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Charge Balance in NO_3^--Fed Soybean

ESTIMATION OF K^+ AND CARBOXYLATE RECIRCULATION

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ABSTRACT

Soybeans (*Glycine max* L. Merr., cv Kingsoy) were grown on media containing NO_3^- or urea. The enrichments of shoots in K^+, NO_3^-, and total reduced N (N_r), relative to that in Ca^{2+}, were compared to the ratios K^+/Ca^{2+}, NO_3^-/Ca^{2+}, and N_r/Ca^{2+} in the xylem saps, to estimate the cycling of K^+, and N_r. The net production of carboxylates (R^-) was estimated from the difference between the sums of the main cations and inorganic anions. The estimate for shoots was compared to the theoretical production of R^- associated with NO_3^- assimilation in these organs, and the difference was attributed to export of R^- to roots. The net exchange rates of H^+ and OH^- between the medium and roots were monitored. The shoots were the site of more than 90% of total NO_3^- reduction, and N_r was cycling through the plants at a high rate. Alkalinization of the medium by NO_3^--fed plants was interrupted by stem girdling, and not restored by glucose addition to the medium. It was concluded that the majority of the base excreted in NO_3^- medium originated from R^- produced in the shoots, and transported to the roots together with K^+. As expected, cycling of K^+ and reduced N was favoured by NO_3^- nutrition as compared to urea nutrition.

Plant roots absorb ions at different rates, so that cation and anion uptakes may not be in equilibrium. The balance of absorbed charges is maintained by net transport of H^+ equivalents (true H^+ transport, or transport of OH^- or of HCO_3^-) (20). In the modern description of the underlying absorption mechanisms, based on chemiosmotic concepts, active, electrogenic H^+ extrusion by the so-called proton pump, energizes cation uptake and OH^-:anion cotransports. Thus, unbalance of ion absorption results in net transport of H^+ equivalents. Furthermore, part of the absorbed cations and anions do not conserve their electric charges. This is the case for NH_4^+, NO_3^-, SO_4^{2-}, and $H_2PO_4^-$ when incorporated in organic molecules. Assimilation of these species results in further net production or consumption of H^+ equivalents in the cells. Plants cope with the production of H^+ or OH^- equivalents associated with transports and metabolism by using the so-called biophysical and biochemical pH-stats (22). The biophysical pH-stats correspond to transport of H^+ or OH^- equivalents out of the cells. The biochemical pH-stats modify the proportions of strong and weak organic acids via carboxylation/decarboxylation mechanisms (8), resulting in an accumulation of organic anions equivalent to the difference between the sums of mineral cations and anions (12).

Nitrogen contents of plant tissues are greater than those of other mineral elements. This implies that the ionic imbalance of absorption depends on whether N is present in the medium as a cation or as an anion (23). OH^- generated by reduction of NO_3^- in the roots may be released in the medium. This kind of cytoplasmic detoxification is not possible in shoots, due to the small volume of leaf apoplast, and to the limited ability of phloem to transport OH^- and HCO_3^- (1, 22). OH^- generated in aerial organs is converted in ionized carboxylic groups, but the calculated accumulation of organic anions is often smaller than the NO_3^- reduction in shoots. A model was proposed by Ben Zioni and co-workers (4) to explain this discrepancy. According to this model, malate synthesized in the leaves is loaded in the phloem and transported together with K^+ into the roots where it is decarboxylated. The HCO_3^- produced is exchanged for NO_3^-, which is transported into the shoots together with K^+. Thus, the transport of K^+ carboxylates by phloem, and K^+ cycling are means by which NO_3^- reduction in shoots controls NO_3^- uptake by roots.

The validity of the Ben Zioni model has been questioned for barley (6), corn (16), tomato (17), and inoculated soybean (cv Ransom) (15), which were reported to accumulate carboxylates in amounts approximately equivalent to NO_3^- reduction in shoots. Castor oil plants excrete more than 50% of the OH^- they produced by assimilating N and S (1, 18, 29). In these plants, shoots were responsible for approximately 40 to 60% NO_3^- assimilation and they returned to the medium approximately 15 to 20% (29) to 60% (1) of the OH^- they produced.

In these experiments, the majority of excreted OH^- originated from roots. Evidence for the cycling of negative charges of NO_3^- and carboxylates between roots and shoots, and for its effect on NO_3^- uptake must be looked for in plants which reduce NO_3^- principally in shoots, in which there is less accumulation of organic anions than there is reduction of NO_3^-. Soybean is thought to assimilate NO_3^- mainly in shoots (2), although the exact proportion is dependent on the cultivar and on plant age (14).

Here we present a study of K^+, Ca^{2+}, NO_3^-, and N_r[1] accumulations in soybean roots and shoots. Briefly, it was assumed that all of them were transported in the xylem, but that only K^+ and N_r could also be transported in the phloem (3, 22). The R^- contents of the tissues were estimated from the differences between the main cations and the main inorganic anions. Comparing the ion and N enrichment rates of shoots to ion and N contents of xylem sap obtained on excised and pressurized roots enabled us to estimate the rates of production of R^- in shoots, of transport into roots, and decarboxylation. The rates of net exchanges of H^+ or OH^- equivalent between the roots and the medium were measured in various conditions corresponding to OH^- excretion, or to inhibition of R^- production, or to inhibition of phloem transport from the shoots to the roots. Taken as a

[1] Abbreviations: N_r, total reduced N; PI, plastochron index; R^-, carboxylic anion; J_{H^+}, H^+ net transport.

whole, the results support Ben Zioni's hypothesis of a net transport of R^- from the shoots to the roots.

MATERIALS AND METHODS

Plant Material. Soybean seeds (*Glycine max* L. Merr. var. Kingsoy) were germinated between wet paper towels at 25°C in the dark. Three days later, seedlings were transferred on aerated nutrient solutions (10 L for 30 plants). The plants were grown in a culture chamber. Temperature was regulated at 25/20°C during the 14/10 h light/dark cycle. Fluorescent discharge lamps provided a photosynthetic photon flux density of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height.

Two culture solutions were used, referred to as NO_3^- and urea media. The pH of fresh solutions was 5.5. The NO_3^- medium contained 2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 0.1 mM Fe-NH_4-EDTA, 50 μM KCl, 30 μM H_3BO_3, 5 μM $MnSO_4$, 1 μM $ZnSO_4$, 1 μM $CuSO_4$ and 0.1 μM $(NH_4)_6Mo_7O_{24}$. In the urea medium KNO_3 and $Ca(NO_3)_2$ were replaced by 2 mM urea, 1 mM K_2SO_4, and 1 mM $CaCl_2$. Solutions were renewed every 3 or 4 d, in order to minimize pH shift (less than 0.4 pH unit in urea medium, and less than 0.3 pH unit in NO_3^- medium) and nutrient depletion (maximum decrease of NO_3^- concentration: 15%). The plant plastochron index (PI) (19) was calculated using a reference length of 3 cm for the central leaflet.

Collection of Xylem Sap. Plants were decapitated 1 cm below the cotyledonary node, epidermis and cortex of the hypocotyl were peeled back to the root junction, and the root systems were introduced into tight pots containing culture solutions. Pressurization of the pots was obtained with a 20%/80% O_2/N_2 mixture. Sap exudates were collected in hemolysis tubes connected to the hypocotyl sections with tygon tubing.

Exudate and Tissue Analysis. Except for the first experiment (ionic balance of 12-d-old plants), tissues were dried at 80°C. In all cases, soluble elements were extracted over 24 h with 0.1 N HCl. Cations, NO_3^-, SO_4^{2-}, and Cl^- were assayed on the soluble fraction. Both the extract and the insoluble material were separately mineralized first with H_2SO_4 plus H_2O_2 at 90°C to ensure NO_3^- volatilization, thereafter with a Kjeldahl digestion, and assayed for total P and N_r. Xylem exudate samples were directly analyzed for K^+, Ca^{2+}, NO_3^-, and, after mineralization for N_r. Cations were assayed by flame spectrophotometry (emission for K^+ and atomic absorption for Ca^{2+} and Mg^{2+}). When measurable, the Cl^- contents were always smaller than the other ionic contents by several order of magnitude, and were disregarded. SO_4^{2-} was measured by turbidity assay (27). NO_3^- was colorimetrically determined after diazotation of the nitrite obtained by reduction on a cadmium column on an automatic analyzer. Total P and N_r were colorimetrically assayed, after formation of phospho-molybdic complex for P, and after Berthelot phenol hypochlorite reaction for N_r.

Transports. H^+ net transport rates were measured using samples of two to three plants installed in pots containing 0.5 L fresh culture medium. The solution was vigorously bubbled with decarbonated air. The rates of net H^+ (or OH^-) exchanges were estimated from the recorded automatic delivery of 10 mN KOH or 10 mN H_2SO_4 necessary in order to maintain the pH of the medium at 5.5 ± 0.02 pH unit (Metrohm pH-stat system) (7). NO_3^- net transport rates were calculated from medium depletion in NO_3^-, using the same device as above. Volume variations of media were periodically estimated from weight losses of pots (0.5–1%/h). Observed decreases in NO_3^- concentration in media (*ca.* 2%/h) were corrected for the volume diminutions to estimate NO_3^- mean uptake rate.

In some experiments, hypocotyls were steam girdled, 1 cm above the collar. Care was taken to ensure that the length of the scalded region did not exceed 1 cm, and to prevent stem bending.

Microscopy. Determinations of the phloem area were made on stem and petiole sections embedded in paraffin after glutaraldehyde fixation. The sections were stained successively with hematoxylin (1% w/v in 10% ethanol plus 10% glycerol), safranine (1% w/v in 50% ethanol), and anilin blue (saturated in ethylene glycol monomethyl ether). They were observed in light microscopy (Visicalc, Reichert), equipped with a Kontron planimeter. Fluorescence of 6(5)carboxyfluorescein was detected on fresh hand cut sections, with Olympus fluorescence microscope (excitation: 465 nm, emission: 515 nm). Electron microscopy observations were made with a Jeol (Jem 100) microscope, after fixation with glutaraldehyde/osmium tetroxyd, embedding in Spurr's resin, and contrast enhancement with uranyl acetate and lead citrate.

RESULTS

Growth and Development. In both NO_3^-- and urea-fed plants, the logarithm of root dry weight was approximately linear with time between 6 and 20 d (Fig. 1). On the contrary, the curves for shoots presented two phases. The first one extended from 6 to 13 d. It corresponded to the extension of the two unifoliate leaves. The cotyledons were green and fully turgid at 6 d, and yellow and shrunken at 13 d (at that time, they had lost 85% of their initial content in total N). During this period, no significant increase in shoot dry weight was observed, because growth was largely supported by remobilization of cotyledon material. Thereafter, quasi simultaneous cotyledon fall and unfolding of the first trifoliate leaf initiated an exponential growth phase (Fig. 1). At 20 d, the third trifoliate leaf was expanding (PI = 3.2). On the urea medium, the unifoliate leaves were senescent. No significant difference appeared in shoot dry weight accumulation between NO_3^- and urea treatments.

The primary phloem area was determined in lower stems of 16- to 18-d-old plants grown on NO_3^- medium (the secondary phloem was neglected). Three estimates were obtained: 4.0×10^{-3}, 5.6×10^{-3}, and 5.8×10^{-3} cm^2 per section. The corresponding section areas were 36.4×10^{-3}, 67.3×10^{-3}, and 45.8×10^{-3} cm^2, respectively. In the petiole of the first trifoliate leaf, the phloem area was 1.1×10^{-3} cm^2 for a section area of 20.4×10^{-3} cm^2. From electron microphotographs, the total area of sieve elements was estimated as not exceeding 12% of phloem area.

Ion and Nitrogen Net Transports in Shoots. In NO_3^--fed, 12-d-old plants (PI = 0.8), shoots amounted to *ca.* 64% of the total fresh weight. The first trifoliate leaf was at 50% full extension,

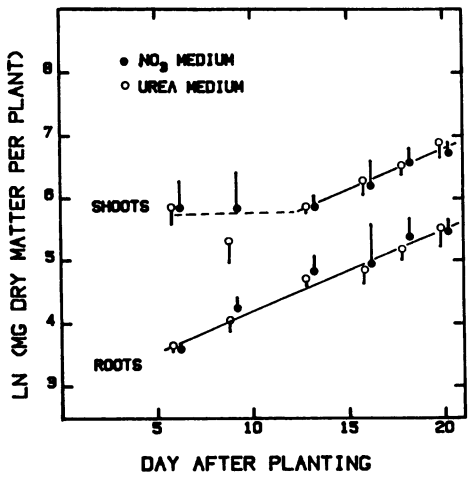

FIG. 1. Dry matter accumulation in shoots and roots of soybean grown either with NO_3^- or with urea as N source. Results are expressed as natural logarithm (means of six plants with 95% confidence limits). To avoid confusion between close points, symbols are shifted 0.2 d before (urea medium) or after (NO_3^- medium) the day of measurement.

and the second one was not completely unfolded. The sum $C = K^+ + Mg^{2+} + Ca^{2+}$ was 1015 ± 77 μeq per plant, and the sum $A = NO_3^- + P^- + SO_4^{2-}$ was 605 ± 67 μeq per plant (means of six plants). The difference $C - A = 410$ μeq per plant is an estimate of the amount of carboxylates. The amount of reduced nitrogen was 1227 ± 114 μmol per plant, of which 137 ± 13 μmol were obtained from the seeds. Thus, NO_3^- reduction was estimated to be 1090 μmol. These results show that only 410/1090 × 100 = 38% of the anionic charge of NO_3^- was conserved by plants after NO_3^- reduction.

Roots contained 20% of total C and 35% of total A of the plants (Table I). The ionic balance was nearly perfect in these organs ($C - A$ was less than 2% of C) (Table I). This was confirmed by titration of the total acidity of water extracts decationized on H^+-form Dowex resin. It was equal to the sum of mineral anions in the extracts (not shown). On the contrary, A accounted for only 47% of C in shoots. This imbalance was mainly due to excess cationic accumulation in shoots as compared to roots, since the two organs had similar contents of A (Table I). Titration of decationized water extracts of the first trifoliate leaf indicated that *ca.* 58% of organic anions were associated with material insoluble in water.

Accumulations of K^+, Ca^{2+}, NO_3^-, and N_r in shoots were periodically assayed between 6 and 20 d in plants growing on NO_3^- medium or on urea medium. When expressed as logarithm of content, the results gave linear graphs as a function of time (Fig. 2 and Table II). On NO_3^- medium, the rate of Ca^{2+} accumulation increased with time more than the rate of K^+ accumulation did. On the contrary, K^+ accumulation was progressively accelerated as compared to Ca^{2+} accumulation on urea medium.

Xylem Transport. Water and ion transport in the xylem, as well as net H^+ transport by roots, were studied on 16-d-old plants (in which shoot growth had reached its exponential phase, as shown in Fig. 1). As estimated from water consumption by intact plants, transpiration was constant throughout the light period. It was 2.5 $ml \cdot h^{-1}$ per plant on NO_3^- medium, and 2.0 $mL \cdot h^{-1}$ per plant on urea medium. Xylem sap was obtained from plants decapitated 6 h after the beginning of the light period. Since the xylem sap composition was to be compared to variations of leaf contents, care was taken to obtain water flow rates as close as possible to the transpiration rates of intact plants. On NO_3^- medium, an exudation rate similar to the transpiration rate was obtained with an applied pressure of 150 kPa. This was not possible on urea medium. At 300 kPa, the exudation rate did not exceed 30% of the transpiration rate, but air was forced through the root. A 250 kPa pressure gave the highest exudation rate without air bubbles. In both media, the concentrations of K^+, Ca^{2+}, NO_3^- and N_r were constant between 5 min and 50 min after pressurization (Fig. 3). In subsequent experiments, xylemic transports were monitored for 24 h by periodically sampling four 16-d-old plants, and collecting the root exudates under pressure between 5 min and 35 min after pressurization. The observed concentrations of K^+, Ca^{2+}, NO_3^-, and N_r in the exudates (Fig. 4), were used to calculate the ratios K^+/Ca^{2+}, NO_3^-/Ca^{2+}, and N_r/Ca^{2+}. As shown in Table III, these ratios were approximately constant during the light period. In the dark, they decreased by 10 to 20% on NO_3^- medium, and they increased by 10 to 20% on urea medium. In control experiments, 2 cm stem segments were cut just under the node of the first trifoliate leaf, 10 h after the beginning of the light period of d 16. Sap samples were obtained by applying a 50 kPa pressure at one end of the segments. The ratios K^+/Ca^{2+} and NO_3^-/Ca^{2+} in the stem sap were close to the ones observed in the root exudate for both NO_3^- and urea-fed plants (Tables III and IV).

Efflux of H^+ or OH^- Equivalents and NO_3^- Uptake by Roots. Net efflux of H^+ or OH^- equivalents was measured with 16-d-old plants grown on NO_3^- medium or on urea medium. In some experiments, plants grown for 11 d on NO_3^- medium were transferred for 5 d on Cl^- medium (similar to NO_3^- medium, except that NO_3^- was replaced by Cl^-). The plants were transferred to the laboratory 4 h after the beginning of the light period and left to equilibrate in the pH-stat device (same photoperiod as in the culture chamber, 300 μmol photon $\cdot s^{-1} \cdot m^{-2}$, 25°C). The recording of H^+ or OH^- net transport began 10 h later.

Plants grown on NO_3^- alkalinized the medium. The OH^- net efflux increased during the light period, and decreased during the dark period, with a 2h lag (Fig. 5). The hypocotyls were girdled 6 h after the beginning of the light period, when the alkalinization rate had reached a relatively high value. The plants remained in the light until the end of the experiment. Comparison of weight losses between pots with control plants, and girdled ones, indicated that transpiration was not affected during the next 12 h. The efficiency of girdling for interrupting the translocation of phloem sap was verified by applying [^{14}C]sucrose (10 mM sucrose, specific activity 370 MBq/mol), to the abraded surface of the first trifoliate leaf. Roots were assayed for radioactivity 8 h later. [^{14}C]-compounds were present in roots of control plants, but not in roots of girdled ones. In other experiments, 1 mM 6(5)carboxyfluorescein (pH 6.3) was applied in place of [^{14}C]sucrose, and fresh sections of stem, hypocotyl and roots were observed with a fluorescence microscope. In control plants, fluorescence could be observed in these organs a few hours after application of the probe to the leaf (initial observations were made at 2 h for stems, and 6 h for roots, and both revealed that the probe was already present). In all instances, 6(5)carboxyfluorescein-associated fluorescence was strictly restricted to phloem tissues. In girdled plants, no fluorescence was observed in hypocotyls below the scalded region, nor in roots, even after 24 h.

Girdling led to a 85% inhibition of the OH^- net efflux within 6 h. Adding 100 mM glucose to the medium immediately resulted

Table I. *Fresh Weight, Ion Contents, and Nitrogen Content of 12-d-Old Plants*

The plants were grown on NO_3^- medium. C: sum of cations; A: sum of inorganic anions.

Organs	Fresh Weight	Ion contents									N_r
		Ca^{2+}	Mg^{2+}	K^+	C	NO_3^-	P^-	SO_4^{2-}	A	$C-A$	
	g/plant	*μeq/g fr wt*									*μmol/g fr wt*
Root	1.77 ± 1.17	11 ± 2	35 ± 5	71 ± 9	116 ± 15	46 ± 4	58 ± 5	11 ± 2	113 ± 8	3 ± 2	124 ± 16
Stem[a]	1.29 ± 0.17	45 ± 5	35 ± 4	121 ± 6	273 ± 20	71 ± 7	47 ± 2	9 ± 1	108 ± 7	165 ± 25	152 ± 23
Leaf 1[b]	0.75 ± 0.18	87 ± 7	54 ± 4	131 ± 10	279 ± 15	31 ± 7	70 ± 11	6 ± 1	109 ± 11	170 ± 24	406 ± 35
Other leaves[c]	1.11 ± 0.16	109 ± 7	59 ± 4	110 ± 13	202 ± 7	30 ± 4	74 ± 13	5 ± 2	127 ± 9	75 ± 14	419 ± 36
Total shoot	3.15 ± 0.46	78 ± 5	48 ± 3	120 ± 9	246 ± 11	47 ± 1	62 ± 8	7 ± 1	116 ± 8	130 ± 18	307 ± 21
Whole plant	5.27 ± 1.09	51 ± 5	43 ± 3	100 ± 7	194 ± 13	46 ± 3	60 ± 6	9 ± 1	115 ± 7	79 ± 17	230 ± 29

[a] Stem with apex, plus petioles. [b] First trifoliate leaf. [c] Unifoliate leaves.

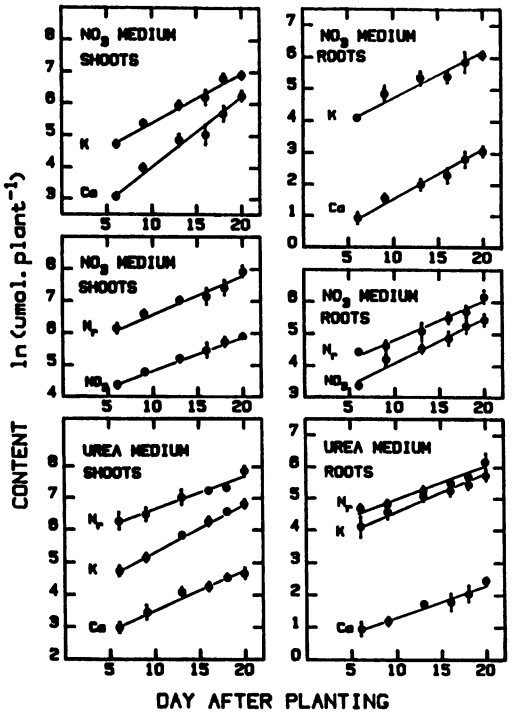

FIG. 2. Accumulations of ions and N in shoots and roots of soybean plants grown either with NO_3^- or with urea as N source. Means of six plants with 95% confidence limits. When nonrepresented, confidence limits are within the size of the symbols. N_r, reduced N. The coefficients of regression lines are given in Table II. The calculated contents (μmol per plant) on d 16 are as follows (respectively Ca^{2+}, K^+, NO_3^-, and N_r). NO_3^- fed plants: shoots, 206, 582, 251, 1519; roots: 12, 265, 141, 252. Urea fed plants: shoots, 74, 522, 0, 1464; roots, 7, 203, 0, 254.

in a H^+ net efflux, which could be interrupted by 0.5 mM vanadate. Mean K^+ absorption rate was estimated from the amount of radioactivity retrieved in the plants at the end of the light period, 5 h after the addition of $^{86}Rb^+$ to the medium. It was 7.1 ± 1.4 μmol·h^{-1}·plant^{-1} in control plants, and 2.0 ± 0.7 μmol·h^{-1}·plant^{-1} in girdled ones.

The effects of girdling and glucose were also studied in the dark. Hypocotyls of NO_3^- plants were girdled 5 h after the beginning of the dark period (at that time, the OH^- net efflux had diminished approximately to its value at the end of the previous dark period). The plants were thereafter maintained in the dark for the remainder of the experiment (Fig. 6). The OH^- net efflux was progressively reduced to zero during the next 10 h, and it was replaced by a H^+ net efflux after glucose was added to the medium. In a control run, girdling was replaced by glucose addition. In this case, the OH^- net efflux remained fixed for more than 14 h at the low value it had reached before glucose addition.

A H^+ net efflux was observed in Cl^- medium during the light period (Fig. 7). H^+ net efflux was immediately interrupted by girdling, and thereafter immediately restored by the addition of 100 mM glucose to the medium (Fig. 7). In all cases, the H^+ net efflux was stopped by 0.5 mM vanadate (not shown). The mean K^+ rates were close to the H^+ efflux rates (10.4 ± 2.8 μmol·h^{-1}·plant^{-1} before girdling, 2.1 ± 0.6 μmol·h^{-1}·plant^{-1} after girdling, and 10.2 ± 3.4 μmol·h^{-1}·plant^{-1} after girdling plus 100 mM glucose). Urea-fed plants acidified the medium at increasing rate during the first half of the light period. The net H^+ efflux then declined, and disappeared 2 h after the beginning of the dark period (Fig. 7).

NO_3^- net uptake was calculated from the decrease of NO_3^- concentration in the medium, corrected for the volume variation due to water consumption. It greatly diminished after girdling. The addition of glucose to the medium after girdling restored NO_3^- uptake, but not OH^- net efflux (Fig. 8).

DISCUSSION

As shown by Armstrong and Kirkby (3) for tomato, measuring the accumulation rates of K^+ and Ca^{2+} in the shoots and the K^+/Ca^{2+} ratio in the xylem sap makes it possible to estimate both xylemic and phloemic transports of K^+. The slopes of the regression lines in Figure 2 give the relative variation rates of the shoot and root contents in K^+, Ca^{2+}, NO_3^-, and N_r (Tab. II). The absolute variations of the contents during d 16 were obtained by multiplying the slopes by the corresponding contents (see legend to Fig. 2). Assuming that Ca^{2+} was not transported in the phloem (21), its xylemic transport was estimated from the Ca^{2+} absolute increase rate in shoots. Xylemic transports of other species were then calculated by multiplying Ca^{2+} transport in the xylem by the ratios of Table IV. The recirculation of K^+ in the phloem was the difference between xylemic transport and accumulation in tops. The same method was used to determine transport of N_r in the phloem in urea-fed plants. The results of this analysis are shown in Figure 9.

We assumed that NO_3^- was not transported in the phloem (9). Thus, the rate of export of N_r from shoots was obtained as the difference between the xylem transport and the accumulation rate of (NO_3^- + N_r). NO_3^- reduction in shoots was obtained by subtracting the NO_3^- accumulation rate from NO_3^- transport in the xylem. It accounted for 93% of the reduction by the whole plant estimated from the sum of N_r increments in shoots and roots (Fig. 9). This figure is substantially higher than the estimations of shoot contribution to NO_3^- reduction in soybean determined from nitrate reductase activity (70% in Ref. 5), or by modeling of N and C circulation (12–34% in Ref. 30). In these investigations, N_r was the major form of N transported in

Table II. *Parameters of Linear Regressions in Figure 1*

Parameter	Shoots				Roots			
	K^+	Ca^{2+}	NO_3^-	N_r	K^+	Ca^{2+}	NO_3^-	N_r
				NO_3^- medium				
Slope	0.153	0.211	0.109	0.114	0.128	0.148	0.139	0.120
Y-intercept	3.918	1.951	3.782	5.502	3.533	0.103	2.723	3.609
r^{2a}	0.978	0.976	0.997	0.959	0.953	0.982	0.968	0.969
				Urea medium				
Slope	0.151	0.121		0.106	0.113	0.101		0.101
Y-intercept	3.842	2.371		5.593	3.503	0.325		3.920
r^{2a}	0.998	0.971		0.969	0.968	0.966		0.921

[a] r^2, correlation coefficient.

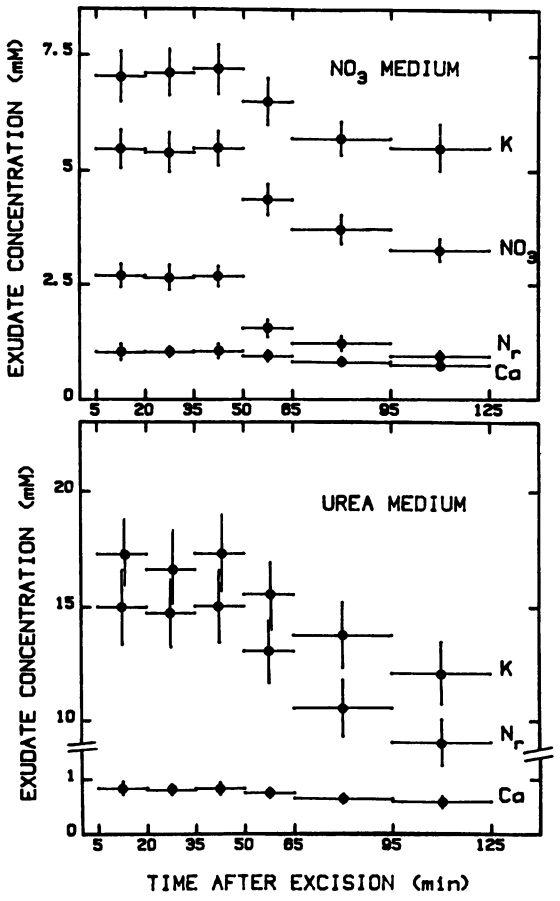

FIG. 3. Concentrations of ions and N in xylemic exudates of NO_3^- fed or urea-fed soybean plants. The excised root systems of 16-d-old plants were sealed in tight pots, in which pressure was raised to 150 kPa (NO_3 medium) or 250 kPa (urea medium). Samples of xylemic sap were periodically collected and assayed. Horizontal bars indicate the period of sampling of exudates. Vertical bars represent 95% confidence limits (four replicates).

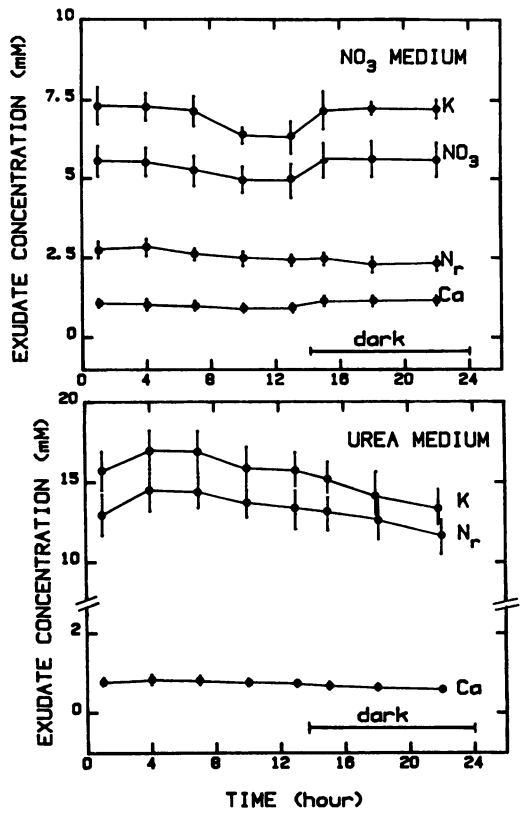

FIG. 4. Circadian evolution of xylemic sap composition of NO_3^--fed or urea-fed soybeans during d 16. Plants were periodically decapitated and the xylemic sap collected as indicated in Figure 3, between 5 and 35 min after pressurization. The origin of the time scale is the beginning of the light period. Means of four plants, with 95% confidence limits.

the xylem sap (50–90% of total N, as compared with 32% in our experiments). Furthermore, it has been shown with ^{15}N that a large fraction of the reduced N in the xylem originated from the shoots. In soybean with 40–60% of xylemic N as N_r, shoot contribution to NO_3^- reduction was estimated to be more than 80 to 90% (25). In NO_3^--fed plants, the cycling of N_r amounted to 40% of total N_r input in shoots (xylem transport plus reduction), and roots recirculated in the xylem the main part of their own N_r input (77%) (Fig. 9). On the contrary, urea nutrition led to a very low N circulation, with 96% of N transported by xylem being accumulated in shoots (Fig. 9). As expected from the fact that K^+ was the main accompanying cation of NO_3^- in xylem (Fig. 4), its transport in shoots was stimulated by NO_3^- nutrition (Fig. 9). Its cycling was also dependent on the N source. On d 16, shoots of NO_3^--fed plants recirculated 193 μmol K^+ per plant (Fig. 9), which represented 33% of their K^+ content (see legend to Fig. 2). This figure was only 21% for plants grown on urea. Clearly, the upward transport of K^+ together with NO_3^-, and the return of the cation back to roots, implies that it was accompanied in the phloem by an anion supplied in a large amount by shoots.

It was assumed that reduction of 1 mol NO_3^- gave 1 mol N_r plus 1 eq R^-. When fed with NO_3^-, soybean retained as organic anions in their tissues only 38% of the negative charges released by NO_3^- reduction (Table I). For instance, the data of Table II for day 16 indicate that 203 μmol NO_3^- per plant were reduced, which theoretically corresponds to an equal production of R^-. The predicted excretion of 127 μeq OH^- per day (62% of 203 μeq) is in good accord with the observed net efflux of OH^- equivalents (110 μeq·plant^{-1} per day, from Fig. 5). The question addressed in this work is the origin of these OH^- equivalents.

R^- was virtually absent from roots (Table I), and NO_3^- reduction in these organs was lower than in shoots by more than one order of magnitude. Thus, it is unlikely that roots contained a source capable of delivering OH^- in sufficient amounts to account for the alkalinization of the medium, and most of the excreted base would have originated in shoots. Furthermore, the absence of R^- from the xylem sap of NO_3^--fed plants indicated that all the OH^- produced by NO_3^- reduction in roots were excreted. For this reason, R^- transport from shoots to the medium was estimated as the difference between total base excretion and OH^- production in roots. It accounted for 89% of total base excretion (Fig. 9).

Plants alkalinized the medium only in the presence of NO_3^- (Figs. 5 and 6): both plants grown on urea, and plants fed with NO_3^- but transferred on N free medium, presented a H^+ net excretion (Fig. 7). The progressive increase of base excretion during the light period, and decrease in dark (NO_3^--fed plants, Figs. 5 and 6), is in accordance with the known kinetics of NO_3^- reduction in soybean (26). During the light period, the inhibition of base excretion after girdling (85%) was as expected from the above estimate concerning the proportional shoot contribution of OH^-. Girdling might have interrupted OH^- excretion by causing an energy shortage in roots rather than by interrupting OH^- delivery from shoots. Indeed, glucose addition after girdling revealed a H^+ net excretion sensitive to vanadate (Figs. 5–7). This indicated that the interruption of phloemic transport of

Table III. *Characteristics of Xylem Exudates*

The ratios are expressed as mol/mol. Light period: 0 to 14 h.

Ratio	Time (h)							
	1	4	7	10	13	15	18	22
				NO_3^--fed plants				
K^+/Ca^{2+}	6.71 ± 0.52	7.00 ± 0.49	7.07 ± 0.42	6.69 ± 0.35	6.63 ± 0.29	6.20 ± 0.37	6.35 ± 0.22	6.16 ± 0.31
NO_3^-/Ca^{2+}	5.08 ± 0.41	5.31 ± 0.47	5.18 ± 0.27	5.21 ± 0.21	5.18 ± 0.19	4.84 ± 0.32	4.92 ± 0.21	4.78 ± 0.20
N_r/Ca^{2+}	2.52 ± 0.20	2.71 ± 0.22	2.56 ± 0.19	2.59 ± 0.21	2.54 ± 0.17	2.13 ± 0.09	1.99 ± 0.11	1.97 ± 0.13
				Urea-fed plants				
K^+/Ca^{2+}	20.13 ± 0.96	19.89 ± 1.01	20.57 ± 0.85	20.31 ± 0.77	21.03 ± 1.05	21.97 ± 2.10	21.88 ± 0.95	22.37 ± 1.09
N_r/Ca^{2+}	16.62 ± 0.82	17.08 ± 1.12	17.52 ± 0.72	17.49 ± 0.67	17.71 ± 1.23	19.01 ± 1.52	19.60 ± 0.87	19.43 ± 1.17

Table IV. *Characteristics of Xylemic Sap of 16-d-Old Plants*

The ratios for exudates are calculated from the data of Table III integrated for 24 h. The stem sap was obtained from stem segments cut 10 h after the beginning of the light period. The sap was extracted by applying 50 kPa pressure at one end of the segments (means of six repetitions and 95% confidence limits). The ratios are expressed as mol/mol.

Method	NO_3^- Medium			Urea Medium	
	K^+/Ca^{2+}	NO_3^-/Ca^{2+}	N_r/Ca^{2+}	K^+/Ca^{2+}	N_r/Ca^{2+}
Root exudate	6.56	5.03	2.36	20.94	17.93
Stem sap	6.55 ± 0.62	5.05 ± 0.47	ND[a]	21.25 ± 1.52	ND

[a] Not determined.

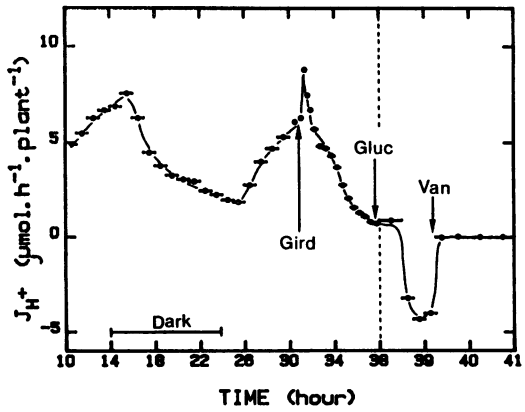

FIG. 5. Net H^+ transport in roots of intact soybean plants grown on NO_3^- medium. The origin of the time scale is the beginning of the light period of d 16. The plants were installed in the experimental device at 4 h. Net H^+ transport rate was monitored with an automatic pH-stat system. Gird, girdling of the base of the stem with water vapor stream; Gluc, addition of 100 mM glucose to the medium; Van, addition of 0.5 mM vanadate to the medium. The vertical dotted line indicates the change of time scale. Negative values of J_{H^+} correspond to H^+ net efflux. Positive ones correspond to a net efflux of OH^- equivalents. The transient acceleration of OH^- efflux after girdling was systematically observed, but its cause is not known.

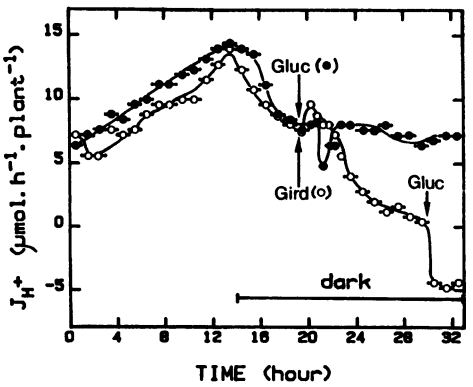

FIG. 6. Net H^+ transport in the roots of intact soybean plants grown on NO_3^- medium. The origin of the time scale is the beginning of the light period of d 16. The plants were installed in the experimental device 20 h before the experiment. Net H^+ transport rate was monitored with an automatic pH-stat system. Gird, girdling of the base of the stem with water vapour stream; Gluc, addition of 100 mM glucose to the medium. Negative values of J_{H^+} correspond to H^+ net efflux. Positive ones correspond to a net efflux of OH^- equivalents. The transient, partial inhibition of OH^- efflux after addition of a glucose solution in the dark was probably an artifact.

carbohydrates inhibited the proton pump activity. However, glucose could not restore base excretion associated with NO_3^- use after it was interrupted by girdling. In the same way, glucose could not stimulate OH^- excretion during the dark period (Fig. 6). From these results, it may be concluded that inhibition of base excretion after girdling was due to interruption of R^- supply rather than to energy shortage.

The velocity of phloem sap seems to vary in a wide range in herbaceous plants, approximately between 1.5 to 150 $cm \cdot h^{-1}$ (28). In soybean, Housley and Fisher (13) have measured velocities comprised between 40 and 55 $cm \cdot h^{-1}$. However, the light intensity in their experiments was 3 to 4 times less than in ours. Since sap velocity depends on light level (28), it is likely that 50 $cm \cdot h^{-1}$ is a minimum value for our plants. With our estimate of the phloem cross sectional area (5×10^{-3} cm^2), and of the area of sieve elements relative to whole phloem tissues (12%), a sap velocity of 50 $cm \cdot h^{-1}$ corresponds to a volume flow of 0.03 $ml \cdot h^{-1}$. Another estimation for phloem sap transport in soybean may be obtained as volume flow, from data presented by Vessey and Layzell (30). In this case, light intensity and photoperiod were similar to ours. In 26-d-old plants, they calculated that phloem sap contained 3.3 $mg \cdot ml^{-1}$ N, and brought 35 mg N to the roots in 96 h. This corresponds to 0.11 $ml \cdot h^{-1}$. Since their plants were 10 days older than ours, this value could be considered as an overestimation of the phloem flow occurring in our experiments. With these extreme estimates, it is possible to

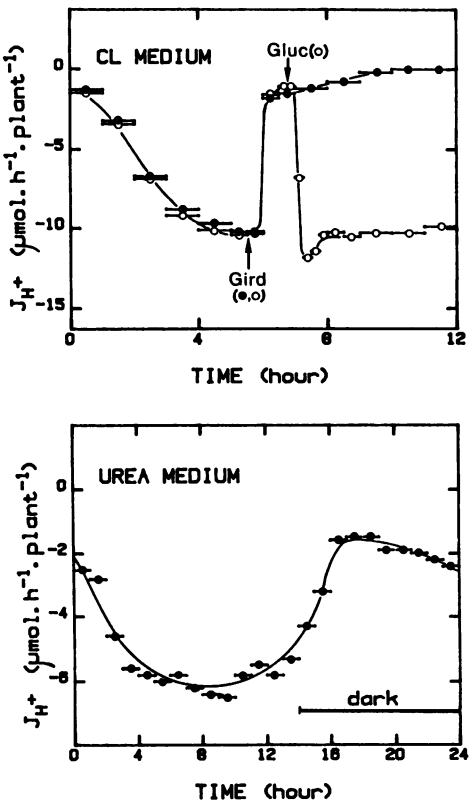

FIG. 7. Net H^+ transport in roots of intact soybeans. The plants were grown on urea medium for 16 d, or on NO_3^- medium for 11 d and on Cl^- (N free) medium for 5 d. The plants were installed in the experimental device 20 h before the beginning of the experiment. The origin of the time scale is the beginning of the light period of d 16. The net H^+ transport rate was monitored with an automatic pH-stat system. Gird, girdling of the base of the stem with water vapor stream; Gluc, addition of 100 mM glucose to the medium. Negative values of J_{H^+} correspond to H^+ net efflux.

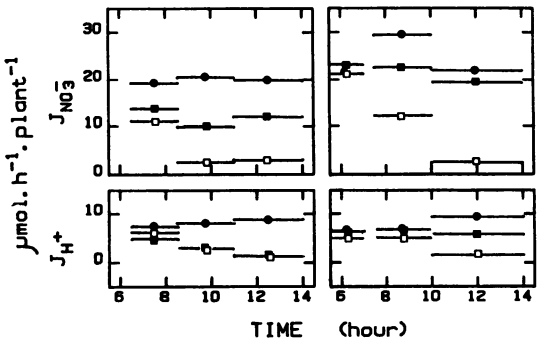

FIG. 8. Net NO_3^- and net H^+ transports in the roots of intact soybean plants grown on NO_3^- medium. The origin of the time scale is the beginning of the light period of d 16. The plants were installed in the experimental device 20 h before the experiments. Net NO_3^- transport rate was calculated from the decrease in medium NO_3^- concentration, corrected for the volume variation. Net H^+ transport rate was monitored with an automatic pH-stat system. (●), control, (■), the hypocotyls were steam girdled and 100 mM glucose was added to the medium at 6 h (left panels) or at 7 h (right panels). (□), girdled hypocotyls, but no glucose in the medium. Positive values of J_{H^+} correspond to a net efflux of OH^- equivalents. The two panels present independent experiments.

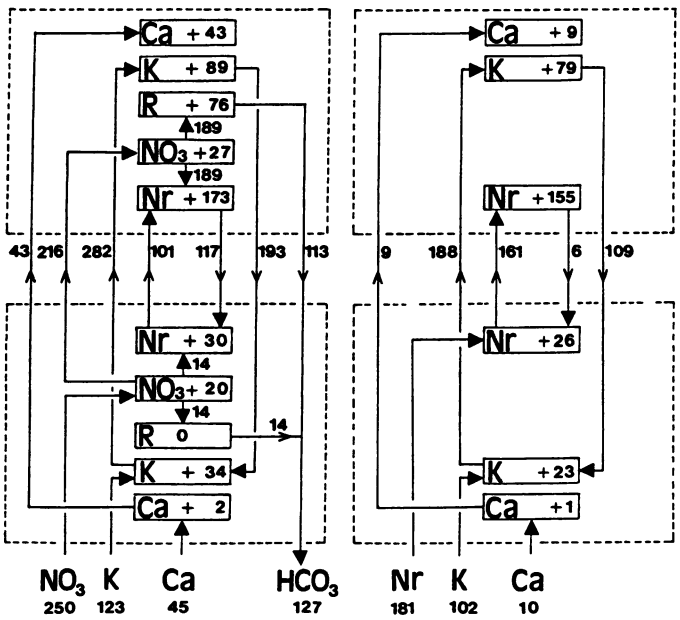

FIG. 9. Balance sheets of net transports. The values are for 16-d-old plants grown on NO_3^- medium (left), or on urea medium (right). The data are expressed as $\mu mol \cdot d^{-1}$ per plant. Upper compartments: shoots; lower compartments: roots.

evaluate the concentration ranges of K^+, R^-, and N_r in phloem sap, which could account for the calculated transports to roots in NO_3^- plants (Fig. 9): 74 to 278 mM K^+, 44 to 161 mN R^-, 45–167 mM N_r. Each one of these ranges encompasses the published concentrations of the corresponding species in the phloem of herbaceous plants (10, 11, 24). Thus, the features of the circulation model in Figure 9 are not unrealistic on an anatomical and physiological basis.

The results of Figure 8 indicate that a large part of NO_3^- uptake may be restored by glucose, after girdling has interrupted OH^- supply. This result suggests that OH^- production by NO_3^- reduction in shoots and conduction into roots by phloem is not essential for NO_3^- uptake NO_3^-.

To summarize, our results support the Ben Zioni hypothesis of a cycling of R^- and K^+ in NO_3^- reducing soybean. Further work is needed to obtain direct indications of an involvement of this mechanism in the control of NO_3^- absorption by roots.

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