ABSTRACT

The objective of this study was to examine the influence of \(N_2\) fixation and \(NO_3^-\)-N and urea-N assimilation on ion balance, uptake, and transport processes in soybean (\textit{Glycine max} L. Merr.).

Inoculated plants were grown in perlite supplied daily with nutrient solutions which contained zero-N, 10 and 20 millimolar \(NO_3^-\)-N, and 10 and 20 millimolar urea-N, and they were sampled 41, 76, and 151 days after transplanting. Total uptake of inorganic cations and anions was determined by analysis of tissue for \(K^+\), \(Ca^{2+}\), \(Mg^{2+}\), \(Na^+\), total N from \(NO_3^-\), total S, \(H_2PO_4^-\), and \(CT^-\). Differences in total inorganic cations (C) and inorganic anions (A) in plant tissue were used to estimate total carboxylate content.

Internal \(OH^-\) generation resulting from excess cation uptake (net \(H^+\) excretion) by the roots accounted for more than 89% of the carboxylate accumulation in \(N_2\)- and urea-fed plants, while \(OH^-\) generation resulting from \(SO_4^{2-}\) reduction accounted for less than 11%. Shoots contained over 89% of the total plant carboxylate content. Malate balanced about 75% of the excess inorganic cationic charge of the xylem sap; allantoin and aspartate balanced most of the remaining charge. These results indicate that carboxylate (primarily malate) are synthesized in roots of \(N_2\)- and urea-fed plants and transported to the shoots in the xylem to maintain charge balance. The high malate concentration resulted in the C/N weight ratio of xylem sap from urea-fed plants being >2.0, even though 83% of the \(N\) was transported as allantoin and alantoic acid which have a C/N ratio of 1.0. The data base for calculating the C/N weight ratio of xylem sap.

The C-to-A uptake ratio for plants supplied 10 millimolar \(NO_3^-\) ranged from 1.24 to 1.57 during development, indicating that internal \(OH^-\) was generated both by excess cation uptake and by \(NO_3^-\) and \(SO_4^{2-}\) reduction. The C-to-A uptake ratio for 20 millimolar \(NO_3^-\)-fed plants ranged from 0.86 to 0.96 during development, indicating a small net \(OH^-\) efflux from the roots for support of excess anion uptake. On a seasonal basis, only 15% of the \(OH^-\) generated during \(NO_3^-\) and \(SO_4^{2-}\) reduction was associated with \(OH^-\) efflux (excess anion uptake), while 85% was associated with carboxylate accumulation. The malate concentration in xylem sap from plants supplied 20 millimolar \(NO_3^-\) was only one-third that of \(N_2\)- and urea-fed plants; however, it did balance 75% of the excess inorganic cationic charge. Potassium, recycling to accommodate excess anion uptake by 20 millimolar \(NO_3^-\)-fed plants, was calculated to involve at most 17% of the \(K^+\) absorbed during the 41- to 76-day growth interval.

It has been shown that total inorganic cations (C) exceed total inorganic anions (A) in the tissue of a number of nonleguminous species (3, 14, 17). Reduction of \(NO_3^-\), and \(SO_4^{2-}\), as well as greater uptake of inorganic cations than of inorganic anions, contributes to the accumulation of excess inorganic cations (C-A) and to the generation of \(OH^-\) within the tissue (17, 23). Cytoplasmic acidity is maintained by transfer of the negative charge associated with the \(OH^-\) to carboxylates, i.e., nonvolatile organic acids and uronic residues of pectic substances (5, 9, 17, 23).

For some species assimilating \(NO_3^-\), essentially equal amounts of inorganic cations and inorganic anions were absorbed, the acidity of the ambient medium remained relatively constant, and the quantity of carboxylates (C-A) in the tissue was equal to the total quantity of reduced \(N\) and organic \(S\) in the tissue (3, 16, 17). Thus, in these plants, the sole fate of \(OH^-\) generated by \(NO_3^-\) and \(SO_4^{2-}\) reduction was to support carboxylate accumulation. For other species supplied \(NO_3^-\), more inorganic anions than inorganic cations were absorbed, the pH of the ambient medium increased, and the quantity of carboxylates (C-A) in the tissue was less than the quantity of reduced \(N\) and organic \(S\) (4, 6, 15). In these instances, two fates are indicated for the \(OH^-\) generated during \(NO_3^-\) and \(SO_4^{2-}\) reduction: exchange to the ambient medium to drive excess anion uptake; and consumption in carboxylate synthesis.

For plants assimilating \(NH_4^+\) and urea, in the absence of the readily permeable \(NO_3^-\) anion, inorganic cation uptake exceeds inorganic anion uptake substantially (3, 12, 17), with a resulting acidification of the rooting medium. The quantity of carboxylate (C-A) in tissue of plants supplied \(NH_4^+\) was only a small fraction of that observed for \(NO_3^-\)-supplied plants and was much less than the quantity of reduced \(N\) and organic \(S\) (3, 12, 17). Most of the \(OH^-\) generated during excess cation uptake is thought to be neutralized by the \(H^+\) produced during subsequent assimilation of \(NH_4^+\) (17, 23). The quantity of carboxylates in tissue of tomato plants assimilating urea was intermediate between that of plants supplied \(NO_3^-\) and plants supplied \(NH_4^+\) (17). Inasmuch as assimilation of urea does not alter the pH of the plant cell cytoplasm, the quantity of carboxylates measured in the tissue was essentially equal to the quantity of excess cation uptake and of \(SO_4^{2-}\) reduced (17).

When leguminous plants are receiving most of their nitrogen from \(N_2\) fixation, a sizeable excess cation uptake should occur with a concomitant acidification of the root rhizosphere and accumulation of tissue carboxylates (13, 22). In addition, such plants must adjust their root metabolism to accommodate charge balance in the upward-flowing xylem fluid. The paucity of information with leguminous plants led us to examine the various ionic balance adjustments which occur in soybean plants obtaining their nitrogen from \(N_2\) fixation, \(NO_3^-\), or urea. A previously proposed conceptual model (13) was used in interpreting the results.

MATERIALS AND METHODS

Plant Culture. Soybean (\textit{Glycine max} L. Merr.) plants were cultured in an unshaded greenhouse from May through mid-
October of 1977. During daylight h, ambient temperatures were kept below 37°C with evaporative cooling. Night temperature was allowed to equilibrate with outdoor temperature by leaving vents open. The RH ranged from 40 to 50% at midday and from 80 to 90% at night.

Seeds of 'Ransom' soybeans were germinated, and all seedlings were inoculated with *Rhizobium japonicum* strain USDA 311b110 and transplanted (two per pot) into 25.4-cm-diameter pots containing Perlite, as described previously (19, 20). Twelve pots of inoculated seedlings were exposed to each of five nutrient treatment treatments and arranged in a randomized complete block design with treatments and sampling dates (at 41, 76, and 151 days after transplanting) randomized within blocks. Seedlings were thinned to one per pot 2 weeks after transplanting.

Nutrient solutions containing N were prepared by adding 10 and 20 mm KNO₃ and 10 and 20 mm urea-N to the N-free nutrient solution previously described by McClure and Israel (19). The initial pH of the treatment solutions ranged from 6.6 to 6.8. The K⁺ concentration of all solutions, including the N-free solution, was made equal to that of the 20 mm KNO₃ solution by adding appropriate amounts of K₂SO₄. From day 1 to day 9, the appropriate nutrient solution (250 ml) was applied twice daily at 0900 and 1500 h. From the 9th day to the 8th week, pots were flushed thoroughly twice daily (0900 and 1500 h) with tap water (1.5 L/flush), and, after the second flushing, appropriate nutrient solution (400 ml) was applied. From the 8th week to final harvest, appropriate nutrient solution (400 ml) was applied after both the morning and afternoon flushings. From the 10th week to late September, pots were also flushed with 1.5 L tap water at noon to meet the high transpiration demand of the plants. Inasmuch as the pH of the root rhizospheres decreased after application of N-free and urea solutions and increased after application of NO₃⁻ solutions, the repeated flushing and application of nutrient solutions were used to prevent long-term exposure of roots to a low or high pH. From the water-holding capacity of the Perlite and the volume of nutrient solution added, the concentrations of nutrients in the solution phase of the growth medium shortly after addition were estimated to be about one-fifth the concentrations in the applied nutrient solutions. As the plants grew, pots were spread to minimize canopy interference. All plants produced branches at the cotyledonal, primary, and lower three or four trifoliate nodes on the main stem.

### Measurement of Tissue Dry Weight and Chemical Composition

At 41- and 76-day samplings, plants were separated into leaflets (including the petiolules), stems plus petioles, roots, and nodules. At the 151-day sampling, seeds and pod walls were also separated. Plant material was dried at 65°C for 72 h, weighed, ground to pass through a 1.0-mm screen, and mixed for 4 h before subsamples were taken for chemical analyses.

The total N content of tissue samples (100 mg) was determined by a Kjeldahl procedure that included a salicylic acid predigestion step to convert NO₃⁻ to NH₄⁺ and that employed a zirconium-copper catalyst (19). After extracting 500 mg tissue in 50 ml H₂O, nitrate-N was determined by a manual modification of the method of Lowe and Hamilton (18). Reduced nitrogen in tissue was calculated by subtracting the NO₃⁻ -N content from the total N content. Ammonium-N content of tissue from 76-day-old plants was determined, so that ash alkalinity values could be corrected. Samples of dry tissue (1 g) were shaken for 2 h in 15 ml distilled H₂O and then allowed to stand overnight. After filtration and dilution to 25 ml, NH₃-N in 2.5 ml aliquots was diffused into 0.5 N HCl and quantified as described previously (20), with the exceptions that 2.0 ml 25 mm sodium tetraborate (pH 10) was used to alkalize the aliquots, and flasks were shaken gently at 50°C for 48 h to obtain quantitative recovery of NH₃.

Although nodulation was inhibited by the application of NO₃⁻ solutions, NO₃⁻-fed plants formed a few nodules that were active in N₂ fixation. Thus, the total N and reduced N contents of tissue of NO₃⁻-supplied plants were derived both from NO₃⁻ uptake and assimilation and from N₂ fixation. The contribution of NO₃⁻ uptake and assimilation to total N and reduced N contents was estimated using the relationship between relative ureide content and assimilation uptake for 1200 h for 37°C.

For determination of Ca²⁺, Mg²⁺, K⁺, Na⁺, and H₂PO₄⁻, the ash from 1.25 g of dry tissue was dissolved in 3 ml 4.0 N HCl. Calcium and Mg²⁺ were determined by atomic absorption, and K⁺ and Na⁺ were determined by flame photometry. Phosphorus was determined spectrophotometrically at 470 nm after reaction with ammonium molybdate-vanadate. Chloride was determined with an automatic chloride titrator.

For total S measurements, 0.25-g samples of dry tissue were ashed in the presence of 5.4 meq Mg(NO₃)₂. The ash was dissolved in 3 ml 4.0 N HCl and determined turbidimetrically (27). Organic S content of tissue was calculated from the organic N content using the relation: organic S = organic N × 0.05 meq organic S/ meq organic N. Inasmuch as the NH₄⁺ content in tissue of 76-day-old plants was small (<5%) relative to the total reduced N concentration, reduced N was taken to be organic N. The ratio of organic S to organic N is the average determined for tissues of a number of leguminous plants supplied adequate sulfur (7).

Sulfate-S was calculated as the difference between total S and organic S. Occasionally the calculated organic S of tissue samples expected to have low SO₄-S content was greater than the measured total S. In these cases, organic S was taken to be equal to total S. Inasmuch as total S content of the tissue was small in relation to nitrogen and total cation contents, errors associated with those estimates had a small impact on the ion balance calculations.

Ash alkalinity of tissue samples from 76-day-old plants was determined by ashing 0.5 g of dry material in the presence of 0.5 meq NaOH at 550°C for 3 h, dissolving the ash in 10 or 20 ml 0.1 N HCl, and titrating the excess acid to pH 5 with 0.1 N NaOH, using methyl red-methylene blue as an indicator. Ash alkalinity values were corrected for NO₃⁻ and NH₄⁺ contents of the tissue, and, thus, should be a measure of total carbonate content (17). Ash alkalinity of tissue from 76-day-old plants was used to verify that the previously described measurements of inorganic cations and anions accounted for the major positively and negatively charged inorganic substances. Excess cation content (C-A) was used as the measure of total carbonate content of tissue from 41and 151-day-old plants.

### Collection and Analysis of Xylem Sap

Sap from 76-day-old plants was collected between 1000 and 1200 h for time periods up to 20 min and kept on ice until transferred to a freezer (−18°C) for storage, as described previously (19, 20). After appropriate dilution, K⁺, Na⁺, Ca²⁺, Mg²⁺, SO₄²⁻, Cl⁻, H₂PO₄⁻, and NO₃⁻ contents of the sap were quantified using procedures described for tissue analyses. Amino acids and ureides (allantoin and allantoic acid) were determined by the use of a colorimetric assay for amino acid content (21).
acid) were separated and quantified using an amino acid analyzer (19). Total N was determined by a summation of N in amino acid, ureide, and NO$_3^-$ fractions, which has been shown to account for essentially all of the N in the sap (19). Malate was determined by the malate dehydrogenase method (11). Total carbon was calculated by summation of carbon associated with amino acid, ureides, and malate.

**RESULTS**

**Yield and Cation-Anion Uptake.** The growth of N$_2$-fed plants was restricted relative to that of plants supplied combined N (Table I), because they experienced a transient N stress as evidenced by reversible yellowing of leaves between the time that cotyledonary N reserves were depleted (2.5 to 3 weeks) and the time that nodules became capable of supplying adequate N for growth (approximately 4 weeks). For N$_2$-fixing and urea-dependent plants, cation uptake exceeded anion uptake 4- to 6-fold throughout the developmental cycle, indicating a considerable net H$^+$ efflux from the roots. The cation-to-anion uptake ratio ranged from 1.27 to 1.57 for plants supplied 10 mM NO$_3^-$, indicating a greatly reduced net H$^+$ efflux as NO$_3^-$ uptake contributed to total anion uptake. The ratio ranged from 0.86 to 0.96 for plants supplied 20 mM NO$_3^-$, indicating a relatively small net OH$^-$ efflux from the roots, as NO$_3^-$ uptake and assimilation accounted for most (82%) of the whole plant N input.

Sulfate and H$_2$PO$_4^-$ were the major anions absorbed by N$_2$-fixing and urea-dependent plants. Nitrate was the major anion absorbed by NO$_3^-$-dependent plants, but its uptake did not markedly affect the uptake of SO$_4^{2-}$ or H$_2$PO$_4^-$.

Potassium plus Ca$^{2+}$ uptake comprised more than 80% of total cation uptake by plants exposed to the different N sources. Regardless of the N source, K$^+$ uptake exceeded Ca$^{2+}$ uptake, but the ratio of K$^+$ to Ca$^{2+}$ uptake was lower for NO$_3^-$-dependent plants than it was for N$_2$- and urea-dependent plants. With all N sources, the ratio of K$^+$ to Ca$^{2+}$ uptake declined as plants ap-

#### Table 1. Influence of Age and N Source on Growth and Inorganic Cation and Anion Uptake by Whole Soybean Plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time after Transplant</th>
<th>Dry weight</th>
<th>Cations</th>
<th>K meq/plant</th>
<th>Ca meq/plant</th>
<th>Mg meq/plant</th>
<th>Na meq/plant</th>
<th>Total meq/plant</th>
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<td>Zero-N (N$_2$ fixation)</td>
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<th>N meq/plant</th>
<th>S meq/plant</th>
<th>P meq/plant</th>
<th>Cl meq/plant</th>
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<th>Cation/Anion Uptake</th>
<th>ratio</th>
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<td>167.0</td>
<td>526.0</td>
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</table>

*a* S considered divalent; P, monovalent.

*b* N from NO$_3^-$ uptake.

*c* Excess anion uptake.
proached maturity.

Cation-Anion Balance. In most instances, the sums of total cations in tissues of 76-day-old plants (K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^+\)) closely balanced (within 10%) the sums of total inorganic anions (NO\(_3^\), SO\(_4^{2-}\), H\(_2\)PO\(_4^-\), and Cl\(^-\)) and total carboxylates, as determined by ash alkalinity (Table II). Agreement was less precise in roots of N\(_2\)- and 20 mm urea-fed plants, where balances were within 25% and 15%, respectively. Leaflet and stem plus petiole tissues contained higher carboxylate concentrations than did root tissue (derived from information in Table II). With all N sources, carboxylates as a percentage of total anions were 68 to 74% in root tissues, 80 to 87% in stem plus petiole tissue, and 86 to 92% in leaf tissue. Inasmuch as urea- and NO\(_3^-\)-N stimulated plant growth, the total quantity of carboxylates in plants supplied these N sources was greater than that in N\(_2\)-fed plants.

**Table II. Effect on N-Source on Cation-Anion Balance of 76-Day-Old Ransom Soybean Plant Tissues**

Values represent the means of four replicates.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Part</th>
<th>Dry Weight g/plant</th>
<th>Cations (meq/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>K</td>
</tr>
<tr>
<td><strong>Zero-N (N(_2) fixation)</strong></td>
<td>Leaflets</td>
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<td>31.7</td>
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<td></td>
<td>Stem + petiole</td>
<td>68.8</td>
<td>72.0</td>
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<td>Root</td>
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<td>10.2</td>
</tr>
<tr>
<td><strong>NO(_3^-), 10 mm</strong></td>
<td>Leaflets</td>
<td>76.5</td>
<td>44.5</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>132.7</td>
<td>141.3</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>25.0</td>
<td>14.8</td>
</tr>
<tr>
<td><strong>NO(_3^-), 20 mm</strong></td>
<td>Leaflets</td>
<td>81.1</td>
<td>48.4</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>136.1</td>
<td>137.1</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>26.7</td>
<td>18.0</td>
</tr>
<tr>
<td><strong>Urea, 10 mm</strong></td>
<td>Leaflets</td>
<td>71.7</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>112.2</td>
<td>114.9</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>21.4</td>
<td>12.4</td>
</tr>
<tr>
<td><strong>Urea, 20 mm</strong></td>
<td>Leaflets</td>
<td>84.3</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>124.3</td>
<td>114.3</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>26.3</td>
<td>13.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Part</th>
<th>Anions (meq/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NO(_3^-)</td>
</tr>
<tr>
<td><strong>Zero-N (N(_2) fixation)</strong></td>
<td>Leaflets</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>NO(_3^-), 10 mm</strong></td>
<td>Leaflets</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>NO(_3^-), 20 mm</strong></td>
<td>Leaflets</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>3.9</td>
</tr>
<tr>
<td><strong>Urea, 10 mm</strong></td>
<td>Leaflets</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Urea, 20 mm</strong></td>
<td>Leaflets</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.8</td>
</tr>
</tbody>
</table>
the total anion charge, while the proportion due to carboxylates decreased from 60% to 33%.

Potassium and Ca²⁺ were the dominant cations in sap from plants exposed to all N sources (Table VI). The K⁺ to Ca²⁺ ratio was lower in sap from plants supplied 20 mM NO₃⁻ than in sap from urea- and N₂-fed plants, as well as those supplied 10 mM NO₃⁻.

The negative charge associated with malate, aspartate, and allantoate closely balanced (within 10%) the excess inorganic cation charge in the xylem sap (Table VI). Regressing the total carboxylate concentration against the excess cation concentration in the xylem sap revealed a linear relationship, \( y = 1.01x + 0.312 \), with an \( R^2 = 0.902 \), indicating that the most important carboxylates in the xylem sap have been taken into account. Malate comprised 76% to 85% of the carboxylate charge in xylem sap from plants exposed to the different N treatments (Table VI). The concentration of malate in sap from N₂-, 10 mM NO₃⁻, and urea-fed plants was 2 to 3 times that in sap from plants supplied 20 mM NO₃⁻.

**Carbon-Nitrogen Balance of Xylem Sap.** Because of the high malate concentration, the carbon in sap from N₂- and urea-fed plants was more than twice that in sap from plants supplied 20 mM NO₃⁻ (Table VII). Forty-eight, 35%, and 17% of the carbon in sap from N₂-fed plants was associated with malate, ureides, and amino acids, respectively. More of the carbon in sap from urea- and 20 mM nitrate-fed plants was present as amino acids (including amides) than as malate. The carbon-to-nitrogen weight ratio of sap from N₂- and urea-fed plants was greater than 2.0, while that of sap from plants supplied 20 mM NO₃⁻ was 1.29. The ureides contained 83% of the total N in sap from N₂-fed plants. The importance of ureides as N transport forms in xylem sap declined as plants were supplied increasing levels of NO₃⁻ and urea (cf. Ref. 19).

**DISCUSSION**

A conceptual model has been proposed to account for the effects of assimilation of different nitrogen sources on ion uptake, translocation, and balance in plants (13). The model assumes that total cation and total anion (inorganic plus organic) charges of plant tissue must be equal (17) and that the pH of the cell cytoplasm must be maintained within close limits (pH 7–8) (23).

An electrogenic H⁺ transport to the ambient medium by a reversible ATPase located in the plasmalemmae of the root cells (14A) is viewed as maintaining both an electrical and an acidity gradient across those membranes (10, 24, 25). Cations enter the tissue in response to the resulting electrical potential gradient (1B) or as an integral part of the pump operation. In addition to the alkalinizing effect in the root cytoplasm resulting from the H⁺ efflux process, cytoplasmic OH⁻ generation occurs when NO₃⁻ is reduced (Ref. 1D; Ref. 6). Sulfate reduction (not shown) also generates OH⁻ (6). Cytoplasmic OH⁻ generated by any of the processes can either serve as counterion for the inward transport of anions (Fig. 1C) or stimulate carboxylate synthesis (Fig. 1E; Refs. 5, 9). The latter prevents dramatic increases in the pH of the cell cytoplasm when generation of OH⁻ exceeds the inward movement of anions. Organic acid decarboxylation (Fig. 1G; Ref. 5) can occur when rapid exchange of NO₃⁻ with cytoplasmic OH⁻ (Fig. 1C) or assimilation of NH₄⁺ (6, 17) causes a decrease in cytoplasmic pH. Excess anion uptake by plants in which NO₃⁻ reduction occurs primarily in the shoot is viewed as being sus-

---

**Table III. Influence of Age and N Source on Carboxylate (C₅A) Accumulation, H⁺ Excretion (Excess Cation Uptake), and NO₃⁻ and SO₄²⁻ Reduction by Whole Soybean Plants**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time after Transplant</th>
<th>H⁺ Excretion</th>
<th>Reduced N from NO₃⁻</th>
<th>Organic S</th>
<th>Carboxylate Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d</td>
<td>meq/plant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero-N (N₂ fixation)</td>
<td>41</td>
<td>14 (93)*</td>
<td>1 (7)*</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>151 (93)</td>
<td>11 (7)</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>455 (90)</td>
<td>49 (10)</td>
<td>504</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻-N, 10 mM</td>
<td>41</td>
<td>20 (49)b</td>
<td>19 (46)b</td>
<td>2 (5)*</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>114 (38)</td>
<td>169 (57)</td>
<td>16 (5)</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>182 (22)</td>
<td>599 (71)</td>
<td>60 (7)</td>
<td>841</td>
</tr>
<tr>
<td>NO₃⁻-N, 20 mM</td>
<td>41</td>
<td>0</td>
<td>41</td>
<td>2</td>
<td>43 (100)c</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>-33 (9)</td>
<td>337</td>
<td>17</td>
<td>321 (91)</td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>-166 (15)</td>
<td>1029</td>
<td>60</td>
<td>923 (85)</td>
</tr>
<tr>
<td>Urea-N, 10 mM</td>
<td>41</td>
<td>28 (93)*</td>
<td>2 (7)*</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>212 (91)</td>
<td>20 (9)</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>505 (89)</td>
<td>65 (11)</td>
<td>570</td>
<td></td>
</tr>
<tr>
<td>Urea-N, 20 mM</td>
<td>41</td>
<td>30 (94)*</td>
<td>2 (6)*</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>224 (91)</td>
<td>22 (9)</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>526 (90)</td>
<td>60 (10)</td>
<td>586</td>
<td></td>
</tr>
</tbody>
</table>

* Values in parentheses for N₂- and urea-dependent plants indicate percentage of total carboxylate accumulation attributed to excess cation uptake and to SO₄²⁻ reduction.

b Values in parentheses for 10 mM NO₃⁻-dependent plants indicate percentage of total carboxylate accumulation attributed to excess cation uptake, to NO₃⁻ reduction, and to SO₄²⁻ reduction.

c Values in parentheses for 20 mM NO₃⁻-dependent plants indicate percentage of total anion charge resulting from NO₃⁻ and SO₄²⁻ reduction associated with OH⁻ efflux (negative H⁺ excretion = excess anion uptake) and with carboxylate accumulation.

---

*Values represent means of four replicates.*
Table IV. Influence of Age and N Source on Distribution of Carboxylates (C-A) in Soybean Plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant Part</th>
<th>Carboxylates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>41 Days</td>
</tr>
<tr>
<td>Zero-N (N₂ fixation)</td>
<td>Leaflets</td>
<td>5.96 (41)*</td>
</tr>
<tr>
<td></td>
<td>Stem + Petiole</td>
<td>7.32 (50)</td>
</tr>
<tr>
<td></td>
<td>Pod wall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shootb</td>
<td>13.28 (91)</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>1.40 (9)</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>14.68</td>
</tr>
<tr>
<td>NO₃-N, 20 mm</td>
<td>Leaflets</td>
<td>17.31 (42)</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>19.55 (47)</td>
</tr>
<tr>
<td></td>
<td>Pod wall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shootb</td>
<td>36.86 (89)</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>4.83 (11)</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>41.69</td>
</tr>
<tr>
<td>Urea-N, 20 mm</td>
<td>Leaflets</td>
<td>13.34 (42)</td>
</tr>
<tr>
<td></td>
<td>Stem + petiole</td>
<td>15.60 (48)</td>
</tr>
<tr>
<td></td>
<td>Pod wall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shootb</td>
<td>28.94 (90)</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>3.32 (10)</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>32.26</td>
</tr>
</tbody>
</table>

* Values in parentheses indicate percentage of whole plant carboxylates.

* Shoot includes leaflets and stems + petioles at 41 and 76 days and these plus pod wall and seed at 151 days.

Table V. Influence of Nitrogen Source on Exudation rate, pH, and Ionic Composition of Xylem Sap from 76-Day-Old Plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Exudation Rate</th>
<th>pH</th>
<th>Ionic Composition of Xylem Sap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g min⁻¹ plant⁻¹</td>
<td>µeq/ml</td>
<td>K</td>
</tr>
<tr>
<td>Zero-N (N₂ fixation)</td>
<td>0.27</td>
<td>6.07</td>
<td>8.10</td>
</tr>
<tr>
<td>NO₃-N, 10 mm</td>
<td>0.39</td>
<td>6.26</td>
<td>7.03</td>
</tr>
<tr>
<td>NO₃-N, 20 mm</td>
<td>0.44</td>
<td>6.13</td>
<td>5.64</td>
</tr>
<tr>
<td>Urea-N, 10 mm</td>
<td>0.26</td>
<td>6.10</td>
<td>7.14</td>
</tr>
<tr>
<td>Urea-N, 20 mm</td>
<td>0.29</td>
<td>6.23</td>
<td>7.03</td>
</tr>
</tbody>
</table>

* Sum of microequivalents of malate, aspartic acid, and allantoic acid, assuming a net negative charge at pH of sap of -2, -1, and -1, respectively.

* Not detected.

Table VI. Influence of Nitrogen Source on the Carboxylate Composition and Charge Balance of Xylem Sap from 76-Day-Old Soybean Plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Malate</th>
<th>Aspartate</th>
<th>Allantoic Acid</th>
<th>Total Carboxylate</th>
<th>C-A</th>
<th>Carboxylate (µeq)</th>
<th>C-A (µeq)</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-N (N₂ fixation)</td>
<td>7.55</td>
<td>0.12</td>
<td>2.23</td>
<td>9.90</td>
<td>9.85</td>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃-N, 10 mm</td>
<td>4.83</td>
<td>0.25</td>
<td>0.76</td>
<td>5.84</td>
<td>6.15</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃-N, 20 mm</td>
<td>2.37</td>
<td>0.15</td>
<td>0.28</td>
<td>2.80</td>
<td>3.03</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea-N, 10 mm</td>
<td>6.49</td>
<td>0.34</td>
<td>1.19</td>
<td>8.02</td>
<td>8.52</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea-N, 20 mm</td>
<td>5.83</td>
<td>0.36</td>
<td>1.00</td>
<td>7.19</td>
<td>8.03</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Contains by decarboxylation of carboxylates (Fig. 1G) which are transferred with accompanying cations (K⁺) in the phloem to the roots (Fig. 1F) following their synthesis in association with leaf NO₃⁻ reduction (1, 2, 6, 8, 15). Transport of cations, organic and inorganic anions, and organic N forms into and out of the xylem (Fig. 1H), and vacuoles also must occur in a manner that allows...
ION BALANCE, UPTAKE, AND TRANSPORT PROCESSES

Table VII. Influence of Nitrogen Source on the C-N Balance of Xylem Sap from 76-Day-Old Soybean Plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>C/N ratio</th>
<th>Total C µg/ml</th>
<th>Malate µg/ml</th>
<th>Ureide %</th>
<th>Amino acids µg/ml</th>
<th>Total N µg/ml</th>
<th>Sap N as Amino acids µg/ml</th>
<th>NO₃⁻ µg/ml</th>
<th>NH₄⁺ µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-N (N₂ fixation)</td>
<td>2.04</td>
<td>381</td>
<td>48</td>
<td>35</td>
<td>17</td>
<td>188</td>
<td>83</td>
<td>16 &lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NO₃, 10 mM</td>
<td>1.84</td>
<td>247</td>
<td>46</td>
<td>22</td>
<td>32</td>
<td>134</td>
<td>48</td>
<td>26 26</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NO₃, 20 mM</td>
<td>1.29</td>
<td>177</td>
<td>32</td>
<td>10</td>
<td>58</td>
<td>138</td>
<td>15</td>
<td>38 47</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Urea, 10 mM</td>
<td>2.27</td>
<td>397</td>
<td>39</td>
<td>19</td>
<td>42</td>
<td>174</td>
<td>49</td>
<td>48 3 3 &lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Urea, 20 mM</td>
<td>2.17</td>
<td>421</td>
<td>33</td>
<td>16</td>
<td>51</td>
<td>193</td>
<td>39</td>
<td>57 4 &lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Table VIII. The Influence of Nitrogen Source on the Carbon Requirement for Maintenance of Ionic Balance in 76-Day-Old Soybean Plants

It was assumed that the carboxylate was malate and that dry matter contained 41% carbon. Values represent means of four replicates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carboxylate (C-A)</th>
<th>Carbon in</th>
<th>Carbon as Carboxylate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carboxylate (C-A)</td>
<td>Dry matter</td>
<td>Carboxylate (C-A)</td>
</tr>
<tr>
<td></td>
<td>Whole plant</td>
<td>Shoot</td>
<td>Whole plant</td>
</tr>
<tr>
<td></td>
<td>meq/plant</td>
<td>g/plant</td>
<td></td>
</tr>
<tr>
<td>Zero-N (N₂ fixation)</td>
<td>162</td>
<td>150</td>
<td>57.1</td>
</tr>
<tr>
<td>NO₃, 10 mM</td>
<td>299</td>
<td>279</td>
<td>95.9</td>
</tr>
<tr>
<td>NO₃, 20 mM</td>
<td>321</td>
<td>295</td>
<td>100.0</td>
</tr>
<tr>
<td>Urea, 10 mM</td>
<td>232</td>
<td>213</td>
<td>84.2</td>
</tr>
<tr>
<td>Urea, 20 mM</td>
<td>246</td>
<td>228</td>
<td>96.4</td>
</tr>
</tbody>
</table>

Stumpf and Burris (26) reported that malonate was the predominant dicarboxylic acid in fresh nodule, root, and leaf tissue of N₂-fixing soybeans. Although we did not measure the malonate content of xylem sap, anion charge associated with malate, aspartic acid, and allantoic acid was sufficient to largely balance excess cations (Table VI), implying that malonate was not a major carboxylate in the xylem. The predominance of malate in the xylem (Table VI) and of malonate in leaf tissue (26) would suggest metabolism of malate upon delivery to the leaves. Apparently, malate that remains in root and nodule tissue would also be metabolized to malonate.

For N₂-fixing plants, the C/N weight ratio of xylem sap was 2.04, even though 83% of the N was transported as ureides which have a C/N ratio of 1. This high C/N ratio resulted from the transport of malate which contained almost 50% of the carbon in sap (Table VII). It is evident that C/N ratios of the N compounds in the xylem sap did not indicate the total upward carbon flow. Failure to consider the carbon in xylem organic acid anions of N₂-fixing plants may lead to significant errors in constructing overall plant carbon balances.

Most (85%) of the OH⁻ formed during NO₃⁻ and SO₄²⁻ reduction in either roots (Fig. 1D) or shoots of plants supplied 20 mM NO₃⁻ was neutralized by carboxylate (C-A) synthesis in either roots (Fig. 1E) or shoots, and only a small amount (15%) was transported (Fig. 1C) to the ambient medium during excess anion (NO₃⁻) uptake (Table III). Nitrate reduction accounted for about 95% of the cytoplasmic OH⁻ generation, and SO₄²⁻ reduction accounted for 5% (Table III). This partitioning of cytoplasmic anion charge (OH⁻) between carboxylate formation and efflux to the ambient medium is similar to that reported for tomato plants supplied adequate NO₃⁻ (16). In 10 mM NO₃⁻-fed plants, excess cation uptake indicated that carboxylate accumulation was associated with OH⁻ generated by net H⁺ excretion (Fig. 1A) in addition to that generated by NO₃⁻ and SO₄²⁻ reduction in roots for maintenance of charge balance.

Large amounts of carboxylates (C-A) were synthesized regardless of the N source (Table II), and more than 89% of these carboxylates accumulated in shoot tissue. However, the processes associated with synthesis and distribution of carboxylates differed appreciably. In N₂- and urea-fed plants, the large proportion of total plant carboxylate content in shoots (Table IV) and the high levels of malate in xylem sap (Table VI) suggest that carboxylates are synthesized in the root cells (Fig. 1E) in response to OH⁻ generation during H⁺ excretion (excess cation uptake) (Fig. 1A). The anion exchange change which balances excess cation uptake is presumably converted from the OH⁻ form to carboxylate (malate) via the PEP carboxylase half of the intracellular pH stat system (Fig. 1E; Ref. 5), thereby permitting maintenance of both ionic balance and pH within the cytoplasm of root cells (23). The malate and associated cations are then deposited in the xylem (Fig. 1H) for transport to the shoot. Carboxylates remain in the shoot tissue balanced by inorganic cations. Inasmuch as nodules are not likely to have any significant involvement in uptake of inorganic cations and anions from the rhizosphere (23), most of the malate observed in the xylem sap is probably synthesized in the root tissue.

Fig. 1. A schematic illustrating interrelationships of processes associated with maintenance of intracellular pH and ion balance in, and ion intake by, higher plants.

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During the 41- to 76-day growth interval, inorganic anion uptake by 20 mm NO₃⁻-fed plants exceeded cation uptake by 32 meq/plant, and total NO₃⁻ and K⁺ uptake were 322 and 179 meq/plant, respectively (Table I). This information can be used to assess the extent of K⁺ recycling. Reduction of 10% (32 out of 322 meq/plant) of the NO₃⁻ which entered the plant in the roots would have generated sufficient OH⁻ to sustain the excess anion uptake (Fig. 1C). On the other hand, if it is assumed that no NO₃⁻ reduction occurred in the roots and that K⁺ is the only cation readily transported in the phloem, about 17% (32 out of 179 meq) of K⁺ ions which entered the plant would have recycled with carboxylate in the phloem (Fig. 1F) to sustain the excess anion uptake. Thus, NO₃⁻-fed soybean plants are similar to NO₃⁻-fed tomato plants (16) in requiring relatively minimal K⁺ recycling.

The requirement for ionic balance and intracellular pH maintenance by carboxylates involves the sequestration of energy in the form of carbon (23). Calculations assuming that plant dry matter is 41% carbon and that malate or citrate is the organic acid in the tissue reveal that, regardless of the N source, 5 to 8% of total plant carbon accumulated is involved in maintenance of ion balance and intracellular pH (Table VIII). Values of 4 to 6% would be obtained if malonate were the organic acid, as it is in the tissue (26). While not great, this is energy which potentially could be used to support other growth processes. Whether alleviation of inorganic cation-anion imbalance would improve growth and productivity would depend on whether carbon supply is the factor limiting growth and whether the means used to improve cation-anion balance involved a comparable energy expenditure.

The results of this greenhouse investigation can be used to make predictions about the behavior of soybean plants in the field. For example, the C-A value for seed from N₂-fixing plants was 0.68 meq/g dry weight. A 3,000-kg per hectare crop of seed with this composition would be associated with acidification of the soil, requiring 102 kg CaCO₃ per hectare for neutralization. Production of soybeans on low N soils, such as those of the Southeastern Coastal Plain, is likely to cause substantial acidification which must be corrected by application of CaCO₃. Implications for effects of rhizosphere acidification on the nodulation process may also be advanced (13, 21).

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