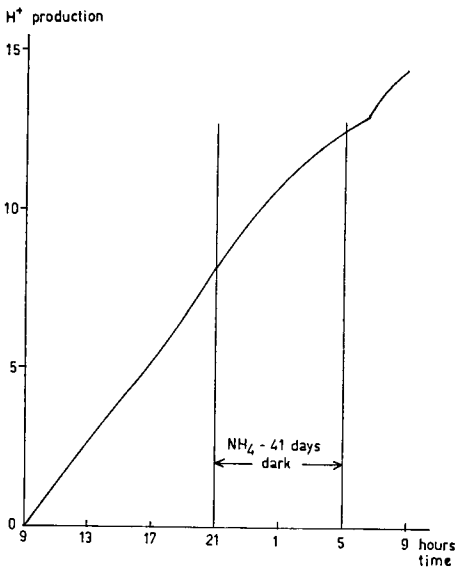
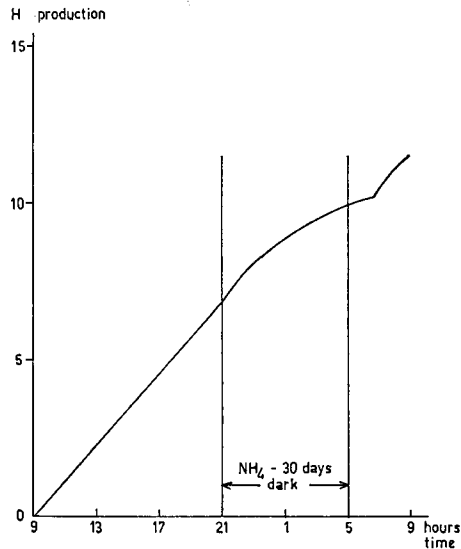
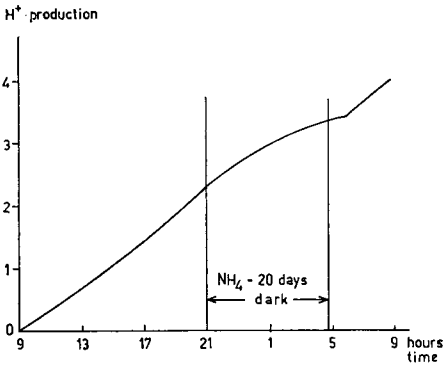


Fig. 8 (top left). Diurnal pattern of  $\text{H}^+$  production of 10 sugar-beet plants (meq), 20 days after transfer to the nutrient solution.  $a = 0.9$  h,  $c = 1.2$  h,  $d = 8.1$  h,  $A = 4.1$  meq,  $B = 1.1$  meq,  $B/A = 27\%$ ,  $\alpha = 0.16$  meq/h,  $\beta = 0.14$  meq/h,  $\gamma = 0.20$  meq/h,  $\delta_{21} = 0.20$  meq/h.

Fig. 9 (top right). Diurnal pattern of  $\text{H}^+$  production of 10 sugar-beet plants (meq), 30 days after transfer to the nutrient solution.  $a = 2.0$  h,  $c = 1.6$  h,  $d = 7.6$  h,  $A = 11.5$  meq,  $B = 3.0$  meq,  $B/A = 26\%$ ,  $\alpha = 0.6$  meq/h,  $\beta = 0.4$  meq/h,  $\gamma = 0.6$  meq/h,  $\delta_{21} = 0.6$  meq/h.

Fig. 10 (left). Diurnal pattern of  $\text{H}^+$  production of 10 sugar-beet plants (meq), 41 days after transfer to the nutrient solution.  $a = 1.8$  h,  $c = 1.6$  h,  $d = 7.8$  h,  $A = 14.4$  meq,  $B = 4.4$  meq,  $B/A = 31\%$ ,  $\alpha = 0.7$  meq/h,  $\beta = 0.5$  meq/h,  $\gamma = 0.6$  meq/h,  $\delta_{21} = 0.7$  meq/h.

The increase in total daily production is shown by Fig. 4. The ratio B : A was close to 8 : 24 indicating a small effect of the dark period on the total daily  $\text{OH}^-$  production. Darkness affects the  $\text{OH}^-$  production obviously early in the subsequent light period.

The curves show that the older plants were, the less darkness affected  $\text{OH}^-$  production.

The diurnal  $\text{H}^+$  production by plants on the ammonium medium for 20, 30 or 41 days is given in Fig. 8, 9 and 10. With increase in age its pattern was fairly constant. A, B and related characteristics  $\alpha$ ,  $\beta$  etc. increase (see Fig. 4), c tends to increase and a tends to decrease. Production of  $\text{H}^+$  ions does not stop completely in or after the dark period. With increasing age  $\text{H}^+$  production of the plants seems to be more affected by darkness. On nitrate,  $\text{OH}^-$  production became less light-dependent in the course of growth.

Compared with the  $\text{OH}^-$  production curves the slope of the curve decreases earlier after darkness (a: 1-2 h for  $\text{NH}_4$ , 2-7 h for  $\text{NO}_3$ ). This reflected in the B : A ratio, which is lower than 8 : 24 for  $\text{NH}_4$  nutrition.

On nitrate medium there is also some decrease in slope in the dark but the slope at 21h00 ( $\delta_{21}$ ) is maintained so long that the B : A ratio is equal to or higher than 8 : 24 because the zero rate of  $\text{OH}^-$  production is found early in the light period. From Fig. 5 to 10 it can be concluded that the  $\text{H}^+$  production rate was more light-affected than  $\text{OH}^-$  production since the effect of darkness is earlier observed, although there is no complete stop in  $\text{H}^+$  production, as for  $\text{OH}^-$  production.

Trends in the curve characteristics with age as described for  $\text{NH}_4$  or  $\text{NO}_3$  nutrition were averaged from all daily recording sheets. It is not useful to represent all daily graphs, which differed appreciably. For instance in the  $\text{NH}_4$  experiment values for a varied between 32 and 128 minutes and for c between 20 and 136 minutes.

### Carboxylates

Any excess of inorganic cations over inorganic anions (C-A) within plants is balanced by carboxylate anions. Table 6 shows contents of the detected carboxylates in the tops, roots and the whole plants.

Table 6. Carboxylates and (C-A) in tops, roots and whole sugar-beet plants grown for 6 weeks on nitrate or ammonium medium. Contents in mcg/kg DM, (C-A) from Table 4.  $\text{NO}_3$ : 38 days, 53 g DM;  $\text{NH}_4$ : 42 days, 57 g DM.

	$\text{NO}_3$			$\text{NH}_4$		
	tops	roots	whole plant	tops	roots	whole plant
Fumarate	8	12	9	12	12	12
Succinate	8	8	8	8	10	8
Malonate	28	16	26	14	16	14
Oxalate	2412	920	2102	1045	990	1037
Malate	60	31	54	42	16	37
Citrate	184	228	193	38	26	35
Sum	2700	1215	2392	1159	1070	1143
(C-A)	3318	1018	2799	992	598	977

The carboxylate content of the plants parallels the (C-A) contents of Table 4, with the highest contents in  $\text{NO}_3$  plants. Tops of nitrate plants have higher carboxylate contents than roots. In many plant species, nitrate metabolism takes place mainly in the leaves (Beevers & Hageman, 1969; Ben Zioni et al., 1971) and a part of the carboxylates generated during nitrate reduction (in sugar-beet plants mainly oxalates) moves downward. Of the carboxylates in the  $\text{NO}_3$  plants 11 % was present in the roots.  $\text{NH}_4$  uptake influences the carboxylate pool so that it is always low due to rapid extraction of keto acids and the formation of organic nitrogen compounds (Wakiuchi et al., 1971).

The synthesis of organic N compounds in the dry matter of nitrate plants (e.g. mmol  $\text{N}_{\text{org}}$  /kg DM) proceeds at a slower rate due to the rate controlling nitrate reduction. The difference between the carboxylate content in tops and roots of  $\text{NH}_4$  plants is small compared with the difference in nitrate plants. This may be because production of organic N is not restricted to the tops in  $\text{NH}_4$  plants. In these plants 20 % of the carboxylates are present in the roots. Lower contents of citrate and malate in  $\text{NH}_4$  plants than in  $\text{NO}_3$  plants may indicate that oxaloacetic acid is an important keto acid in the nitrogen assimilation process in sugar-beet plants. The high oxalate contents in the  $\text{NH}_4$  plants show that oxalate is also readily synthesized in ammonium nutrition.

### Carbohydrates

Total water-soluble carbohydrates, glucose, fructose and sucrose in different plant parts and in whole plants grown on  $\text{NO}_3$  or  $\text{NH}_4$  medium are listed in Table 7. It is clear that ammonium has a depressive effect on the water-soluble carbohydrate content in both plant parts. As there is no notable concentration effect caused by growth difference between the media, this observation confirms the idea that  $\text{NH}_4$  nutrition leads to a depletion of carbohydrate reserves in the plant (Kirkby & Hughes, 1970; Mulder, 1956). Differential soluble-carbohydrate consumption under the two N regimes may explain why  $\text{OH}^-$  production becomes less and  $\text{H}^+$  production more light-affected with increase in plant age. Concentrations of reducing sugars in the plant are not altered as much as the total soluble-carbohydrate pool by the kind of nitrogen supply. Of course, the test plants are not comparable with full-grown sugar-beet plants, but it should be noticed that nitrate plants contain more than twice as much soluble carbohydrates as ammonium plants. Sucrose content is also higher in nitrate nutrition.

Table 7. Total water-soluble carbohydrates, glucose, fructose and sucrose content (% of DM) of tops, roots and of whole sugar-beet plants grown for a period of about 6 weeks on nitrate or ammonium medium.  $\text{NO}_3$ : 38 days, 53 g DM;  $\text{NH}_4$ : 42 days, 57 g DM.

	$\text{NO}_3$			$\text{NH}_4$		
	tops	roots	whole plant	tops	roots	whole plant
Glucose	0.58	0.85	0.63	0.38	0.40	0.38
Fructose	0.27	0.40	0.30	0.49	0.33	0.47
Glucose + fructose	0.85	1.25	0.93	0.87	0.73	0.85
Sucrose	4.24	4.28	4.25	2.98	2.51	2.90
Total	10.3	8.9	10.0	4.7	3.9	4.6

*Free nitrogenous compounds*

The content of ammonium ions, free glutamine, asparagine and amino acids is given in Table 8. Contents in DM and as % of total N are higher in ammonium nutrition than in nitrate nutrition and most of the free N is in the amides. With  $\text{NH}_4$  the value of the glutamine/asparagine quotient is higher than with nitrate. The content of ammonium ions in both tops and roots may be high and comparable to values for sulphate, sodium and calcium in plant parts.

It is clear that  $\text{NH}_4$  should be included in the calculation of (C-A) particularly in  $\text{NH}_4$  plants. In contrast to the situation in gramineous plants like rice (Zsoldos, 1962) and wheat (Breteler, unpublished data) the  $\text{NH}_4$  content in the tops is higher than in the roots in dry as well as in fresh material.

$\text{NH}_4$  and the free nitrogenous metabolites are considerably higher in tops than in roots of  $\text{NO}_3$  plants, while they are on the same level in tops and roots of  $\text{NH}_4$  plants, suggesting that nitrate is metabolized by the aerial parts. The data on free N compounds and carboxylates (Table 6) show that the carboxylate content and the free amino acid and amide content in tops of  $\text{NO}_3$  plants are three times as high as in roots.

It is also clear that a higher organic N content is associated with a lower carbohydrate content, because the latter compounds supply C skeletons for the synthesis of the amino acids.

Table 8. Ammonium, soluble amides and amino acids in tops, roots and in whole 6-week old sugar-beet plants grown on nitrate or ammonium medium. Content in meq  $\text{NH}_4^+$ /kg DM, mmol/kg DM (amides) and mmol N/kg DM (amino acids and amides).  $\text{NO}_3$ : 38 days, 53 g DM;  $\text{NH}_4$ : 42 days, 57 g DM.

	$\text{NO}_3$			$\text{NH}_4$		
	tops	roots	whole plant	tops	roots	whole plant
$\text{NH}_4$	27	15	25	123	74	114
Glu. $\text{NH}_2$	63	10	52	141	149	143
Asp. $\text{NH}_2$	76	38	68	47	55	48
Glu. $\text{NH}_2$ +Asp. $\text{NH}_2$	139	48	120	188	204	191
Glu. $\text{NH}_2$ /Asp. $\text{NH}_2$	0.83	0.26	0.71	3.00	2.71	2.95
Amino acids and amides	317	102	272	—	—	471*
Amino acid-N and amide-N as % of total N	10	3	9	—	—	13

\* As no data were available, the contents in whole plants of additional experiments are represented (Breteler, 1973).

**Discussion***Plant nutritional aspects*

The relatively small difference in dry matter production on nutrient solutions with ammonium and nitrate in the present experiment is due to the control of the nutrient solution pH. In most other experiments without pH control sugar-beets and other plants

yielded less dry matter on  $\text{NH}_4$  medium than on  $\text{NO}_3$  medium. The present results could not support the view that there exists a normal (C-A) content, required for optimum growth (van Tuil, 1965; Dijkshoorn, 1964; de Wit et al., 1963), because the low relative growth rate indicates that growth was far from optimum and did not change with the large difference in carboxylate content between treatments.

Chouteau (1963) found that tobacco plants grown on  $\text{NH}_4$  had the same dry matter production as on  $\text{NO}_3$ , if he added 12 meq  $\text{HCO}_3^-$  per litre. The pH was adjusted with acid so that there was a certain control of acidity. Other techniques for plant growth at controlled pH have been described by Audus (1949), Pitman (1970), Kirkby & Hughes (1970), Weissman (1972) and others.

None of the described techniques unifies the advantage of pH maintenance with continuous registration of the hydrogen or hydroxyl ion production.

Eq. 4 shows that of the 247 meq  $\text{H}^+$  released by 10 plants, 203 originate from organic nitrogen and 8 from apparently unmetabolized  $\text{NH}_4$  ions, 86 % being due to uptake of  $\text{NH}_4$  ions and 14% to the uptake of non-nitrogenous cations over anions. Dijkshoorn & Ismunadji (1972) found for the shoots of rice plants grown on  $\text{NH}_4$  medium  $\text{N}_{\text{org}} = 320$  meq and (C-A) = 63 meq per 4 plants, which means that although free ammonium ions were not taken into account 84 % of the corresponding acidification was caused by  $\text{NH}_4$  absorption. In other plants (e.g. oil palm and corn) we found this percentage always between 85 and 90.

Therefore the  $\text{H}^+$  production is roughly equivalent to the  $\text{NH}_4$  uptake and this is the more true if the excess uptake of non-nitrogenous cations of anions varies in proportion to the  $\text{NH}_4$  uptake, so that the proportion of total acidification caused by  $\text{NH}_4$  uptake is constant. This would make the equipment for pH control a recording ammonium electrode, helpful in research on  $\text{NH}_4$  uptake.

There is no reliable ion specific electrode for ammonium, the present electrodes are still too sensitive to  $\text{K}^+$  and  $\text{H}^+$  (Simon et al., 1970).

Reading  $\text{NH}_4$  uptake for  $\text{H}^+$  production allows the following conclusions.

1. Ammonium uptake per plant increases with time (Fig. 3) and the daily  $\text{NH}_4$  uptake per plant reaches a maximum between 5 and 6 weeks after transfer to the nutrient solution (Fig. 4). Decreased ammonium uptake may be related to the onset of beet growth and internal redistribution of nitrogen.
2. The ammonium uptake rate decreases in the dark period (1–2 hours after darkness) but not to zero. About 1½ hours after reillumination the effect of the dark is overshadowed by the effect of light (Fig. 8, 9 and 10). Under the present experimental conditions the  $\text{NH}_4$  uptake per plant per hour was almost constant in the light period.
3. In a normal diurnal cycle the sugar-beet plants did not completely stop  $\text{H}^+$  production ( $\text{NH}_4$  uptake). This result is contradictory to the view of Prianishnikov (1951) that young sugar-beet plants are strongly dependent on carbohydrate of light for their ammonium absorption because of their carbohydrate poverty. In gramineous plants like corn, perennial ryegrass and wheat (Breteler, unpublished data) a decrease to zero rate of  $\text{H}^+$  production in the dark period was observed under exactly the same climatological conditions.

Recently some authors (Pitman, 1970, and others) related  $\text{H}^+$  production by plants to the ion uptake process. For this purpose the experimental technique I described offers facilities, because it can be used in small scale and short-term experiments as well (Breteler, unpublished data).

Pitman found a hydrogen ion production by excised low-salt barley roots from 50 to  $250 \times 10^{-6}$  meq per g fresh root per hour at pH 5.3 and  $600 \times 10^{-6}$  meq at pH 4.2.

Maximum  $H^+$  production in the present experiments was 20 to 30 meq per 10 plants per day at pH 5.5 corresponding with about  $400 \times 10^{-6}$  meq per g fresh root per hour. This figure fits in between the two maximum rates measured by Pitman. As far as the production of  $OH^-$  is concerned there is no possibility (Eq. 3) of relating the curves to nitrate uptake. It is known that in sugar-beet plants grown with nitrate the contents of organic nitrogen and (C-A) are roughly equivalent (Houba et al., 1971), in other plant species the possibility of relating  $OH^-$  release to some extent with  $NO_3$  uptake may be considered. Of course one must realize that the  $H^+$  or  $OH^-$  production is never caused by the uptake of one single ion but by the difference in positively charged and negatively charged ions. It was observed that with the increase of age the  $OH^-$  production became less and the  $H^+$  production affected by darkness.

Michael et al. (1970) found that the carbohydrate content of sugar-beet roots decreased with age. A great difference in soluble-carbohydrate content (Table 7) under influence of the two N forms after about six weeks growth was observed. These findings suggest a strong mutual effect between the uptake of nitrogen in the form of  $NH_4$  or  $NO_3$  and the carbohydrate reserve.

A question that rises is: what is the effect of respiration on the apparent  $H^+$  or  $OH^-$  production? At pH 5.50 nearly all the carbon dioxide released by root respiration is in the form of  $H_2CO_3$  and not in the form of  $HCO_3^-$  and a possible effect of respiration remains small.

If  $CO_2$  production had an effect than the pH value should have changed because of the interference of  $CO_2$  from the air when the system runs without plants. This change in pH was never observed.

#### *Practical aspects*

Some practical aspects are summarized in Table 9. The properties of the mature sugar-beets were predicted from characteristics of the 6-week old plants, used in the present experiments.

Of course it must be realized that dry matter yields under field conditions have been reported to be unequal between the two considered nitrogen sources and that fertilizer leaching, nitrification and soil acidification interferes. It is concluded that taking all aspects of  $NO_3$  and  $NH_4$  nutrition together nitrate is a better N source for beet sugar

Table 9. Predictions for some properties of sugar-beets grown on  $NH_4$  or  $NO_3$  based on the results of the present water culture experiments. + = favourable; — = unfavourable.

Feature	$NO_3$	$NH_4$
Dry matter yield	+	+
Sugar content	+	—
Sugar yield	+	—
Harmful N content	+	—
Na and K content	—	+
Oxalate content	—	+
$NO_3$ content	—	+



production than ammonium. This conclusion was drawn long ago in agricultural practice.

For related crops not grown for sugar, however, it is possible that, depending on the specific quality characteristics,  $\text{NH}_4$  may be as good a nitrogen source as  $\text{NO}_3$ .

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