

Nitrate Uptake and Assimilation by Wheat Seedlings during Initial Exposure to Nitrate¹

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ABSTRACT

Nitrate uptake, reduction, and translocation were examined in intact, 14-day-old, nitrogen-depleted wheat (*Triticum vulgare* var. Knox) seedlings during a 9-hour exposure to 0.2 mM Ca(NO₃)₂. The nitrate uptake rate was low during the initial 3-hour period, increased during the 3- to 6-hour period, and then declined. By the 3rd hour, 14% of the absorbed nitrate had been reduced, and this increased to 36% by the 9th hour. Shoots accumulated reduced ¹⁵N more rapidly than roots and the ratio of reduced ¹⁵N to ¹⁵N-nitrate was higher in the shoots. A significant proportion of the total reduction occurred in the root system under these experimental conditions. Accumulation of ¹⁵N in ethanol-insoluble forms was evident in both roots and shoots by the 3rd hour and, after 4.5 hours, increased more rapidly in shoots than in roots.

An experiment in which a 3-hour exposure to 0.2 mM Ca(¹⁵NO₃)₂ was followed by a 12-hour exposure to 0.2 mM Ca(¹⁴NO₃)₂ revealed a half-time of depletion of root nitrate of about 2.5 hours. A large proportion of this depletion, however, was due to loss of ¹⁵N-nitrate to the ambient ¹⁴N-nitrate solution. The remaining pool of ¹⁵N-nitrate was only slowly available for reduction. Total ¹⁵N translocation to the shoot was relatively efficient during the first 3 hours after transfer to Ca(¹⁴NO₃)₂ but it essentially ceased after that time in spite of significant pools of ¹⁵N-nitrate and α-amino-¹⁵N remaining in the root tissue.

Many higher plant species (3, 9, 10, 14, 15), as well as tobacco cell cultures (6) and *Penicillium chrysogenum* (5), exhibit an apparent induction pattern of nitrate uptake. In each instance the rate of uptake increased substantially after a period of exposure to nitrate. The induction of nitrate reductase in corn roots (18, 23) parallels the increase in rate of nitrate uptake (9), and nitrate translocation to the xylem, as well as nitrate uptake and reduction, was restricted by protein-synthesis inhibitors (9, cf. 12). It follows that, upon exposure to nitrate, plants should develop enhanced capabilities for nitrate uptake, reduction in roots, translocation, and reduction in shoots. To examine the developmental patterns of these processes, two

experiments were conducted. The first involved exposure of nitrogen-depleted plants to nitrate solutions highly enriched in ¹⁵N; the results show the effectiveness with which absorbed nitrate (a) accumulated in roots and shoots, (b) was reduced, and (c) was incorporated into protein during a 9-hr period. In the second experiment, a 3-hr exposure to highly enriched ¹⁵N-nitrate was followed by an additional 12 hr in ¹⁴N-nitrate, permitting an assessment of the fate of the initially absorbed ¹⁵N-nitrate during the early phases of recovery from the nitrogen-depleted state.

MATERIALS AND METHODS

Wheat seed (*Triticum vulgare* var. Knox) were kept moist with 10⁻⁴ M CaSO₄, germinated 3 days in darkness, and transferred to small plastic cups containing holes in the bottom through which the roots were threaded. Each cup contained six seedlings and henceforth will be referred to as a culture. Roots of the required numbers of cultures were placed in 13-liter plastic tanks containing nutrients at one-fifth the concentrations given by Hoagland and Arnon (7) for minus nitrogen solutions. The plants were grown in these solutions for 11 days in a controlled environment chamber. Sixteen hr of light and 8 hr of darkness were used. The light intensity was 194 hectolux at plant height, and temperature was 24 ± 2 C and 17 ± 2 C during the light and dark periods, respectively. These growth conditions produced seedlings low in nitrogen and high in carbohydrate content (16). Thus the seedlings would be expected to absorb and assimilate nitrate nitrogen at a rapid rate, provided the required transport, reduction, and assimilatory systems were present.

Both experiments were conducted in the growth chamber at 25 C and 194 hectolux. For the first experiment, cultures were exposed to aerated 0.2 mM Ca(¹⁵NO₃)₂ containing 95 atom % ¹⁵N and were harvested at 0, 3, 4.5, 6, and 9 hr. Each harvest consisted of 32 cultures. For the second experiment all cultures were exposed to 0.2 mM Ca(¹⁵NO₃)₂ with 97.5 atom % ¹⁵N for 3 hr. Then 28 cultures were harvested while the remainder were placed in 0.2 mM Ca(¹⁴NO₃)₂. Subsequently, harvests of 28 cultures each were made at 4.5, 6, 9, 12, and 15 hr after initiation of the experiment. All solutions were adjusted initially to pH 6.5 with Ca(OH)₂, and adjustments to pH 6.5 were made with Ca(OH)₂ and H₂SO₄ every 15 to 30 min. At the designated times, cultures were removed from the treatment solution, rinsed thoroughly with deionized H₂O, separated at the root-shoot junction, blotted with cheesecloth to remove excess water, weighed, quickly frozen, and lyophilized. At each harvest, plants were divided at random into two groups, permitting duplicate analyses of all components.

The lyophilized tissue was extracted with 70% ethyl alcohol and the insoluble residue was analyzed for total N and ¹⁵N. The ethyl alcohol-soluble material was evaporated to dryness, and

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the resulting residue was brought into solution with chloroform-H₂O (1:1). The chloroform fraction, containing nitrogen in the form of lipoprotein, Chl, etc., was analyzed for total nitrogen and ¹⁵N. However, at any given time this fraction constituted less than 1% of the total ¹⁵N, and the data have not been included here. The water fraction contained nitrate and soluble reduced nitrogen constituents. These were separated, after acidifying to pH 2.9, by passage through a cation exchange resin (Dowex 50 × 8). The effluent from the column contained nitrate. The reduced nitrogen fraction, containing primarily amino acids, was eluted from the columns with 1.2 N K₂SO₄. Both fractions were analyzed for total N and ¹⁵N.

Nitrate nitrogen was determined by the phenoldisulfonic acid method (11). When determination of ¹⁵N-enrichment in nitrate was required, nitrate was reduced to ammonia with Devarda's metal. Ammonium + amide and α-amino nitrogen were determined by a selective distillation method (1), and other reduced nitrogen components were determined by micro-Kjeldahl procedure (19). The quantity of ¹⁵N in the samples was determined mass spectrometrically (22).

To facilitate comparisons of reduction, assimilation, and translocation rates with uptake rates, all ¹⁵N data, including that in shoots, are presented on the basis of unit dry weight of root tissue. "Uptake" or "absorption" refers to the total ¹⁵N recovered in the entire plant, whereas "accumulation" or "incorporation" refers to the quantity of ¹⁵N present in any of the specified tissue fractions; "reduced ¹⁵N" includes all of the non-nitrate fractions. Average dry weight per culture of shoots and roots, respectively, were 0.180 and 0.129 g for experiment I and 0.163 and 0.103 g for experiment II.

RESULTS

EXPERIMENT I

Nitrate Uptake, Accumulation, and Reduction. The slower nitrate uptake during the first 3 hr relative to the next 3 hr (Fig. 1A) is typical of the apparent induction pattern of nitrate

uptake noted previously with wheat (10, 14, 15). The subsequent decline in rate has also been observed with Ca(NO₃)₂ solutions (14).

Nitrate accumulated in both roots and shoots throughout the experiment (Fig. 1, B and C). Efficient translocation was evident; by the 3rd hr the shoots contained 41% of the absorbed ¹⁵N, primarily as nitrate (Fig. 2). During the remainder of the experiment, however, the percentage of the absorbed ¹⁵N which was translocated to the shoots increased to only 48% (Fig. 2C). Reduction of the entering nitrate was evident by the 3rd-hr (Fig. 1, B and C), reduced ¹⁵N being present in both roots and shoots. Following that time, reduction increased substantially, with more reduced ¹⁵N accumulating in the shoots than in the roots (Fig. 1, B and C). The percentage of entering nitrate which had been reduced rose from 14% at the 3rd hr to 36% by the 9th hr (Fig. 2D). Throughout the experiment, the shoots differed from the roots in having a greater proportion of their total ¹⁵N present in the reduced form (Fig. 1, B and C).

Accumulation of Reduced ¹⁵N Fractions. The majority of the reduced ¹⁵N accumulated in the α-amino-N and insoluble N fractions (Fig. 2). The ammonium + amide fraction constituted less than 3% of the total, and the majority of this fraction was recovered in the root tissue (Fig. 2). Accumulation in the α-amino-N fraction of the roots declined toward the end of the experiment, indicating an approach to saturation. This trend was not as evident in the shoots. Accumulation as insoluble ¹⁵N in the shoots exceeded that in the roots after 4.5 hr (Fig. 2, B vs. C). At the end of the experiment, nearly equal proportions of the absorbed nitrate of the entire plant were present as α-amino-¹⁵N and insoluble ¹⁵N (16 and 17%, respectively; Fig. 2A).

EXPERIMENT II

Nitrate Accumulation. The pattern of nitrate (¹⁵N + ¹⁴N) accumulation in roots and shoots is presented in Figure 3. A marked increase in nitrate accumulation by roots occurred during the 12- to 15-hr period. Whether this was due to an

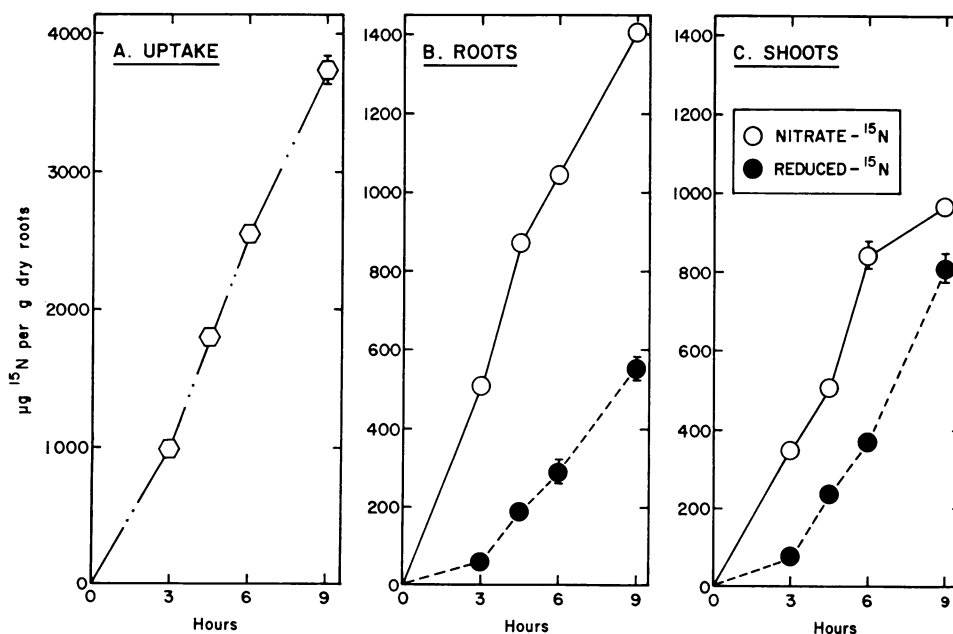


FIG. 1. Total uptake of ¹⁵N-Nitrate (A), accumulation of the absorbed ¹⁵N as ¹⁵N-Nitrate and reduced ¹⁵N in roots (B), and shoots (C) during continuous exposure to Ca(¹⁵NO₃)₂ (experiment I). The vertical lines through symbols indicate the range of replicate values when they exceed the size of the symbol.

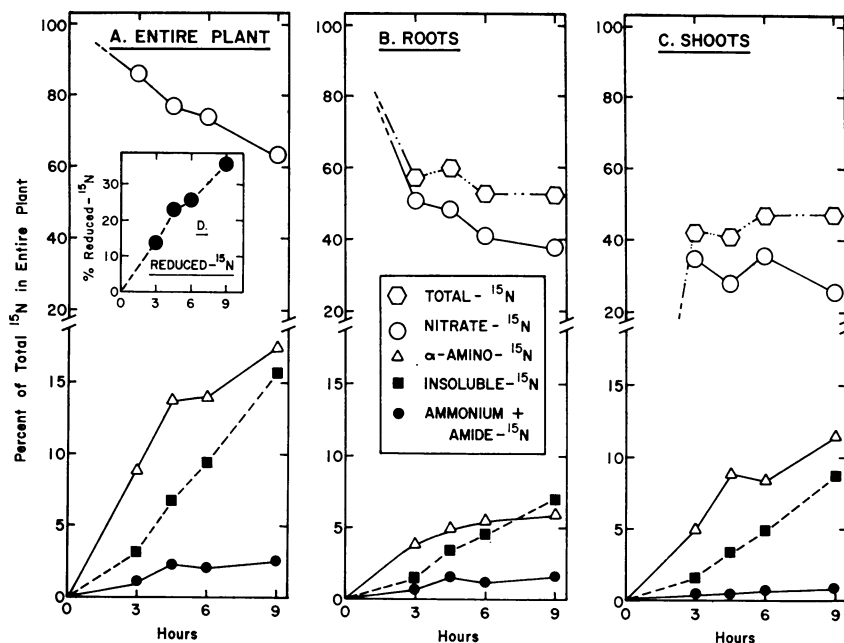


FIG. 2. Percentage of total ¹⁵N taken up which was present in various nitrogen fractions of the entire plant (A), roots (B), shoots (C), and percentage of total ¹⁵N which was reduced in the entire plant (D) during continuous exposure to Ca(¹⁵NO₃)₂ (experiment I).

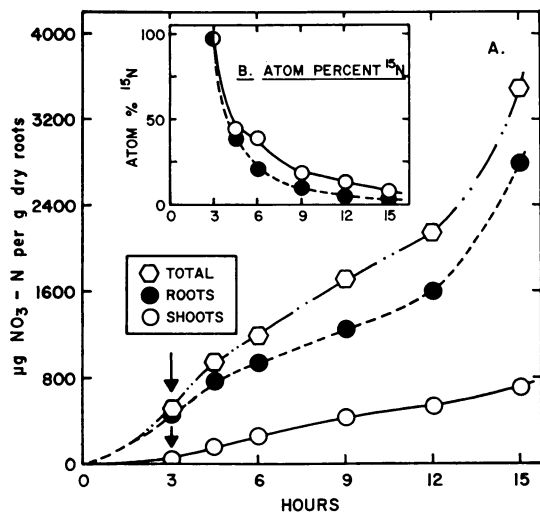


FIG. 3. Accumulation of nitrate, and atom per cent ¹⁵N of the nitrate, in roots and shoots during exposure to Ca(¹⁵NO₃)₂ for 3 hr (arrow) followed by Ca(¹⁴NO₃)₂ for 12 additional hr. The Ca(¹⁵NO₃)₂ contained 97.5 atom % ¹⁵N (experiment II).

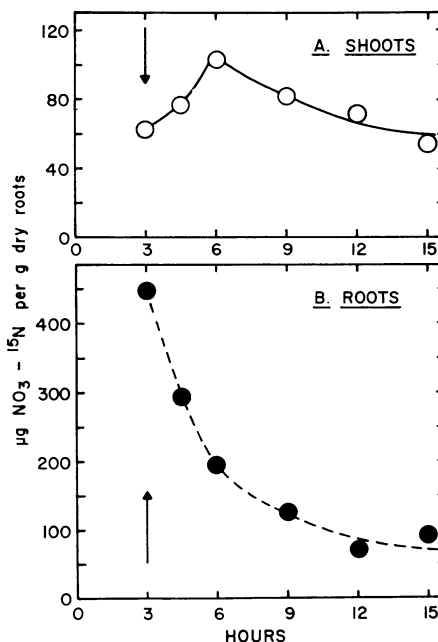


FIG. 4. Distribution of ¹⁵N-nitrate in roots and shoots during the 12-hr exposure to Ca(¹⁴NO₃)₂ following a 3-hr exposure (arrow) to Ca(¹⁵NO₃)₂ (experiment II).

increase in the rate of nitrate uptake or to a decrease in its rate of reduction during this period cannot be determined from the present data, but previous evidence (14) indicates that an abrupt increase in the uptake rate would not be expected. The atom per cent ¹⁵N of the tissue nitrate decreased rapidly after transfer from ¹⁵N-nitrate to ¹⁴N-nitrate (Fig. 3B), the decrease being more rapid in the roots than in the shoots. More than half of the ¹⁵N-nitrate present in the roots at 3 hr was lost during the next 3 hr (Fig. 4B). Some of the ¹⁵N-nitrate accumulated in the shoots (Fig. 4A) but more than 30% of the ¹⁵N absorbed during the 3-hr exposure to ¹⁵N was lost to the ambient solution during the subsequent 12-hr exposure to ¹⁴N-nitrate (Fig. 5). Figure 6 summarizes the relative distribution of ¹⁵N in various plant fractions during the displacement period.

About 16% of the total ¹⁵N present at 3 hr remained as nitrate in the plants at the end of the experiment (Fig. 6A) while 35% had accumulated in the insoluble N fraction (Fig. 6D). The α-amino-¹⁵N and the ammonium + amide ¹⁵N of the roots decreased steadily during the ¹⁴N displacement period (Fig. 6, B and C). In the shoots, both of these fractions increased during the 3- to 6-hr period. After 6 hr, there was a rapid decline in α-amino-¹⁵N while the ammonium + amide ¹⁵N remained relatively constant (Fig. 6, B and C). Accumulation of ¹⁵N in the insoluble fraction of the shoots exceeded that of roots during the displacement period (Fig. 6D).

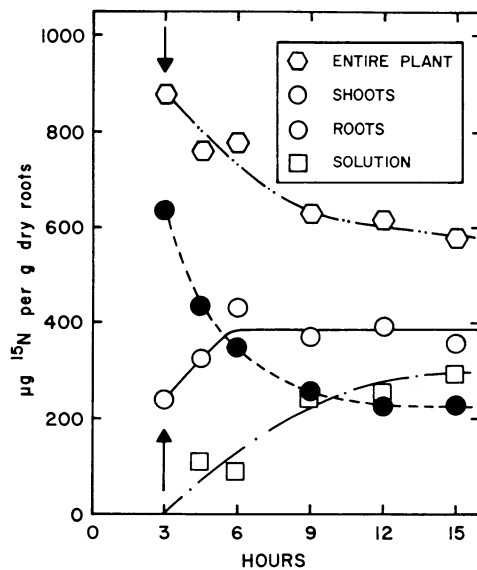


FIG. 5. Total ^{15}N in the plants, distribution of ^{15}N between roots and shoots, and loss of ^{15}N to the ambient solution during the 12-hr exposure to $\text{Ca}(^{15}\text{NO}_3)_2$ following a three-hr exposure (arrow) to $\text{Ca}(^{14}\text{NO}_3)_2$ (experiment II). (Symbol for roots should be ●.)

DISCUSSION

Nitrate Accumulation, Reduction, and Translocation. Wheat seedlings similar to those used in these investigations were devoid of nitrate, had barely detectable nitrate reductase activities (13), and were initially limited in their capacity to absorb nitrate (10, 14, 15). Such plants may be expected to increase in nitrate reductase activity (2) and concurrently in nitrate uptake upon first exposure to nitrate, as has been demonstrated with dark-grown seedlings (9), tobacco cell suspension cultures (6), and *Penicillium chrysogenum* (5). However, *in vitro* measurements of nitrate reductase activity do not necessarily reflect the actual rates of nitrate reduction occurring in the tissue (*cf.* 25). One purpose of these studies was to obtain unequivocal values for the quantities of the entering nitrate which were reduced and assimilated during the initial exposure to nitrate.

In experiment I, 14% of the absorbed nitrate had been reduced during the first 3 hr (Fig. 2D). Of the nitrate which was reduced during this period, 24% had been incorporated into the ethyl alcohol-insoluble fraction (Fig. 2A). Corresponding values for the first 3 hr of experiment II were 40% and 29%. The data show that significant reduction and incorporation of the absorbed nitrate into protein took place during the period prior to attainment of the maximal nitrate uptake rate.

Definitive conclusions on the proportion of the total ^{15}N -nitrate reduction which occurred in the roots of experiment I is not possible because of the likelihood of translocation of soluble reduced ^{15}N nitrogen from the roots to the shoots (8, 20, 21, 24). During the four measurement periods of experiment I, rates of accumulation of reduced nitrogen in roots were 47, 44, 43, and 37% of the rates of accumulation of reduced nitrogen in the entire plant. These are minimal estimates of the relative quantities reduced in the roots and would be increased by the extent to which translocation of soluble reduced ^{15}N from roots to shoots exceeded the reverse process during each period. Since appreciable downward translocation of soluble reduced ^{15}N seems unlikely in the relatively short times of these experiments, it is probable that a sizeable, but steadily decreasing, proportion of the total nitrate reduction occurred in the root tissue.

^{15}N -Nitrate Reduction and ^{15}N Translocation from Previously Absorbed ^{15}N -Nitrate. Calculations, using the smoothed lines in Figures 5 and 6, show that about $365 \mu\text{g}$ of $^{15}\text{N} \text{ g}^{-1}$ was recovered in the reduced N fractions of the entire plant in experiment II during the 3-hr exposure to ^{15}N -nitrate. However, during the next 3 hr (after transfer to ^{14}N -nitrate) only $75 \mu\text{g}$ of $^{15}\text{N} \text{ g}^{-1}$ as nitrate was reduced in the roots or shoots even though the sum of the ^{15}N -nitrate in both tissues was $510 \mu\text{g}$ of $^{15}\text{N} \text{ g}^{-1}$ at the start of the displacement period. Therefore, a substantial portion of the previously absorbed ^{15}N -nitrate was not readily available for subsequent reduction (*cf.* 4, 9).

During the 3-hr exposure to ^{15}N , translocation to the shoot ($235 \mu\text{g}$ of $^{15}\text{N} \text{ g}^{-1}$) accounted for 27% of the total ^{15}N absorbed by the root. Within the limits of the experimental methodology, subsequent translocation of ^{15}N was essentially complete within the first 3 hr after exposure to ^{14}N -nitrate, but efflux of ^{15}N -nitrate to the ambient solution continued after this time (Fig. 5; *cf.* ref. 17). Part of the recently absorbed ^{15}N -nitrate apparently was more readily available for efflux than for reduction or translocation. During the first 3 hr of the displacement period, approximately $150 \mu\text{g}$ of $^{15}\text{N} \text{ g}^{-1}$ moved from the roots to the shoots. This is equivalent to 26% of the soluble ^{15}N present in the roots at the beginning of the displacement period. Thus, in contrast to the decline in ^{15}N -nitrate reduction, ^{15}N translocation remained relatively high during the initial 3 hr in ^{14}N -nitrate.

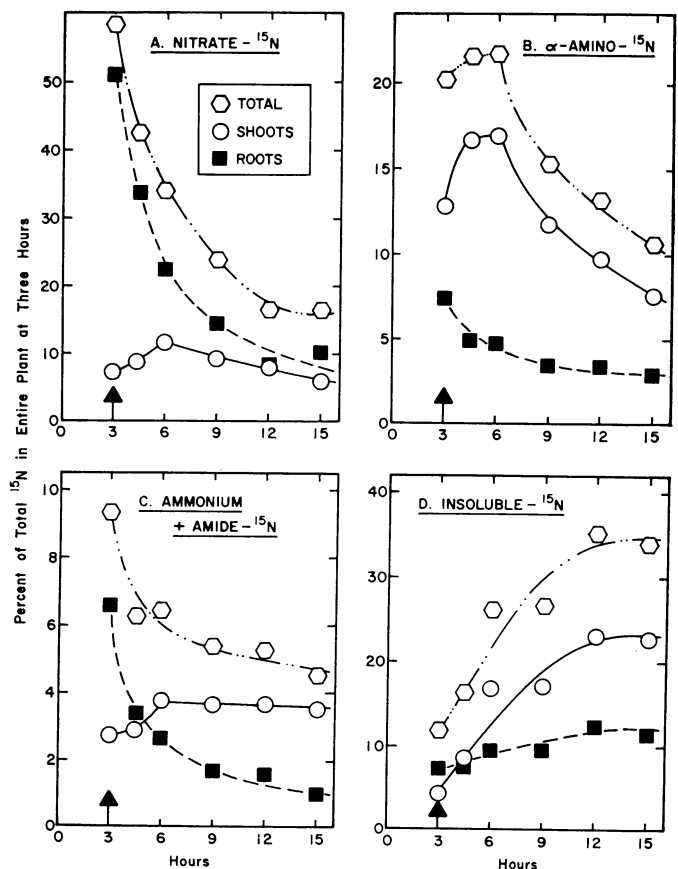


FIG. 6. Percentage distribution of ^{15}N present in the entire plant into various nitrogen fractions during the 12-hr exposure to $\text{Ca}(^{15}\text{NO}_3)_2$ following a 3-hr exposure (arrow) to $\text{Ca}(^{14}\text{NO}_3)_2$ (experiment II). At 3 hr, the atom per cent excess ^{15}N values for the roots and shoots, respectively, were 7.71 and 7.59 for ammonium + amide nitrogen, 3.61 and 8.21 for α -amino nitrogen, and 0.79 and 0.24 for insoluble nitrogen.

The smoothed lines in Figures 5 and 6 also permit calculation of the minimal and maximal values for translocation of previously absorbed nitrate and reduced nitrogen to the shoots during the first 3 hr of exposure to ^{14}N -nitrate. The minimal amount translocated as ^{15}N -nitrate was the increase in ^{15}N -nitrate of the shoots, or $40\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$. During the same period, the roots decreased in reduced ^{15}N by $45\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$ so at least this amount was translocated to the shoots in the form of soluble reduced ^{15}N . Since total ^{15}N translocation was $150\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$, at least 25% of the total ^{15}N translocated was in the form of soluble reduced ^{15}N . This is a minimal value and assumes no ^{15}N -nitrate reduction in the root system after transfer to the ^{14}N -nitrate solution. The absolutely maximal value for ^{15}N -nitrate available for translocation was $150 - 45 = 105\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$, whereas the ^{15}N -nitrate available for translocation was that present in the roots at the 3rd hr ($450\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$) minus that lost to the ambient solution during the 3- to 6-hr period (approximately $125\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$), or $325\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$. It follows that the maximal ^{15}N -nitrate translocated, as a percentage of that available for translocation was $(105/325)100 = 32\%$ during this period.

At the start of exposure to ^{14}N -nitrate, there was $125\ \mu\text{g}$ of soluble reduced $^{15}\text{N}\ \text{g}^{-1}$ in the root tissue. During the next 3 hr, the insoluble fraction of the roots increased only by about $15\ \mu\text{g}$ of $^{15}\text{N}\ \text{g}^{-1}$. Again assuming no reduction of ^{15}N -nitrate in the roots during the chase period, there would have been $110\ \mu\text{g}$ of soluble reduced $^{15}\text{N}\ \text{g}^{-1}$ available for translocation. Since on the same assumptions, at least $45\ \mu\text{g}$ of soluble reduced $^{15}\text{N}\ \text{g}^{-1}$ was translocated, the minimal soluble reduced ^{15}N translocated, as a percentage of that available for translocation, was more than 40%. A fairly efficient translocation of soluble reduced ^{15}N during the first 3 hr after transfer to ^{14}N -nitrate is clearly indicated under these experimental conditions. After this time, however, there was an abrupt cessation in translocation.

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