Plant Physiol. (1985) 78,768–773
0032-0889/85/78/0768/06/$01.00/0

Intracellular pH Regulation during NO_3^- Assimilation in Shoot and Roots of *Ricinus communis*

Received for publication January 22, 1985 and in revised form May 1, 1985

MARINUS L. VAN BEUSICHEM*, ROBERT BAAS[1], ERNEST A. KIRKBY, AND JACOB A. NELEMANS
Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, De Dreijen 3, NL 6703 BC Wageningen, The Netherlands (M.L.v.B., R.B., J.A.N.); *and Department of Plant Sciences, The University of Leeds, Leeds LS2 9JT, United Kingdom* (E.A.K.)

ABSTRACT

Ricinus communis **L. was used to test the Dijkshoorn-Ben Zioni hypothesis that NO_3^- uptake by roots is regulated by NO_3^- assimilation in the shoot. The fate of the electronegative charge arising from total assimilated NO_3^- (and SO_4^{2-}) was followed in its distribution between organic anion accumulation and HCO_3^- excretion into the nutrient solution. In plants adequately supplied with NO_3^-, HCO_3^- excretion accounted for about 47% of the anion charge, reflecting an excess nutrient anion over cation uptake. *In vivo* nitrate reductase assays revealed that the roots represented the site of about 44% of the total NO_3^- reduction in the plants. To trace vascular transport of ionic and nitrogenous constituents within the plant, the composition of both xylem and phloem saps was thoroughly investigated. Detailed dry tissue and sap analyses revealed that only between 19 and 24% of the HCO_3^- excretion could be accounted for from oxidative decarboxylation of shoot-borne organic anions produced in the NO_3^- reduction process. The results obtained in this investigation may be interpreted as providing direct evidence for a minor importance of phloem transport of cation-organate for the regulation of intracellular pH and electroneutrality, thus practically eliminating the necessity for the Dijkshoorn-Ben Zioni recycling process.**

Following the work of Dijkshoorn (7–9), Ben Zioni *et al.* (2), and more recently Kirkby and Armstrong (21), there is a generally currently held view in the literature that NO_3^- reduction in the shoot may control NO_3^- uptake by the roots. This proposed process, which incorporates the recirculation of K^+ within the plant and provides the plant with a pH-regulating mechanism, may be described as follows: K^+ and NO_3^-, the ions taken up in largest amounts by the root, are translocated in the xylem to the shoot, where for every NO_3^- ion reduced an equivalent of malate is formed (1). Some of the K^+ originally accompanying this NO_3^- is then transferred together with malate from the shoot via the phloem to the root system. Here the malate is oxidized and decarboxylated, and the HCO_3^- produced is released stoichiometrically in exchange for further uptake of NO_3^-. The K^+ remaining in the root, together with this NO_3^-, is transported upwards and the cycle repeated. The mechanism necessitates an excess uptake of nutrient anions over cations by the plant, in accordance with the net HCO_3^- excretion from the roots originating from NO_3^- reduction in the shoot. The often-observed increase in the pH in NO_3^- cultures is a well-established phenomenon (17, 23, 33).

Findings consistent with the above mechanism have been presented by a number of authors (2, 3, 14, 22). Detailed analytical data, however, are lacking to provide unequivocal evidence for the occurrence of this Dijkshoorn-Ben Zioni recycling process. In particular, the organic constituents of xylem and phloem saps have not been investigated thoroughly in the experiments cited above. Of the evidence available, there is some indication that the concentration of malate in the phloem sap is well below that of K^+ (15, 24) and hence not fully in accordance with the Dijkshoorn-Ben Zioni model. The occurrence of the process has also been called into question by members of the plant nutrition group at Wageningen Agricultural University, who concluded that in a number of very different species the amount of NO_3^- reduced in the roots is adequate to account for excess nutrient anion over cation uptake and hence HCO_3^- excretion (11, 13, 20, 34). Results of split-root experiments with maize in which $^{86}Rb^+$ was used as a physiological substitute for $^{42}K^+$ (19), and of experiments with sorghum in which K^+ contents of shoots were considered in relation to the dry tissue-pH (12) confirmed the absence of the necessity of the xylem-phloem recirculation scheme. To establish whether the proposed Dijkshoorn-Ben Zioni model does occur, detailed evidence of the origin of the excreted HCO_3^- ions is required. This implies that the precise location of NO_3^- (and SO_4^{2-}) assimilation and the fate of the anion charge arising from these reduction processes should be investigated thoroughly.

The purpose of the present study was to test the Dijkshoorn-Ben Zioni model in castor oil plants, using more detailed analyses of xylem and phloem saps than had previously (21) been carried out. The castor oil plant was chosen as it enabled the separation of both xylem and phloem saps from the same plant at the same growth stage. The analyses of these saps provide an indication of the upward and downward movement of ions which can be applied to the model. Additionally, this species takes up a large excess of nutrient anions over cations when NO_3^- as the sole source of nitrogen is supplied in adequate amounts (20, 21). There is thus a relatively large HCO_3^- excretion into the nutrient medium which is a necessary factor in testing the hypothetical model.

MATERIALS AND METHODS

Plant Cultivation. Castor oil seeds (*Ricinus communis* L. var. Gibsonii) were surface-sterilized with 5% H_2O_2 (v/v) for 3 min. The disinfected seeds were washed intensively with demineralized water and germinated in moist quartz sand at room temperature. On the appearance of the first leaves, 14 d after ger-

[1] Present address: Institute for Ecological Research, Department of Dune Research, Duinzoom 20A, NL 3233 EG Oostvoorne, The Netherlands.

mination, the seedlings were transferred to aerated nutrient solutions held in two 150-L containers (surface, 130 dm^2; total height, 15 cm), each container supporting nine plants. The whole system was set up in a phytotron where the experimental conditions were: temperature, 18°C; dew point, 16°C; photoperiod, 16 h d^{-1}; light intensity, 60 w m^{-2}. For the first 6 d, the plants were grown in half-strength nutrient solutions to prevent detrimental effects on plant growth. After this period, full-strength nutrient solutions were used which had the following composition: $Ca(NO_3)_2$ (2 mM/L), KNO_3 (1 mM/L), KH_2PO_4 (1 mM/L), K_2SO_4 (1.5 mM/L), $MgSO_4$ (1 mM/L). The micronutrients were supplied as: Fe-EDTA (4.60 mg Fe/L), $MnSO_4 \cdot 10H_2O$ (0.50 mg Mn/L), H_3BO_3 (0.50 mg B/L), $ZnSO_4 \cdot 7H_2O$ (0.05 mg Zn/L), $CuSO_4 \cdot 5H_2O$ (0.02 mg Cu/L), and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.01 mg Mo/L). At the same time, the environmental conditions were changed as follows: day temperature, 27°C (dew point, 23°C); night temperature, 19°C (dew point 16°C); photoperiod, 16 h d^{-1}; light intensity, 120 w m^{-2}. The plants were allowed to grow a further 30 or 34 d under these conditions. The nutrient solutions were completely renewed every 6 d. Throughout the whole experiment, the acidity of each of the nutrient solutions was kept constant at pH 5.50 by a pH-meter with pH-stat equipment (Radiometer PHM 64/TTT 60) operating an automatic burette (Radiometer ABU 13). The burette contained 0.5000 M HNO_3 plus 0.5000 M KNO_3. The amounts of acid necessary to keep the pH of the nutrient solution at the adjusted value were recorded continuously.

Collection of Samples. After 36 or 40 d of growth, phloem and xylem saps were collected from the plants for analysis. All sap collections started 3 h after the onset of the photoperiod. Phloem sap was obtained from diagonally cut incisions in the bark of six plants as described by Hall *et al.* (16). The total sample was collected over a 3-h period using capillary tubes. After each sample collection during this 3-h period, the sap was stored in closed plastic vials to restrict evaporation. Immediately after the sampling period, the saps were weighed and stored at −20°C prior to analysis. Xylem sap was collected continuously over a 135-min period using Pasteur pipettes after decapitating three plants about 4 cm above the uppermost root node. Sap exuded during the first 15 min after decapitation was discarded because of possible phloem contamination. The samples collected during the period between 15 and 135 min after decapitation were weighed and stored for analysis in closed plastic vials at −20°C immediately after sampling.

After sap collection, the plants were harvested and divided into leaves, stems plus petioles, and roots. Immediately after determination of the fresh weights, each of these organs was cut into pieces of 0.5 to 1.0 cm^2 and two homogenous 2.0-g subsamples were taken for an *in vivo* NRA[2] assay. Subsequently, other subsamples of the roots were washed in 10 mM HCl for 1 min and then rinsed twice with demineralized water in order to remove ions accumulated in the free space. All plant samples were dried at 70°C for at least 24 h and individual dry weights of leaves, stems plus petioles, and roots were recorded. Dried subsamples of the leaves and the stems plus petioles were bulked prior to grinding for analysis.

Analytical Methods. NRA in leaves, stems plus petioles, and roots was determined by an anaerobic *in vivo* procedure (6) based on Jaworski's method (18). Total N, $H_2PO_4^-$, K^+, Ca^{2+}, and Mg^{2+} were determined after wet digestion of dried subsamples in a H_2SO_4-Se-salicylic acid mixture with three additions of H_2O_2 (25). In the diluted digests, N and P were measured spectrophotometrically by the indophenol-blue method (27) and after reaction with ammonium molybdate-ascorbic acid (36), respectively. Potassium and Ca^{2+} were determined by flame photometry, and Mg^{2+} by atomic absorption spectrometry. For the total S determination, subsamples were digested in a $HClO_4$-H_2O_2 mixture (26), followed by measuring the turbidity in the diluted digests after reaction with $BaCl_2$ (31). For the determination of Cl^-, NO_3^-, and SO_4^{2-}, dried subsamples were extracted with demineralized water. In the filtered extracts, Cl^- was determined coulorimetrically with an Ag anode at constant current, NO_3^- by automatic spectrophotometry after reduction to NO_2^- on a Cu-Cd column, and SO_4^{2-} turbidometrically as described above.

Xylem and phloem saps were analyzed for total N, pH, inorganic cations, inorganic anions, amides, amino acids, citrate, malate, succinate, oxalate, fumarate, formate, and ascorbate. For the total N determination, 0.2 ml was digested at 360–380°C in 1 ml H_2SO_4:salicylic acid (30:1, v/w) and 0.2 g Se-mixture (Merck 8030) after nitration at room temperature for at least 2 h (10). Nitrogen in the diluted digests and inorganic ions in the diluted saps were determined as described above. Carboxylates were determined by enzymic procedures, all based on the increase or decrease of the absorption of NAD(P)H (340 nm) as a result of specific enzymic oxidation or reduction of the substrate. All enzymic procedures were provided by Boehringer-Mannheim GmbH. Amino acids and NH_4^+ were assayed on an amino acid analyzer. Amides were determined by steam distillation after partial hydrolysis of the amide groups (35). All results represent means of at least three replicates.

RESULTS

Ion Uptake and Chemical Composition. Table I shows the yields and chemical composition of shoots and roots of the castor oil plants at both harvest dates. In both shoots and roots, the (C − A) values, which have been shown to be related about stoïchiometrically to the amount of carboxylates (17), are much lower than the values for the assimilated anions (N + S). This indicates that part of the anion charge arising from NO_3^- and SO_4^{2-} reduction was not neutralized by *de novo* synthesis and subsequent accumulation of organic acid anions, but must have been excreted as HCO_3^- ions from the roots into the nutrient medium. This phenomenon is clearly demonstrated in Table II showing the ion uptake pattern by whole castor oil plants. At both harvests, anion uptake exceeded cation uptake by far. The calculated excess nutrient anion over cation uptake corresponded very well with the amounts of excreted HCO_3^- recorded by automatic titration. About 47% of the total assimilated (N + S) charge was recorded as HCO_3^- excretion into the nutrient solution.

Nitrate Reductase Activity. The *in vivo* NRA in the different tissues of the castor oil plant is shown in Table III. At both harvests, the enzyme activity was highest in the leaves. The activity per g fresh weight of both stems plus petioles and roots was about 40% of that in the leaves. Based on an average activity per plant part for both harvests, the root tissue accounted for 44% while leaves and stems plus petioles represented the site of 36 and 20%, respectively, of the total NRA.

Ionic Composition of Xylem and Phloem Sap. An accurate assessment of the contribution of phosphate and the different organic anions to the ionic balance in the xylem and phloem saps was made by calculating the degree of dissociation of these compounds. Calculated values were based on standard (published) pK values and the prevailing pH of the saps.

Table IV shows the ionic composition of the xylem saps. The pH in these saps ranged from 5.53 to 5.84. Between 91 and 97% of the total cationic charge was balanced by inorganic anions. This close xylary inorganic cation-anion balance is commonly observed under conditions of adequate NO_3^- supply and has been demonstrated in a diversity of species (4, 8, 21, 34, 37).

[2] Abbreviation: NRA, nitrate reductase activity.

Table I. *Yield and Chemical Composition of Shoots and Roots of Castor Oil Plants Grown for 36 or 40 d in a Nutrient Solution with NO_3^- as the Sole Source of Nitrogen*

Time after Transplant	Plant Part	Dry Wt	Cations (C)				Anions (A)					(C − A)	Assimilated (N + S)[a]
			K	Ca	Mg	Total	H_2PO_4	Cl	NO_3	SO_4	Total		
d		*g/plant*	*meq/100 g*										
36	Shoots	25.6	102	92	28	221	19	3	46	12	80	142	244
	Roots	8.0	171	28	46	245	37	5	105	12	159	86	220
40	Shoots	30.5	99	85	28	212	18	2	44	11	75	137	229
	Roots	9.8	173	28	49	249	37	5	110	14	166	84	204

[a] Total N − NO_3^- + total S − SO_4^{2-}.

Table II. *Cation and Anion Uptake, NO_3^- and SO_4^{2-} Assimilation, and HCO_3^- Excretion by Whole Castor Oil Plants after 36 or 40 d Growth in a Nitrate Solution*

	Time after Transplant	
	36 d	40 d
	meq/plant	
Potassium	39.7	47.1
Calcium	25.8	28.7
Magnesium	10.9	13.2
Total cations	76.4	89.0
Phosphate	7.8	9.0
Chloride	1.1	1.0
Nitrate	94.4	107.9
Sulfate	9.9	10.8
Total anions	113.2	128.7
Excess anion uptake	36.8	39.7
Recorded HCO_3^- excretion	38.1 (48)[a]	41.3 (46)
Assimilated (N + S)	80.1	89.8

[a] Numbers in parentheses indicate the percentage of HCO_3^- excretion of the total assimilated (N + S) charge.

The small excess inorganic positive charge was found to be compensated by aspartate, glutamate, citrate, malate, and succinate.

The ionic composition of the phloem saps is shown in Table V. The phloem sap pH ranged from 7.53 to 7.82 which is consistent with earlier findings (15, 29, 32). Only 61 to 68% of the total cationic charge was balanced by inorganic anions, predominantly by $H_2PO_4^-$ and SO_4^{2-}. Surprisingly, a small amount of NO_3^- was also detected. The main organic anions present were aspartate and glutamate which accounted for about 45% of the excess inorganic positive charge. The remaining charge was compensated by citrate, malate, succinate, oxalate, formate, and ascorbate. At both harvests, malate represented only 10% of the K^+ in the phloem.

Both xylem and phloem saps proved to be free from fumarate. In all saps, total cations were closely balanced by inorganic anions plus carboxylates (Tables IV and V), indicating that all major contributing compounds had been taken into account.

Long Distance Transport of Nitrogenous Compounds. Table VI shows the partitioning of nitrogenous compounds in the xylem saps. Nitrate was the most important nitrogen carrier, accounting for between 49 and 56% of the total nitrogen transport. The main organic compounds involved in upward nitrogen transport were asparagine and glutamine, contributing 36 to 41% to the total xylary nitrogen translocation. The partitioning of nitrogenous compounds in the phloem saps of these plants is shown in Table VII. The total N content of phloem sap was much higher than that in xylem sap (Table VI). Most of the N was transported as amides and other non-ionic amino acids (Table VII; data on individual amino acids not presented).

In both xylem and phloem saps, a good agreement was found between the sum of the determined nitrogenous compounds and the results of the total N determinations (Tables VI and VII), indicating that all major contributing compounds had been taken into account.

DISCUSSION

To prevent an increase in internal pH in plant tissue, the anion charge arising from the assimilation of NO_3^- and SO_4^{2-} must be directed toward organic anion accumulation in the vacuoles or excreted as HCO_3^- from the roots into the nutrient medium. Our results indicate that in castor oil plants, adequately supplied with NO_3^-, this HCO_3^- excretion accounted for about 47% of this anion charge (Table II). Similar results were obtained by Kirkby and Armstrong (21) with the same plant species. Since anion uptake was much in excess of cation uptake (Table II), one important condition for the Dijkshoorn-Ben Zioni model to play a significant role as a NO_3^- uptake control mechanism is satisfied in the castor oil plant.

Whether it is necessary to invoke the recycling model depends entirely on the origin of the excreted HCO_3^- ions. For the Dijkshoorn-Ben Zioni process to occur, NO_3^- reduction must be localized primarily in the shoot. The ideal model (2) even suggests that all the organic N in the shoot (and possibly that in the roots as well) is the product of NO_3^- assimilation in the shoot, and that initially all of the net OH^- generated in this assimilation is neutralized by organic anion (malate) synthesis through the operation of the biochemical pH-stat (28). From the present data, it can be concluded that in the castor oil plant this latter criterion for the occurrence of the model is not satisfied. This is evident from NRA measurements which revealed that the root system accounted for about 44% of the total NO_3^- reduction (Table III). It is recognized that the enzyme activities, considered in relation to total fresh weight, may provide only a rough indication of the importance of the various plant parts in NO_3^- reduction. However, since it was established that results of these *in vivo* NRA tests provide a good estimate for the rate of *in situ* reduction (5), the present results allow the conclusion to be drawn that in the castor oil plant the root tissue represented the site of just under half of the total NO_3^- reduction. These observations clearly demonstrate that lower levels of NO_3^- in the leaves than in the roots (Table I) and a major role of NO_3^- as a carrier of electronegative charge in the xylem sap (Table IV) are not sufficient to conclude that NO_3^- reduction occurs primarily in the upper plant parts as was formerly believed (21). Additionally, it has been reported that the relative content of NO_3^- and reduced N in the xylem exudate might provide an overestimate of the percentage of freshly absorbed NO_3^- in the roots (30). In the present experiment, xylem sap analysis for nitrogenous com-

Table III. *Nitrate Reductase Activity in Leaves, Stems and Petioles, and Roots of NO_3^--Supplied Castor Oil Plants, and Distribution of the Enzyme Activity over These Organs Allowing for the Fresh Weight Yields of the Individual Plant Parts*

Plant Part	36 d after Transplant			40 d after Transplant		
	μM/g fresh wt·h	*μM/ organ·h*	%	*μM/g fresh wt·h*	*μM/ organ·h*	%
Leaves	1.070	80.14	38.0	0.941	66.72	34.8
Stems + petioles	0.393	39.54	18.7	0.387	38.47	20.0
Roots	0.421	91.46	43.3	0.394	86.68	45.2
Whole plant		211.14	100.0		191.87	100.0

Table IV. *Cationic and Anionic Composition of Xylem Exudates Collected from NO_3^--Supplied Castor Oil Plants*

	Time after Transplant	
	36 d	40 d
	meq/L	
Potassium	21.9	14.8
Sodium	0.1	0.2
Calcium	17.7	13.6
Magnesium	4.9	4.2
Ammonium	0.0	0.0
Total cations	44.6	32.8
Phosphate	2.9	3.0
Chloride	0.8	0.7
Nitrate	32.4	19.4
Sulfate	7.2	6.8
Total inorganic anions	43.3	29.9
Aspartate	0.3	0.3
Glutamate	0.7	0.6
Citrate	2.0	1.4
Malate	0.7	0.7
Succinate	0.1	0.2
Oxalate	0.0	0.0
Formate	0.0	0.0
Ascorbate	0.0	0.0
Fumarate	0.0	0.0
Total organic anions	3.8	3.2
Total anions	47.1	33.1

Table V. *Cationic and Anionic Composition of Phloem Saps Collected from NO_3^--Supplied Castor Oil Plants*

	Time after Transplant	
	36 d	40 d
	meq/L	
Potassium	104.7	86.6
Sodium	1.6	1.0
Calcium	5.6	5.3
Magnesium	7.7	7.1
Ammonium	1.5	1.2
Total cations	121.1	101.2
Phosphate	34.0	27.9
Chloride	4.7	3.9
Nitrate	6.5	4.1
Sulfate	36.9	25.8
Total inorganic anions	82.1	61.7
Aspartate	6.6	6.1
Glutamate	10.6	9.9
Citrate	5.1	5.2
Malate	10.8	8.8
Succinate	1.4	1.6
Oxalate	1.5	1.0
Formate	0.1	0.1
Ascorbate	1.8	3.0
Fumarate	0.0	0.0
Total organic anions	37.9	35.7
Total anions	120.0	97.4

pounds (Table VI) and *in vivo* NRA measurements (Table III) yielded similar estimates for root reduction of absorbed NO_3^-, confirming the above conclusion.

In Figure 1, A and B, relevant data from the Tables I, II, and III are assembled to illustrate the fate of the electronegative charge arising from total NO_3^- and SO_4^{2-} reduction at the two harvest dates. These schemes clearly demonstrate that at both harvests 80% of the NO_3^- and SO_4^{2-} reduced in the roots resulted in HCO_3^- excretion, assuming that the distribution of NO_3^- and SO_4^{2-} reduction follows a similar pattern over the plant organs. The low organic anion (C − A) levels in the roots are in accordance with this high proportion of HCO_3^- excretion (Table I) as is also the lack of evidence of any major xylem transport of organic anions resulting from NO_3^- and SO_4^{2-} reduction in the roots (Table IV). The distribution of the products of NO_3^- and SO_4^{2-} assimilation in the shoots was very different, since a large amount of the electronegative charge was directed toward organic anion accumulation (Table I), leaving only a small fraction for ultimate HCO_3^- excretion (Fig. 1). This means that only a small portion of the organic anions produced in the biochemical pH-stat needed to be translocated in the phloem to the roots to be decarboxylated by the second half of the pH-stat with regeneration of HCO_3^- ions. These considerations are consistent with the phloem sap analyses, since relatively low concentrations of organic anions were found in relation to the K^+ levels (Table V). The present results also clearly demonstrate that malate does not

Table VI. *Total Nitrogen Contents and Partitioning of Nitrogenous Compounds in Xylem Exudates Collected from NO_3^--Supplied Castor Oil Plants*

	Time after Transplant	
	36 d	40 d
	mmol N/L	
Asparagine	2.5	2.4
Glutamine	18.5	13.9
Aspartate	0.3	0.3
Glutamate	0.7	0.6
Other amino acids	2.8	2.7
Nitrate	32.4	19.4
Ammonium	0.0	0.0
Sum	57.2	39.3
Total N	58.2	40.7

Table VII. *Total Nitrogen Contents and Partitioning of Nitrogenous Compounds in Phloem Saps Collected from NO_3^--Supplied Castor Oil Plants*

	Time after Transplant			
	36 d		40 d	
	mmol N/L			
Asparagine	28.8		23.3	
Glutamine	142.3		114.7	
Aspartate	6.6		6.1	
Glutamate	10.6		9.9	
Other amino acids	14.0		11.0	
Nitrate	6.5		4.1	
Ammonium	1.5		1.2	
Sum		210.3		170.3
Total N		216.8		174.9

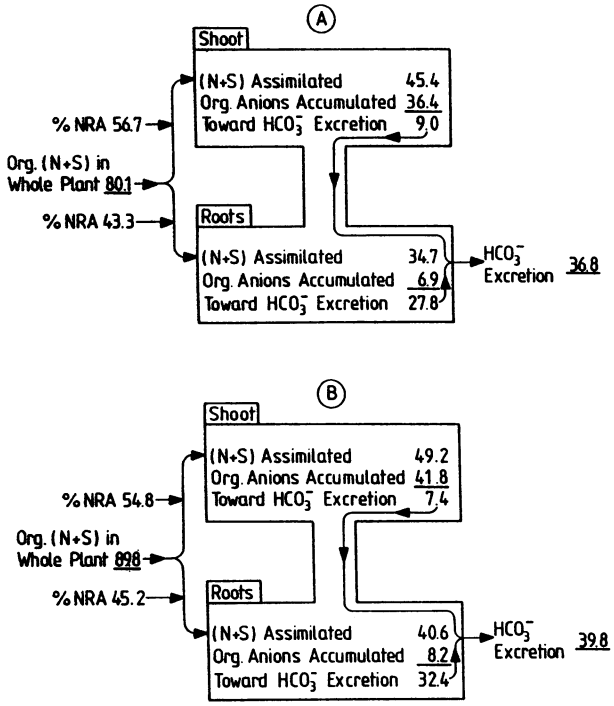

FIG. 1. Distribution of anion charge arising from NO_3^- and SO_4^{2-} assimilated in shoot and roots between organic anion accumulation and (ultimate) HCO_3^- excretion by castor oil plants grown for 36 (A) or 40 (B) d in a nitrate solution. Organic anion accumulation estimated by (C − A). All values expressed in meq/plant.

play a dominant role in internal pH regulation as was previously accepted for the castor oil plant (21). Since the roots represented the site of a considerable portion of the total NO_3^- and SO_4^{2-} reduction in the plant and that only between 19 and 24% of the total HCO_3^- excretion originated from reduction in the shoot (Fig. 1), the conclusion is justified that the Dijkshoorn-Ben Zioni model is only of minor importance as a mechanism to control NO_3^- uptake by the roots and regulation of intracellular pH in the shoot. It is tempting to speculate that the recycling model is invalid for all plant species.

LITERATURE CITED

1. BEN ZIONI A, Y VAADIA, SH LIPS 1970 Correlations between nitrate reduction, protein synthesis and malate accumulation. Physiol Plant 23: 1039–1047
2. BEN ZIONI A, Y VAADIA, SH LIPS 1971 Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiol Plant 24: 288–290
3. BLEVINS DG, AJ HIATT, RH LOWE, JE LEGGETT 1978 Influence of K on the uptake, translocation, and reduction of nitrate by barley seedlings. Agron J 70: 393–396
4. BRETELER H, CH HÄNISCH TEN CATE 1978 Ionic balance of root-shoot nitrate transfer in dwarf bean. Physiol Plant 42: 53–56
5. BRETELER H, CH HÄNISCH TEN CATE 1980 Fate of nitrate during initial nitrate utilization by nitrogen-depleted dwarf bean. Physiol Plant 48: 292–296
6. BRETELER H, CH HÄNISCH TEN CATE, P NISSEN 1979 Time-course of nitrate uptake and nitrate reductase activity in nitrogen-depleted dwarf bean. Physiol Plant 47: 49–55
7. DIJKSHOORN W 1958 Nitrate accumulation, nitrogen balance and cation-anion ratio during the regrowth of perennial rye grass. Neth J Agric Sci 6: 211–221
8. DIJKSHOORN W 1971 Partition of ionic constituents between organs. *In* RM Samish, ed, Recent Advances in Plant Nutrition, Vol 2. Gordon and Breach Science Publishers, New York, pp 447–476
9. DIJKSHOORN W, DJ LATHWELL, CT DE WIT 1968 Temporal changes in carboxylate content of rye grass with stepwise change in nutrition. Plant Soil 29: 369–390
10. EASTIN EF 1978 Total nitrogen determination for plant material containing nitrate. Anal Biochem 85: 591–594
11. FINDENEGG GR, WG KELTJENS 1984 Does nitrate reduction in the shoot regulate NO_3^- uptake by roots? *In* WJ Cram, K Janáček, R Rybová, K Siegler, eds, Membrane Transport in Plants. Academia, Praha, pp 491–492
12. FINDENEGG GR, M SALIHU, NA ALI 1982 Internal self-regulation of H^+-ion concentration in acid-damaged and healthy plants of *Sorghum bicolor* (L.) Moench. *In* A Scaife, ed, Plant Nutrition 1982, Vol 1. Commonwealth Agricultural Bureaux, Slough (United Kingdom), pp 174–179
13. FINDENEGG GR, ML VAN BEUSICHEM, WG KELTJENS 1984 Regulation of inner pH: coordination of nitrogen assimilation and synthesis of organic acids. Fourth Congress of the Federation of European Societies of Plant Physiology (Strasbourg), pp 463–464
14. FROST WB, DG BLEVINS, NM BARNETT 1978 Cation pretreatment effects on nitrate uptake, xylem exudate and malate levels in wheat seedlings. Plant Physiol 61: 323–326
15. HALL SM, DA BAKER 1972 The chemical composition of *Ricinus* phloem exudate. Planta 106: 131–140
16. HALL SM, DA BAKER, JA MILBURN 1971 Phloem transport of ^{14}C-labelled assimilates in *Ricinus*. Planta 100: 200–207
17. HOUBA VJG, F VAN EGMOND, EM WITTICH 1971 Changes in production of organic nitrogen and carboxylates (C − A) in young sugar-beet plants grown in nutrient solutions of different nitrogen composition. Neth J Agric Sci 19: 39–47
18. JAWORSKI EG 1971 Nitrate reductase assay in intact plant tissues. Biochem Biophys Res Commun 43: 1274–1279
19. KELTJENS WG 1981 Absorption and transport of nutrient cations and anions in maize roots. Plant Soil 63: 39–46
20. KELTJENS WG 1982 Nitrogen metabolism and K-recirculation in plants. *In* A Scaife, ed, Plant Nutrition 1982, Vol 1. Commonwealth Agricultural Bureaux, Slough (United Kingdom), pp 283–287
21. KIRKBY EA, MJ ARMSTRONG 1980 Nitrate uptake by roots as regulated by nitrate assimilation in the shoot of castor oil plants. Plant Physiol 65: 286–290
22. KIRKBY EA, MJ ARMSTRONG, JE LEGGETT 1981 Potassium recirculation in tomato plants in relation to potassium supply. J Plant Nutr 3: 955–966
23. KIRKBY EA, K MENGEL 1967 Ionic balance in different tissues of the tomato plant in relation to nitrate, urea, or ammonium nutrition. Plant Physiol 42: 6–14
24. MENGEL K, H-E HAEDER 1977 Effect of potassium supply on the rate of phloem sap exudation and the composition of phloem sap of *Ricinus communis*. Plant Physiol 59: 282–284
25. NOVOZAMSKY I, VJG HOUBA, R VAN ECK 1983 A novel digestion technique for multi-element plant analysis. Commun Soil Sci Plant Anal 14: 239–248
26. NOVOZAMSKY I, R VAN ECK 1977 Total sulphur determination in plant material. Z Anal Chem 286: 367–368
27. NOVOZAMSKY I, R VAN ECK, JC VAN SCHOUWENBURG, I WALINGA 1974 Total nitrogen determination in plant material by means of the indophenol-blue method. Neth J Agric Sci 22: 3–5
28. RAVEN JA, FA SMITH 1976 Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol 76: 415–431
29. RICHARDSON PT, DA BAKER, LC HO 1982 The chemical composition of cucurbit vascular exudates. J Exp Bot 33: 1239–1247
30. RUFTY TW, RJ VOLK, PR MCCLURE, DW ISRAEL, CD RAPER 1982 Relative content of NO_3^- and reduced N in xylem exudate as an indicator of root reduction of concurrently absorbed $^{15}NO_3^-$. Plant Physiol 69: 166–170
31. TABATABAI MA, JM BREMNER 1970 A simple method of determining sulfur in plant materials. Agron J 62: 805–806
32. TAMMES PML, J VAN DIE 1964 Studies on phloem exudation from *Yucca flaccida* Haw. Acta Bot Neerl 13: 76–83
33. VAN BEUSICHEM ML 1981 Nutrient absorption by pea plants during dinitrogen fixation. 1. Comparison with nitrate nutrition. Neth J Agric Sci 29: 259–272
34. VAN BEUSICHEM ML 1983 Xylary charge distribution and nitrogen transport

in *Pisum sativum* L. during dinitrogen fixation or nitrate nutrition. Z Pflanzenphysiol 109: 449–458

35. VAN BEUSICHEM ML, JJ NEETESON 1982 Urea nutrition of young maize and sugar-beet plants with emphasis on ionic balance and vascular transport of nitrogenous compounds. Neth J Agric Sci 30: 317–330

36. VAN SCHOUWENBURG JC, I WALINGA 1967 The rapid determination of phosphorus in presence of arsenic, silicon and germanium. Anal Chem Acta 37: 271–274

37. WALLACE A, AM ABOU-ZAMZAM, E MOTOYAMA 1971 Cation and anion balance in the xylem exudate of tobacco roots. Plant Soil 35: 433–438