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The influence of nitrate nutrition on H^+ efflux by young rape plants (*Brassica napus* cv. emerald)

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Summary Changes in pH around the roots of young rape plants (*Brassica napus* cv. emerald) were studied using a nutrient film technique that allowed part or whole of the root system to be subjected to specific nutrient treatments. The rapidity and direction of pH change was assessed by imbedding absorbing roots in a thin film of agar containing bromocresol purple. When nitrate-fed plants were deprived of all sources of nitrogen at 15 or 17 days old, the release of H ions from the roots was immediate and uniformly distributed over the root length. When nitrate was witheld from half of the root system of nitrate-fed plants, the roots deprived of nitrate immediately started to produce H ions even though the nitrate-fed half of the root system continued to supply the whole of the plant with nitrate. However, the rate of H ion production in plants partly supplied with NO_3 was less than in plants completely deprived of NO_3. It is suggested that malate produced in the shoots, following nitrate reduction, may be redistributed to the roots deprived of nitrate. There, HCO_3 produced by the decarboxylation of the malate masks some of the expected H ion efflux.

Introduction

Interactions between plant roots and the soil in which they are growing have recently received an increasing amount of attention. In particular, the ability of plants to change the pH of the rhizosphere is important since the availability to the plants of many elements is affected by the pH. This change in soil pH may be confined to a very restricted area, only a few millimetres surrounding an individual root[10]. This effect has been known for over a century, one of the earliest reports being that of Sachs (1859)[22] who demonstrated that exudates from roots could etch a polished marble slab. But the causes of the effect remain unresolved.

Lowering of pH has been found to be associated with iron and zinc deficiences[2,10,21] and the uptake of nitrogen as ammonium[20]. Nitrogen in the nitrate form seems almost universally to lead to an increase in pH[3,4,5]. It has also been shown that the amounts of acid or base liberated by roots is associated with the excess uptake of cations over anions or of anions over cations respectively. Our earlier work[6,7] demonstrated

that nitrate-fed, phosphate deficient rape plants could reduce the rhizosphere pH 1.5–2 units.

Preliminary work showed that it was possible to demonstrate rapid changes in the pH of solutions circulating through small flowing nutrient systems in which rape plants were growing. We therefore decided to investigate the relationship between NO_3 ion concentration in the solution bathing the roots (with all other nutrients being adequately supplied) and the net H ion and HCO_3 ion efflux from these roots in a flowing nutrient film system. We also wished to know which areas of the root extrude HCO_3 ions or H ions and a colorimetric method was used to demonstrate this. Other workers[1,4,9] have found that HCO_3 ion production may be associated with the activity of the enzyme nitrate reductase. No information was available on the location of this enzyme in rape and, therefore, the activity of the enzyme was measured in the roots and leaves.

Experiment 1.

The effect of NO_3 removal on H ion or HCO_3 ion release from roots

Materials and methods

The experiment was carried out in a controlled environment cabinet at a temperature of 25°C, a photoperiod of 16 hours and a light intensity of 180 μ E m^{-2} s^{-1} P.A.R. The plants were grown in a nutrient film system described in an earlier paper[14]. Briefly, the apparatus consisted of shallow "Perspex" trays each divided into two compartments or channels through which a thin film of nutrient solution was circulated at 9 cm^3 min^{-1}. In this experiment identical solutions ran through both channels. The reservoir for each tray contained 500 cm^3 of nutrient solution at an initial pH of 6.5. The composition of the nutrient solution was (*M*): NO_3 2×10^{-3}, SO_4 1.5×10^{-3}, PO_4 10^{-4}, Cl 5.5×10^{-4}, Ca 5×10^{-4}, K 3.5×10^{-3}, Mg 3.5×10^{-4}, Mn 5×10^{-4}, Cu 5×10^{-7}, Zn 5×10^{-7}, B 2.5×10^{-8}, Fe(E.D.T.A.) 2.5×10^{-8}, Na 5×10^{-8}. Minus nitrate solutions contained additional KCl at 2×10^{-3} *M*.

The nutrient solution was changed daily and the volume remaining was measured and restored to 500 cm^3 with distilled water to compensate for loss by evaporation, before measurement of the pH. Amounts of HCO_3 ion or H ion produced by the roots were determined by titration to pH 6.5 with 0.01 *M* HCl or 0.01 *M* NaOH using an automatic titrator.

8 trays were used in this experiment and the following experimental treatments were imposed:

1. Control, full nutrient solution: no plants, 1 tray:
2. Control, nutrient solution minus nitrate: no plants, 1 tray.
3. Full nutrient solution: 2 plants per tray, 2 trays.
4. Nitrate supply withheld from plants at 15 days: 2 plants per tray, 2 trays.
5. Nitrate supply withheld from plants at 17 days: 2 plants per tray, 2 trays.

Since it has been shown[4] that plants in flowing culture can maintain adequate total nitrogen levels when supplied with very low concentrations of nitrate, the plants were completely deprived of nitrate in treatments 4 and 5 to induce deficiency.

The experiment finished when the plants were 21 days old.

Nitrogen assay of plants

Soluble nitrogen extract Two g samples of plant material dried at 80°C were ground to pass through a 1 mm sieve, mixed with 50 cm^3 double distilled water and shaken for 30 minutes. The filtrate was analysed for nitrate using a Technicon Series II autoanalyser[11].

Total nitrogen 0.1 g samples of dried plant material were digested by the method of Thomas, Sheard and Moyer[24]. The NH_4 ion concentration of these digests was determined using the autoanalyser[8].

Demonstration of HCO_3 ion and H ion efflux by the roots

The method was based on that developed by Weisenseel, Dorn and Jaffe[25]. When the plants were 14 days old, one lateral was led into the second channel of each tray, where they developed a well spaced root system. An aqueous solution of Bromocresol purple in 1% agar was prepared in either complete nutrient solution at pH 4.8 (yellow) or in the minus nitrate nutrient solution at pH 6.2 (purple). Agar just warm enough to remain liquid (approx 35°C) was quickly poured over the roots to a depth of 1 mm, when it immediately set. The colour changes round the roots were then observed for several hours. This procedure was carried out at the end of the experiment when the plants were 21 days old.

Four sets of roots were used:

1. Roots grown with nitrate throughout the experiment: covered with agar + NO_3
2. Roots grown with nitrate throughout the experiment: covered with agar — NO_3
3. Roots grown for 5 days without nitrate: covered with agar + NO_3
4. Roots grown for 5 days without nitrate covered with agar — NO_3

Nitrate reductase assay

The *in vivo* assay was based on that used by Stewart, Lee and Orebanjo as modified by Osbourne and Whittington[19]. 0.2 g of fresh leaf or root material was taken, 3 hours after the start of the photoperiod. After washing the roots with double distilled water and blotting dry, the plant material was chopped into small pieces and put in a boiling tube with 5 cm^3 of a mixture of 0.1 *M* KNO_3, 1.5% propanol and 0.1 *M* phosphate buffer at pH 7.8. The tubes were kept in ice until all samples in a batch were ready (up to 30 min) and were then evacuated in a desiccator for two 2 min periods. All samples were replicated four times. One replicate of each sample was plunged into boiling water for 5 min immediately after evacuating, the remaining replicates were wrapped in aluminium foil to exclude the light and shaken in a water bath at 30°C for 30 min. The reaction was stopped by boiling the tubes for 5 min. The tubes were then cooled and the nitrite produced determined. 1 cm^3 of 1% sulphanilamide in 25% v/v HCl and 1 cm^2 0.02% N-1-Naphthyl ethylenediamine dihydrochloride were added to 1 cm^3 aliquots of the sample solution. The colour was developed for 15 min and then measured at 540 nm on a spectrophotometer.

Results and discussion, Experiment 1.

HCO_3 ion and H ion production by roots

Fig. 1 shows the cumulative HCO_3 ion or H ion production by the roots on a daily basis. All plants fed plus nitrate solution produced HCO_3 ions in steadily increasing amounts as the plants grew older. However, as soon as the plus nitrate solution was replaced by a minus nitrate solution the roots started to produce H ion and the rate of production also increased with time. The switch from HCO_3 ion to H ion production was so rapid (see below) that very little change in the nitrogen status of the plant could have occurred, suggesting that the

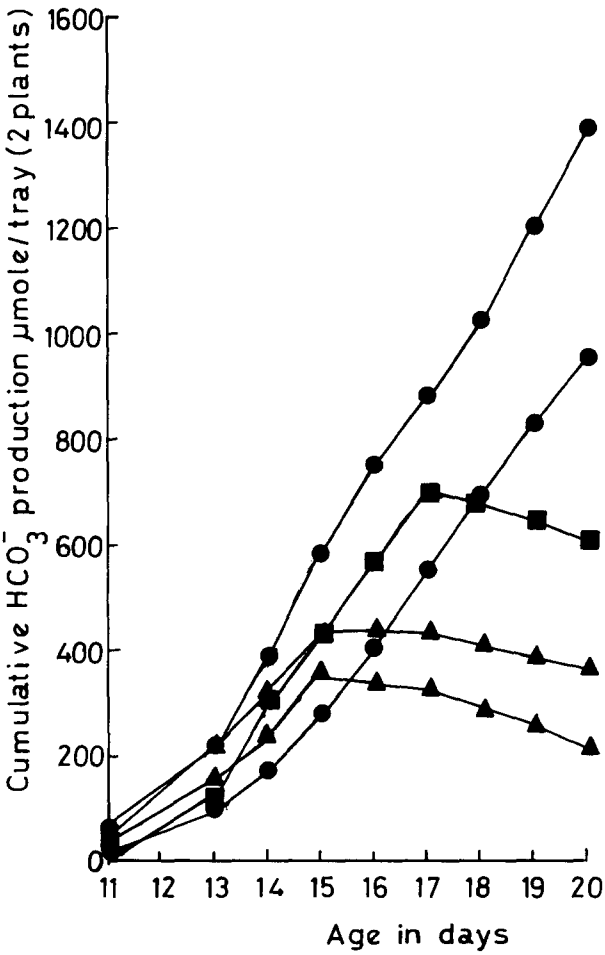

Fig. 1. Experiment 1. Cumulative daily HCO_3 ion production. • plants given NO_3 ion throughout the experiment, ▲ plants deprived of NO_3 ion at 15 days, ■ plants deprived of NO_3 ion at 17 days.

effect is a local one brought about by the change in the environment of the roots. Plants derpived of nitrate at 15 days produced little acid (3–5 μmol) during the first 24 hours of deprivation, but steadily increasing amounts were produced as the plants grew older. Plants deprived of nitrate at 17 days however, produced approximately the same amount of H ions (25 μmol) in the first 24 hours as the plants which had already been deprived of nitrate for 2 days.

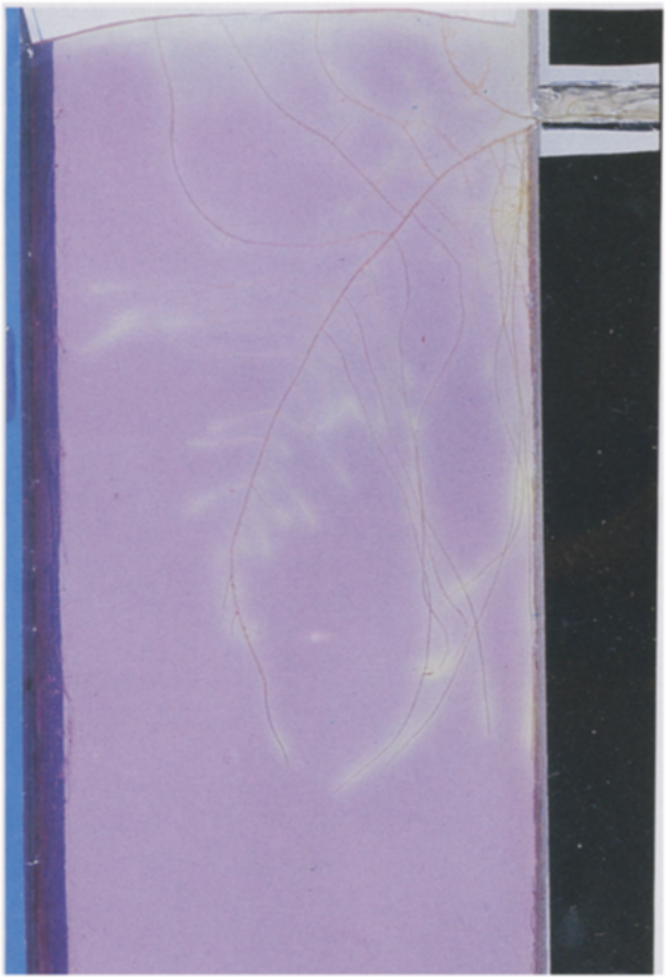

Plate 1. Lateral roots of a plant deprived of nitrate for 5 days, embedded in agar containing bromocresol-purple and nutrient solution minus nitrate. Purple areas indicate the original pH of the agar of 6.2. Yellow zones around the root show lowering of the pH due to H ion production by the root.

Colorimetric demonstration of HCO_3 ion and H ion production

Photograph 1 is of laterals, isolated at 14 days, but subjected to the same experimental conditions as the rest of the roots. Photograph 1 was taken 20 min after pouring minus nitrate agar over roots grown without nitrate for 5 days. All the roots are surrounded by a yellow zone indicating the presence of acid, with possible areas of increased production at the root tips. Thus it can be seen that the change over from HCO_3 ion production to H ion production is extremely rapid. Covering roots from a plant which had been supplied with nitrate

throughout the experiment, with minus nitrate agar, also produced a zone of yellow surrounding the entire length of the root. Plus nitrate agar covering roots deprived of nitrate for 5 days, produced purple zones all round the roots 2 hours after the agar was poured, purple zones indicating the production of HCO_3. Plus nitrate roots covered with plus nitrate agar also produced purple zones all round the roots within 20 minutes of pouring the agar. The production of acid by the entire root surface contrasts with the findings of work on acid production by iron deficient plants where acid is produced only at the root tips.

Nitrogen status of the plants

The concentration of nitrogen was the same in the roots and shoots of plants supplied with nitrate. When plants were deprived of nitrate, concentration decreased in both roots and shoots but more rapidly in the shoots. Since H ion production started immediately the root was deprived of nitrate, it seems unlikely that this resulted from the decrease in the nitrogen status of the plant.

Table 1. Experiment 1, Total nitrogen in rape plants at 20 days

Treatment	% N in shoot	% N in root
NO_3^- throughout experiment	3.4 +/− 0.6*	3.4 +/− 0.1
Minus NO_3^- 3 days before harvest	1.3	2.1
Minus NO_3^- 5 days before harvest	1.3 +/− 0.1	2.6 +/− 0.3

* means and S.E.'s

Nitrate reductase assay

Table 2 shows the nitrate reductase activity (N.R.A.) in the plants at the end of the experiment. The assay shows clearly that nitrate reductase is found only in rape leaves. Plants grown with nitrate showed a high level of nitrate activity in the leaves, while in plants deprived of nitrate for 3 or 5 days there was no detectable activity. Leaves from a plant deprived of nitrate for 5 days and then given approximately

Table 2. Experiment 1. Nitrate reductase activity in rape plants at 20 days (μ mol NO_2^--N/g fresh wt/hr)

Treatment	N.R.A. leaves	N.R.A. roots
NO_3^- throughout experiment	5.8 +/− 1.0*	0
Minus NO_3^- for 3 days before assay	0	0
Minus NO_3^- for 5 days before assay	0	0
Minus NO_3^- for 5 days but 40 μmol of NO_3^- 18 h before assay	1.3 +/− 0.007	0

* means and S.E.'s

40 μmol of nitrate in agar, showed a low level of nitrate reductase activity in the leaves 18 hours after the nitrate had been supplied to the root. Further evidence that in rape the leaves are the major site of N.R.A. was obtained from analysis of xylem sap (Hedley unpubl.) which contained a high level of nitrate in nitrate-fed plants.

Experiment 2.

The effect of removal of nitrate from half the root system

The results of the first experiment suggested that H ion production was independent of the nitrogen status of the plant, but was induced by the absence of nitrate in the immediate environment of the root. A split root experiment to test this hypothesis was carried out in which part of the root was supplied with nitrate and part was not.

Materials and methods

The growing conditions were the same as in the first experiment. Seedlings, produced in the way described in an earlier paper[18], were put into trays with their roots divided equally between the two channels of the tray. Six trays were used in this experiment and the following experimental treatments imposed.

1. Control, full nutrient solution: no plants, 1 tray.
2. Control, nutrient solution minus nitrate: no plants, 1 tray.
3. Both halves of root system supplied with full nutrient solution: 3 plants per tray, 2 trays.
4. Both halves of the root system supplied with full nutrient solution until 14 days when one half of the root system was deprived of nitrate for the remaining 7 days of the experiment: 3 plants per tray, 2 trays.

On day 14 when the minus nitrate treatment was started, one plant was removed from each tray. These plants were analysed for total cation and anion content.

Root lengths were measured each day using Newman's method as modified by Marsh[13,17]. The nutrient solution was changed daily, the change in pH measured and the H ion or HCO_3 ion production was calculated.

At the end of the experiment the plants were analysed for total N, K, Ca, Mg and P using the digest described for experiment 1. In addition S and Na were determined using Mottersheads digestion and a modification by Sansum and Robinson of Garrido's turbidimetric method for sulphur determination[23].

Cl was extracted from dried plant material with 10% v/v HNO_3 and the filtrate analysed on the autoanalyser. P was measured using the method of Murphy and Riley[14], Ca and Mg were measured by atomic absorption spectroscopy, Na and K by emission spectroscopy using a Unichem SP 90A Series 2 spectrophotometer.

Results and discussion, Experiment 2

Plant growth

Up to day 14 all the plants were supplied with nitrate. There was no significant difference between the relative rates of root elongation during this time. Fig. 2 shows examples of the change of root length per plant

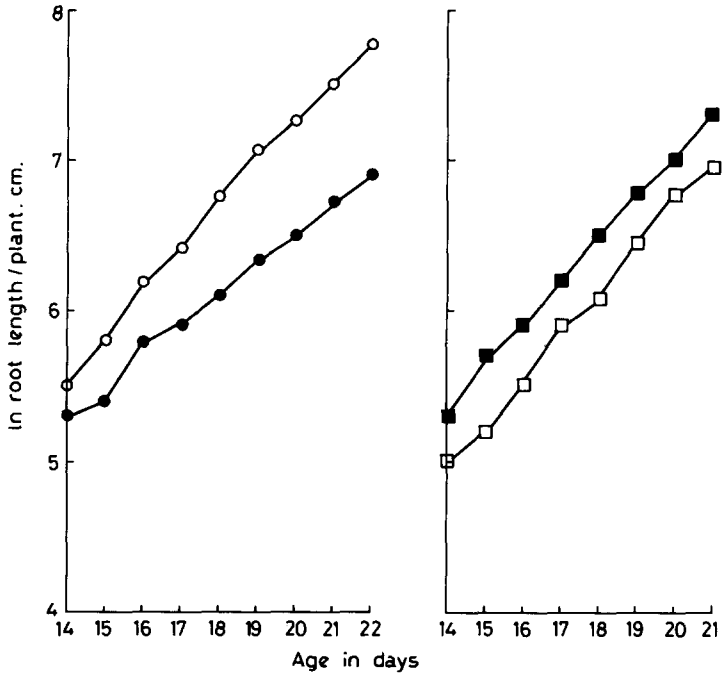

Fig. 2. The change with time of ln mean root length per plant. (a) *Open* and *closed* symbols, two halves of the same root system, ○ given NO_3 ion throughout, ● deprived of NO_3 ion from day 14 onwards. (b) □,■ two halves of the same root system both given NO_3 ion throughout. Linear regressions fitted to this data were:

ln mean root length (cm) = a + bt

	a	b	r^2		a	b	r^2
○	1.6	.28	.99	□	1.5	.27	.99
●	2.5	.20	.99	■	0.98	.28	.99

with time. Fig. 2a shows the growth of 2 halves of the same root system, one half of which was supplied with nitrate the other half was not; Fig. 2b shows the growth of two halves of the same root system, both of which were supplied with nitrate. The relative elongation rate (ln root length/time) was approximately constant for plus and minus roots so that linear regressions could be fitted to the data in Fig. 2. Using the data for the whole experiment, linear regressions and confidence limits, there was no difference between the rate of elongation of roots for which the whole root system was supplied with nitrate (0.305 +/− 0.015 cm cm^{-1} day^{-1}), and the rate of elongation of nitrate-fed roots which were attached to other roots not supplied with nitrate (0.325 +/− 0.045 cm cm^{-1} day^{-1}). However, depriving half of a root system of nitrate reduced the rate of elongation of those roots to 0.200 +/−0.008 cm cm^{-1} day ^{1}. even though the plant was a whole was well supplied with N from the other half of the root system.

The decrease in rate of elongation of the minus nitrate roots was mainly due to fewer laterals being produced, an average of 2.1 per cm compared with 4.2 per cm of nitrate-fed roots at the end of the experiment.

H ion and HCO_3 ion production

Fig. 3 shows the cumulative H ion and HCO_3 ion production by the roots on a daily basis. As in experiment 1 all root systems supplied with nitrate produced HCO_3 ion. However in contrast to experiment 1 H ions were not detectable in the first 24 hours after removal of nitrate, but the rate of HCO_3 ion production was markedly reduced and H ion production became apparent after a further 24 hrs (day 16). The rate of

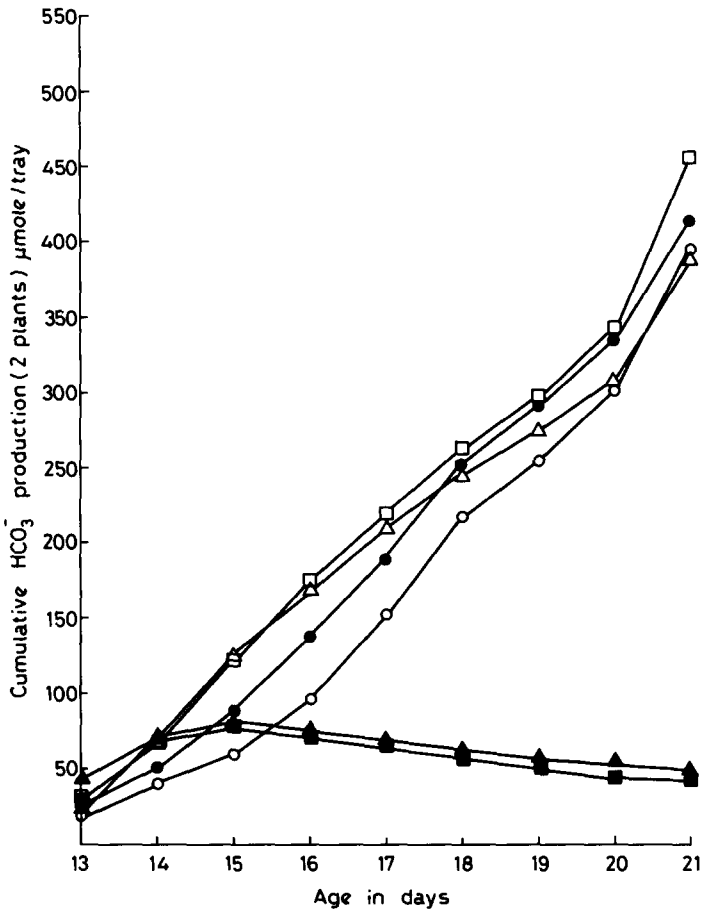

Fig. 3. Experiment 2. Cumulative daily HCO_3 *ion production. Open* and *closed* symbols, two halves of the same root system. ○, ● both given NO_3 ion throughout the experiment. △ and □ given NO_3 ion throughout. ▲ and ■ deprived of NO_3 ion on day 14.

Table 3. Experiment 2. Total nitrogen concentration in rape plants at 32 days

Treatment	% N in shoot	% N in root
All roots given NO_3^-	3.9 +/− 0.14*	3.6 +/− 0.2
Half root system deprived of NO_3^- at day 14	2.8 +/− 0.33	3.1 +/− 0.1 (+NO_3^-) 3.7 +/− 0.4 (−NO_3^-)

* Means and S.E.'s

H ion production did not increase with time but remained constant at 5–7 μmol per day even though the root length was increasing (*cf.* Fig. 2).

N status of plants

Table 3 shows the % N found in the shoots and roots from the two treatments. Analysis of variance showed no significant difference between the % N in shoots and roots. In addition, there was no significant difference between the % N in the halves of the root system receiving nitrate and deprived of nitrate. This suggests that the minus nitrate roots received nitrogen from the plus nitrate roots via the shoot.

Cation anion balance

Table 4 shows the total uptake of cations and anions per g dry wt of the total plant material between days 14–22. Depriving half of the root system of nitrate resulted in less N in the plant but also in a significant decrease in the K and Cl uptake. The plants did not compensate for the lack of nitrate by increasing the uptake of any other anion. Daily uptake of all major ions by the plants was calculated from the depletion of the nutrient solution at the end of each 24 hours. The nutrient solution was analysed by the same methods described earlier for plant analysis. K ion uptake by the minus nitrate roots decreased and eventually a net loss occurred from these roots, but the attached nitrate-fed roots continued to take up K ions in the normal amounts. For the plants with all roots supplied with nitrate there was excellent agreement between excess anions taken up and HCO_3 ions released. For plants with half the roots deprived of nitrate the agreement was not so good, but within experimental error.

General discussion

These experiments demonstrate that the release of HCO_3 ion from roots is sensitive to NO_3 supply. Nitrate-fed plants release HCO_3 ions, which raise the pH of the medium, but when the supply of NO_3 is reduced to zero, H ions are produced. The production of H or HCO_3 ions appears to depend primarily upon the nitrate level of the solution

Table 4. Experiment 2. Total cation/anion uptake, total HCO_3 ion/H ion production by rape plants, days 14–22

Treatment	Uptake, days 14–22 (meq/g dry wt)										Excess anion uptake	Total HCO_3^- and H^+ produced, days 14–22 (meq/g dry wt)		
	K^+	Ca^{2+}	Mg^{2+}	Na^+	Total ΣC	NO_3^-	PO_4^-	SO_4^{2-}	Cl^+	Total ΣA	ΣA−ΣC	HCO_3^-	H^+	Net HCO_3^-
Plants with all roots given NO_3^-	1.59† ± 0.03	1.07 ± 0.02	0.59 ± 0.2	0.02 ± 0.009	3.27 ± 0.08	2.49 ± 0.1	0.23 ± 0.007	1.03 ± 0.3	0.45 ± 0.01	4.2 ± 0.07	0.93 ± 0.10	0.97 ± 0.10	—	0.97 ± 0.10
Plants with half roots $-NO_3^-$	1.17 ± 0.11	0.93 ± 0.10	0.49 ± 0.1	0.01 ± 0.005	2.60 ± 0.18	1.7 ± 0.5	0.2 ± 0.03	0.88 ± 0.2	0.34 ± 0.05	3.13 ± 0.36	0.53 ± 0.20	0.71 ± 0.14	0.053 ± 0.005	0.66 ± 0.14
Sig. value	***	N.S.	N.S.	N.S.		*	N.S.	N.S.	*					

† Means and S.E.'s

surrounding the root, rather than N status of the whole plant, and is almost instantaneous. However, uptake of nitrate in one part of the root system may influence the net H ion production in another part deprived of nitrate. For example, plants wholly deprived of nitrate in experiment 1 produced H ions at a steadily increasing rate which reached 25 μmoles per day per tray within 2 days. Plants for which only part of the root system was deprived of nitrate (Expt. 2) produced H ions at a constant rate of only 6 μmoles per day per tray. We could not detect any nitrate reductase activity in the roots of rape plants. Thus for plants in which nitrate is reduced in the shoot, the Ben Zioni[1] model of nitrate assimilation may provide a satisfactory explanation for the decreased H ion efflux from the nitrate deprived root system. This postulates that there is a stoichiometric relationship between nitrate supply to the shoot and malate production. The malate is then transported via the phloem to the root where it is decarboxylated and the CO_2 and OH^- ions produced are excreted as HCO_3 ion. If some of the malate, produced in the shoots following absorption of nitrate by the nitrate-fed roots, was directed to the minus nitrate roots, HCO_3 ion resulting from decarboxylation of this malate could mask some of the H ion production by these minus roots. This release of HCO_3 ion could be coupled with K ion efflux, which might account for the loss of K recorded for the minus nitrate roots.

The ability of different parts of the root system to secrete H ion or HCO_3 ion according to the nitrate supply available has an adaptive significance for plants growing in poor soil. Some nutrients, notably phosphate, occur in soils in forms unavailable to plants at neutral and higher pHs, but become increasingly available as the pH is lowered[24]. The ability of rape roots to release H ion is particularly significant since its onset is rapid once nitrate has disappeared, but reversible if nitrate is resupplied, and occurs before any damaging change in the nitrogen status of the plant has taken place.

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References

1 Ben Zioni A, Vaadia Y and Lips S H 1971 Nitrate uptake by roots as regulated by nitrate reductase products of the shoot. Physiologia Plant. 24, 288–290.

2. Bienfait H F, Van der Bliek A M and Bino R J 1982 Different regulations on ferric reduction and acidification of the medium by roots of Fe-stressed plants in a rhizostat. J. Plant Nutr. 5. No 3, 447–450.

3. Breteler H 1973 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. Neth. J. Agric Sci. 21, 227–244.

4. Clement C R, Hopper M J and Jones L H P 1978. The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. J. Exp Bot. 29, 453–464.
5. Crooke W M and Simpson W E 1971 Determination of ammonium in Kjeldahl digests of crops by an automated procedure. J. Sci. Fd Agric. 22, 9–10.
6. Deane-Drummon Celia E 1982 Mechanisms for nitrate uptake into barley (*Hordeum vulgare* L cv Fergus) seedlings grown at controlled nitrate concentration in the nutrient medium. Plant Sci. Letters 24, 79–89.
7. Grinsted M J, Hedley M J, White R E and Nye P H 1982 Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. emerald) seedlings I pH changes and the increase in P concentration in the soil solution. New Phytol 91, 19–29.
8. Hedley M J, Nye P H and White R E 1982 Plant-induced changes in the rhizosphere of rape (*Brassica napus* var emerald) seedlings II The origin of the pH change. New Phytol. 91, 31–44.
9. Kirkby E A and Armstrong M J 1980 Nitrate uptake by roots as regulated by nitrate assimilation in the shoot of Castor Oil plants. Plant Physiol. Lancaster, 65, 286–290.
10 Landsberg E 1982 Shoot root interactions in the regulation of H^+efflux from Fe-stressed sunflower roots. J. Plant Nutr. 5, No. 4–7, 415–432.
11 Litchfield M H 1967 The automated analysis of nitrite and nitrate in the blood. Analyst 92, 132–136.
12 Marschner H and Rombald V 1983 In vivo measurement of root induced pH changes at the soil-root interface: Effect of plant species and nitrogen source. Z Pflanzenphysiol. 111, 241–251.
13 Marsh B 1971 Measurement of length in random arrangement of lines. J. Appl. Ecol 8, 265–267.
14 Moorby H and Nye P H 1983 A nutrient film technique for simultaneous measurement of root growth and nutrient uptake. Plant and Soil 70, 151–154.
15 Moorby H and Ny P H 1984 The effect of temperature variation over the root system on root extension and phosphate uptake by rape. Plant and Soil (in press).
16 Murphy J and Riley J P 1962 A modified single solution method for determination of phosphate in natural waters. Analytica Chimica acta 27, 31–36.
17 Newman E I 1966 A method of estimating the total length of root in a sample. J. Appl. Ecol 3, 139–145.
18 Nye P H 1981 Changes in pH across the rhizosphere induced by roots. Plant and Soil 61, 7–26.
19 Osbourne B A and Whittington W J 1981a Eco-physiological aspects of interspecific and seasonal variation in nitrate utilization in the genus *Agrotis*. New Phytol. 87, 595–614.
20 Riley D and Barber S A 1971 Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root induced pH changes at the root soil interface. Soil. Sci. Soc. Amer. Proc. 35, 301–306.
21 Romheld V and Marschner H 1981 Rhythmic iron stress reactions in sunflower at sub-optimal iron supply. Physiologia Plant. 53, 347–353.
22 Sachs von J 1860 Bot. Ztg. p118.
23 Sansum Linda and Robinson J B D 1974 Wet digestion, manual and automated analysis of total sulphur in plant material. Comm. Soil Science Plant Analysis 5, 365–383.
24 Thomas R L, Sheard R W and Moyer J R 1967 Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. Agron J. 59, 240–243.
25 Weisenseel M H, Dorn A and Jaffe L F 1979 Natural H ion currents traverse growing roots and root hairs of barley. Pl Physiol. Lancaster, 64, 512–518.
26 White R E 1980 Retention and release of phosphate by soil and soil constituents. Soils and Agriculture (Ed. P B Tinker). Critical Reports on Applied Chemistry, Vol 2, 71–114 Blackwell Scientific Publications, Oxford.