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Effects of Altered Carbohydrate Availability on Whole-Plant Assimilation of $^{15}NO_3^{-1}$

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ABSTRACT

An experiment was conducted to investigate the relative changes in NO_3^- assimilatory processes which occurred in response to decreasing carbohydrate availability. Young tobacco plants (*Nicotiana tabacum* [L.], cv NC 2326) growing in solution culture were exposed to 1.0 millimolar $^{15}NO_3^-$ for 6 hour intervals during a normal 12 hour light period and a subsequent period of darkness lasting 42 hours. Uptake of $^{15}NO_3^-$ decreased to 71 to 83% of the uptake rate in the light during the initial 18 hours of darkness; uptake then decreased sharply over the next 12 hours of darkness to 11 to 17% of the light rate, coincident with depletion of tissue carbohydrate reserves and a marked decline in root respiration. Changes also occurred in endogenous $^{15}NO_3^-$ assimilation processes, which were distinctly different than those in $^{15}NO_3^-$ uptake. During the extended dark period, translocation of absorbed ^{15}N out of the root to the shoot varied rhythmically. The adjustments were independent of $^{15}NO_3^-$ uptake rate and carbohydrate status, but were reciprocally related to rhythmic adjustments in stomatal resistance and, presumably, water movement through the root system. Whole plant reduction of $^{15}NO_3^-$ always was limited more than uptake. The assimilation of ^{15}N into insoluble reduced-N in roots remained a constant proportion of uptake throughout, while assimilation in the shoot declined markedly in the first 18 hours of darkness before stabilizing at a low level. The plants clearly retained a capacity for $^{15}NO_3^-$ reduction and synthesis of insoluble reduced-^{15}N even when $^{15}NO_3^-$ uptake was severely restricted and minimal carbohydrate reserves remained in the tissue.

Activities of processes involved in NO_3^- assimilation by plants are influenced by carbohydrate availability and the associated generation of energy. Uptake of NO_3^- into the root symplasm from the rhizosphere against an electrochemical gradient (5) and NO_3^- transport out of the root symplasm into the xylem (15, 26, 31) require metabolic energy. Also, a continual supply of reducing equivalents, ATP, and carbon skeletons is required for sustained enzymatic assimilation of NO_3^- into protein and nucleic acids (3, 19).

Although each component of the NO_3^- assimilatory system is energy dependent, the processes may be differentially sensitive to a carbohydrate limitation. It has been consistently observed, for example, that NO_3^- reductase activity and *in vivo* NO_3^- reduction are restricted to a greater extent than NO_3^- uptake when carbohydrate availability declines. This was true in experiments with excised roots of dwarf bean (10), intact roots of wheat and corn seedlings (15), and detached leaves of barley (2).

The relative responses of NO_3^- assimilatory processes to decreasing carbohydrate availability in intact, rapidly growing plants have not been examined in detail. The experiment described here was undertaken for that purpose. Natural fluctuations in carbohydrate (energy) status occur during the daily light/dark cycle, and carbohydrate reserves become severely depleted with extended darkness (17). Limitations in carbohydrate supply likely are, in large part, responsible for the decreased rates of NO_3^- uptake (6, 11, 25), and reduction (1, 27) observed in darkness. Accordingly, our experiment involved exposure of young tobacco plants to $^{15}NO_3^-$ for 6 h intervals during a normal 12 h light period and during a following 42 h period of extended darkness. The approach allowed assessment of the relative sensitivities of NO_3^- assimilation activities in plants with widely varying carbohydrate availabilities.

Of particular interest in this experiment was the regulation of NO_3^- transport from the root into the xylem. Previous studies have revealed that a larger proportion of the NO_3^- taken up by roots is translocated to the shoot in light than in darkness (22, 24, 29). The observation raises the possibility that xylem transport of NO_3^- is highly sensitive to a carbohydrate limitation. An alternative explanation is that the lower rate of xylem transport in darkness is closely associated with decreased water movement through the root and vascular system. Since stomatal opening and closure, and transpiration, continue to oscillate rhythmically in periods of extended darkness (13, 16), coincident with depletion of carbohydrate reserves, the relative involvement of the two regulatory factors could be distinguished under the treatment conditions imposed.

MATERIALS AND METHODS

Plant Culture

Seeds of tobacco (*Nicotiana tabacum* [L.], cv NC 2326) were germinated on a soil mixture in 170 mL plastic pots located in a greenhouse. The seedlings were watered daily (AM), received one-half strength Hoagland solution twice weekly, and were exposed to natural sunlight. After 7 weeks,

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72 seedlings were selected for uniformity and placed into three 115-L continuous flow, hydroponic culture systems. The culture systems were located in a controlled-environment growth room programmed for 28°C/22°C during the 12/12 h light/dark cycle. A photosynthetic photon flux density of 1100 ± 50 μmol m^{-2} s^{-1} (at canopy; height) was provided during the 12-h light period from a combination of high pressure sodium and metal halide lamps. The environmental conditons used were sufficient to sustain net photosynthetic rates which exceed those of tobacco plants grown in the greenhouse or field.

The culture solution temperature was 24 ± 1.0°C, and the solution pH was maintained at 5.8 ± 0.2 by automatic additions of 0.2 N H_2SO_4. Nutrient concentrations in solution were 1.0 mM NO_3^-, 0.1 mM $H_2PO_4^-$, 1.1 mM K^+, 1.0 mM Ca^{2+}, 1.0 mM Mg^{2+}, 1.0 mM SO_4^{-2}, 17 μM B, 3 μM Mn, 0.3 μM Zn, 0.1 μM Cu, 0.04 μM Mo, and 18 μM Fe as ferric diethylenetriamine pentaacetate (Fe-DPTA, CIBA-Geigy Corp., Greensboro, NC).[2] The solutions were changed every 2 d to avoid depletion effects.

Experimental Conditions

The experiment began on d 12 after transplant into the hydroponic system. Starting at the beginning of the light period, sets of four plants were exposed to solutions containing $^{15}NO_3^-$ for 6 h intervals over the following 54 h. Four randomly chosen plants were removed from the solutions in which they were growing and placed into a separate solution (also in a 115-L continuous flow culture sytem) containing an identical nutrient composition except with 1.0 mM $^{15}NO_3^-$ (99 atom % ^{15}N) substituted for 1.0 mM $^{14}NO_3^-$. The treatment interval of 6 h represented a compromise strategy in pursuing the objectives of this study. The 6 h exposure to $^{15}NO_3^-$ was sufficient time for adequate ^{15}N incorporation into all N fractions of the root and shoot for analytical accuracy, while the time was sufficiently short to minimize cycling of soluble reduced-^{15}N from the shoot to the root. At the end of each 6 h exposure period, plants were harvested, with shoots and roots separated and frozen promptly at −20°C. During the initial 24 h of the experimental period, environmental conditions were the same as those existing previously, with 12 h of light and 12 h of darkness; during the following 30 h, the lights remained off with air temperature maintained at 22°C. Thus, plants were kept in darkness continuously for 42 h. The mean dry weights of shoots and roots of plants sampled during the experiment were 1.74 ± 0.05g and 0.51 ± 0.02g, respectively.

At each sample time, root tips (5 mm) from the four plants being harvested were excised, and respiration measured using a YSI O_2 monitor and Clark-type O_2 electrode. The rate of O_2 depletion was determined while the root tips were submerged in nutrient solution. Throughout the 54 h experimental period, stomatal resistance of leaves of randomly selected plants in the $^{14}NO_3^-$ solutions was measured using a LI-COR 1600 steady state porometer. The leaves always exceeded 14 cm in length and were (0–12 h) or had been (12–54 h) fully exposed to the light source.

Tissue Analysis

The tissue samples were analyzed for carbohydrates. After being freeze-dried, weighed, and ground, tissue was extracted with hot 80% ethanol, and the supernatant enzymically analyzed for sucrose (refer to Ref. 16). The particulate fraction, containing starch, was suspended in 1.0 mL of 0.2 N KOH and placed in boiling water for 30 min. After cooling, the pH was adjusted to 5.5 with 200 μL of 1.0 N acetic acid. To each sample, 1.0 mL of dialyzed amyloglucosidase solution (from *Aspergillus niger* [Sigma], 70 units/mL in 50 μM Na-acetate buffer, pH 4.5) was added and the tubes incubated at 55°C for 30 min. After digestion, the tubes were placed in boiling water for 1 min, centrifuged, and the glucose in the supernatant was analyzed using hexokinase and glucose-6-P dehydrogenase (16).

The samples also were analyzed for NO_3^- and soluble and insoluble reduced ^{15}N, abbreviated SRN[3] and IRN, respectively. Tissue was extracted with methanol:chloroform:water (13:4:3, v/v/v). Following separation of the chloroform from the methanol:water fraction, the chloroform was added back to the tissue residue, with this constituting the insoluble reduced N fraction. Total nitrogen in the insoluble N fraction was determined by Kjeldahl digestion and colorimetric analysis of NH_4^+ (refer to Ref. 29). The NH_4^+ in the remaining digest was recovered by diffusion and the atom percent ^{15}N determined mass spectrometrically using a freeze-layer procedure (35).

The methanol-water fraction was analyzed for NO_3^- and soluble reduced-N. After the methanol was evaporated, an aliquot was removed and NO_3^- determined using a manual modification of the method of Lowe and Hamilton (18). The atom percent ^{15}N of the NO_3^- fraction was determined by mass spectrometry using a nitric oxide procedure (34). Nitrate remaining in the water fraction was volatilized by addition of peroxide and H_2SO_4 (23), and the remaining soluble reduced N and atom percent ^{15}N determined as in analysis of the insoluble reduced N.

Data Presentation

Carbohydrate and root respiration data are plotted in figures at the end of each 6 h ^{15}N exposure interval, the time at which plants were harvested. The N data are plotted at the midpoint of each interval and expressed as a rate. In each figure, variability is shown either as a single LSD .05, when variances were homogeneous, or as separate standard errors unless values were less than individual data symbols.

RESULTS

Substantial changes in the carbohydrate status of root and shoot tissues occurred during the experimental period (Fig. 1). Carbohydrate levels, and presumably energy availability, were maximal during the light period. In the root, amounts of soluble sugars gradually declined during the normal 12 h

[2] The use of trade names in this publication does not imply endorsement by the United States Department of Agriculture or the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[3] Abbreviations: SRN, soluble reduced nitrogen; IRN, insoluble reduced nitrogen; R_s, stomatal resistance.

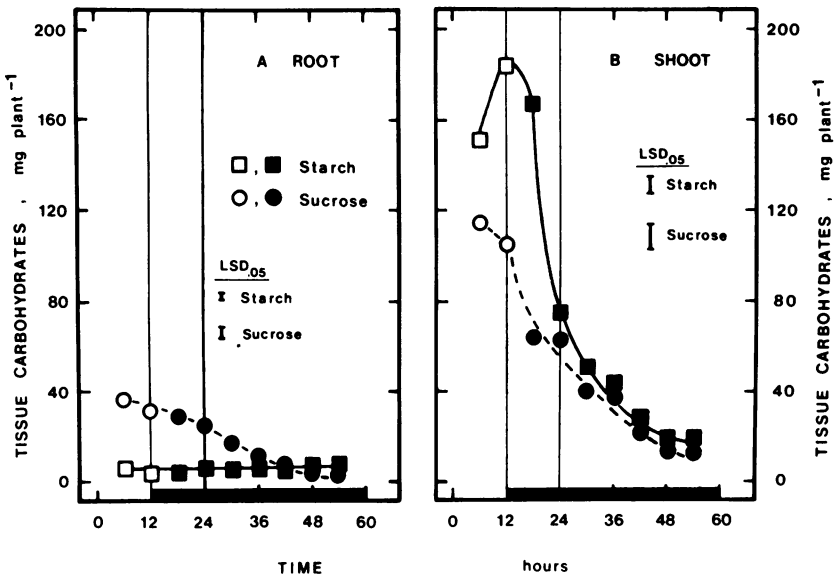

Figure 1. Changes in the total amounts of starch and sucrose in roots and leaves of tobacco plants during a normal light period and 42 h of continuous darkness.

dark period and the subsequent 30 h of extended darkness (Fig. 1A). In shoot tissues, total amounts of starch and soluble sugars decreased sharply in the initial 12 h of darkness and more gradually thereafter (Fig. 1B).

Root tip respiration, another general indication of the plant energy status, was maximal during the light period and decreased in darkness (Fig. 2). The pattern of decrease was somewhat different from that of total carbohydrate, as only a small decrease in respiration rate occurred in the initial 12 h of darkness. The rate dropped sharply during the next 18 h and then tended to stabilize.

Plant uptake of $^{15}NO_3^-$ was greatest during the light period (Fig. 3A). The rate decreased during the following 18 h of darkness to 71 to 83% of that in the light, and thereafter uptake decreased markedly until stabilizing at 11 to 17% of the rate in the light during the last three sample intervals. The changes in uptake were not due to altered root growth, as root dry weight did not vary significantly during the experiment (data not shown). The pattern of changes in whole plant $^{15}NO_3^-$ reduction (Fig. 3A) and apparent translocation of ^{15}N to the shoot (Fig. 3B; estimated from net ^{15}N accumulation in the shoot), resembled the pattern of changes in $^{15}NO_3^-$ uptake. The similarity is expected since uptake provides substrate for reduction and for translocation out of the root into the xylem. The relative restriction of the processes, nevertheless, was consistently different. Compared to the maximal rates occurring in the light, extended darkness and the associated carbohydrate/energy stress consistently affected $^{15}NO_3^-$ reduction > ^{15}N translocation > $^{15}NO_3^-$ uptake.

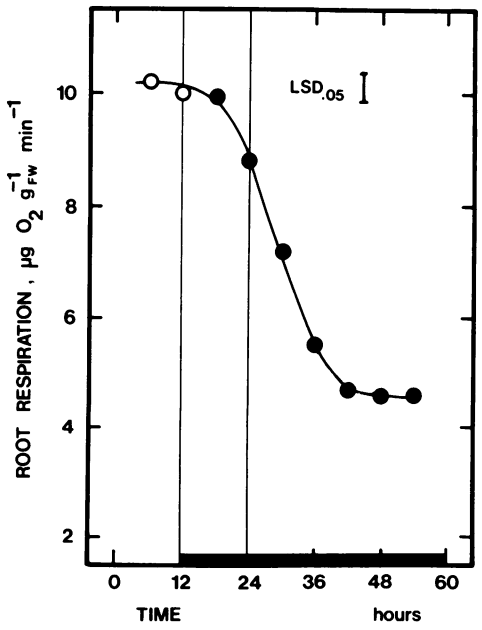

Figure 2. Changes in root respiration of tobacco plants during a normal light period and 42 h of continuous darkness.

A consistent pattern of change occurred in the accumulation of ^{15}N in the different nitrogen fractions in the root and shoot (Fig. 4). Following the light period, there was an increase in $^{15}NO_3^-$ accumulation, sustained for 12 h of darkness in the root and 18 h in the shoot, which coincided with lower ^{15}N incorporation into reduced N fractions. Incorporation of ^{15}N into reduced N fractions in the shoot was more severely limited. After that time, ^{15}N in all fractions declined (as uptake of $^{15}NO_3^-$ decreased, *cf.* Fig. 3A), and then tended to stabilize during the later sample intervals.

Relative changes among the various N assimilation processes are most apparent when ^{15}N in each fraction is expressed as a percent of the total ^{15}N accumulated by plants during each exposure interval (*i.e.* $^{15}NO_3^-$ uptake, Fig. 3A). If all processes were effected equally, than the percent distribution of ^{15}N among fractions and plant parts would remain relatively constant.

When ^{15}N translocation to the shoot (Fig. 3B) is expressed as a percent of uptake, for example, marked changes are apparent (Fig. 5). Translocation of ^{15}N decreased in the initial 12 h of the dark period, but then increased and continued to vary rhythmically thereafter, ranging from 45 to 65% of $^{15}NO_3^-$ uptake. The rhythmic adjustments in translocation were reciprocally related to alterations in R_s until the last three sample intervals. The methodology in this experiment

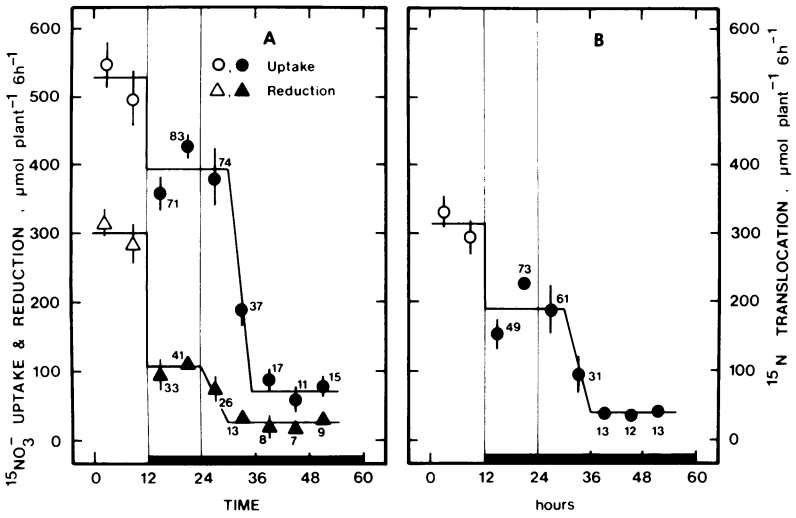

Figure 3. (A) Net uptake and whole plant reduction of $^{15}NO_3^-$ and (B) apparent translocation of ^{15}N to the shoot in tobacco plants exposed to $^{15}NO_3^-$ for 6 h intervals during a normal light period and 42 h of continuous darkness. Reduction represents the total ^{15}N in SRN and IRN fractions. Numbers adjacent to each data point represent data expressed as a percent of the mean rate occurring in the light for each process.

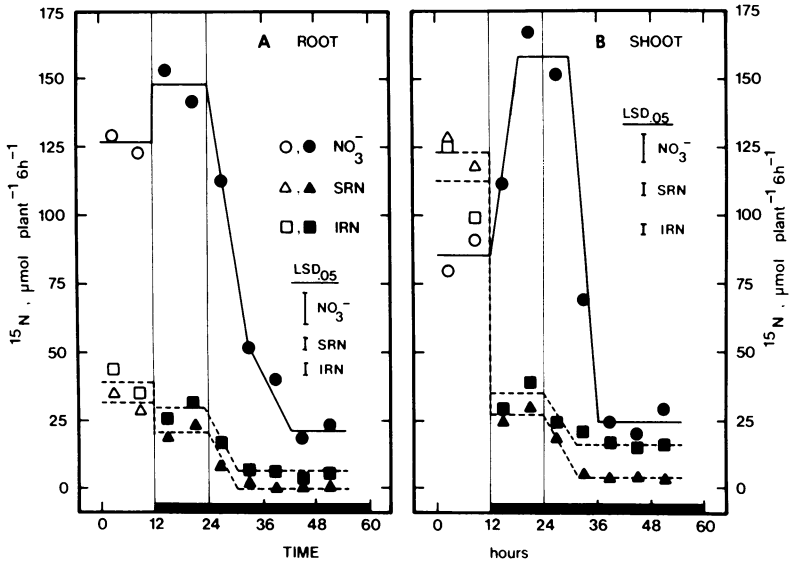

Figure 4. Distribution of ^{15}N among various N fractions in the root and shoot of tobacco plants exposed to $^{15}NO_3^-$ for 6 h intervals during a normal light period and 42 h of continuous darkness.

did not include determination of the form in which ^{15}N was transported in the xylem to the shoot.

Alterations also were evident in whole-plant $^{15}NO_3^-$ reduction (Fig. 6). Whole-plant $^{15}NO_3^-$ accumulation was elevated and reduced-^{15}N accumulation lowered after the initial light period, indicating that absorbed $^{15}NO_3^-$ was assimilated less efficiently in darkness. Assimilation efficiency did change somewhat during darkness, however, as reduced-^{15}N was relatively stable in the initial 12 h of darkness (h 12–24) at about 28% of $^{15}NO_3^-$ uptake, then decreased to 20% for the next 12 h, and later increased and stabilized at 36% of uptake during the last two sample intervals.

The accumulation of ^{15}N in different N fractions in the root and shoot, again expressed as a percent of the $^{15}NO_3^-$ taken up by the plant, is shown in Figure 7. Accumulation of $^{15}NO_3^-$ was variable in both tissues during the treatment intervals. In the root (Fig. 7A), changes in $^{15}NO_3^-$ accumulation were inversely related to coincident adjustments in ^{15}N translocation (*cf.* Fig. 5). Insoluble reduced-^{15}N in the root remained relatively constant at 7 to 9% of the $^{15}NO_3^-$ taken up throughout the experiment (Fig. 7A). In the shoot, however, ^{15}N in the insoluble reduced fraction decreased in the initial 18 h of darkness (h 12–30), and then increased sharply (Fig. 7B).

DISCUSSION

Markedly different amounts of carbohydrates were present in plants during the 54 h experiment. The changes, which presumably are associated with differing energy status, can be separated into general phases (Fig. 1). The first, the most favorable energetically, occurred in the light when sucrose and starch were being accumulated extensively in shoot tissues (*cf.* 17), and root respiration and NO_3^- uptake were maximized. Another phase was discernible during the initial 12 h of darkness, the normal dark period. Utilization of carbohydrate reserves, the shoot being the main source, was most rapid during this time, and root respiration and NO_3^- uptake maintained at relatively high rates. A third phase was distinguishable over the next 12 h of darkness (h 24–36). Remaining shoot carbohydrate was depleted at a slower rate, while root respiration and NO_3^- uptake sharply declined. A last recognizable phase extended over the last 18 h of darkness (h 36–

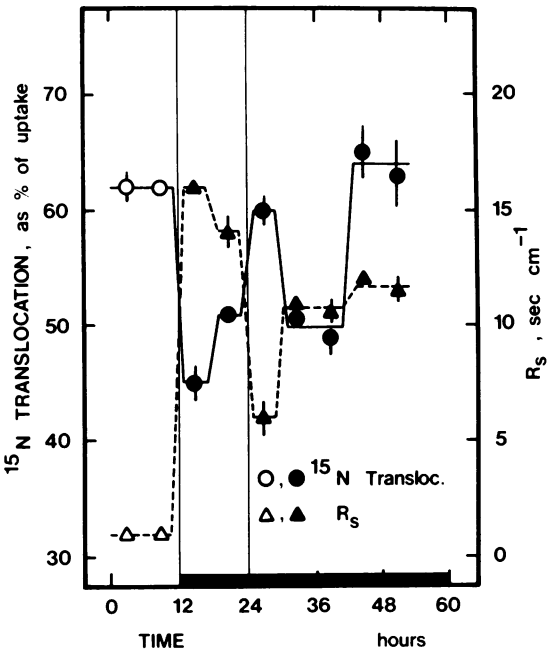

Figure 5. Apparent translocation of ^{15}N to the shoot, expressed as a percent of the total ^{15}N accumulated (*i.e.* $^{15}NO_3^-$ uptake, Fig. 3A), and R_s of leaves of tobacco plants exposed to $^{15}NO_3^-$ for 6 h intervals during a normal light period and 42 h of continuous darkness.

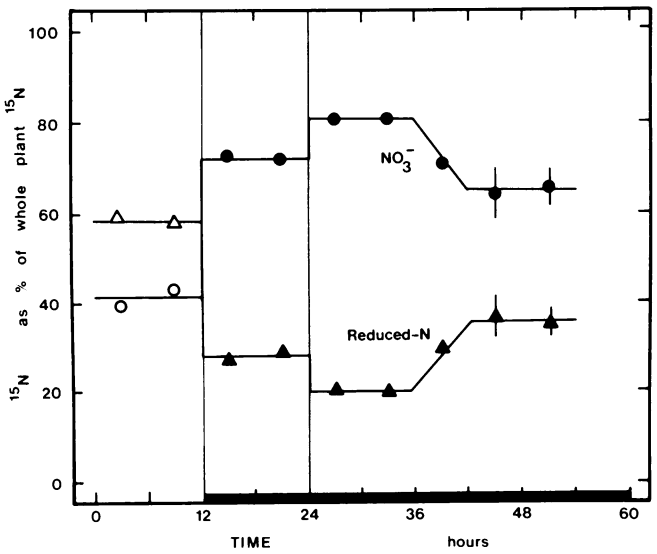

Figure 6. Whole plant $^{15}NO_3^-$ and reduced-^{15}N in tobacco plants exposed to $^{15}NO_3^-$ for 6 h intervals during a normal light period and 42 h of continuous darkness. Reduced ^{15}N represents the total ^{15}N in SRN and IRN fractions, which is equivalent to $^{15}NO_3^-$ reduction. All data are expressed as a percent of the total ^{15}N accumulated, *i.e.* $^{15}NO_3^-$ uptake (Fig. 3A).

54), as carbohydrate levels and rates of root respiration and NO_3^- uptake remained low and somewhat stable. The lack of complete utilization of shoot carbohydrate during the last sample intervals implies that the remaining carbohydrate was sequestered in storage areas, possibly in the stem, and relatively unavailable for metabolism.

NO_3^- Uptake

In general terms, a primary effect of limited carbohydrate availability on plant assimilation of exogenous $^{15}NO_3^-$ was exerted through the restriction of $^{15}NO_3^-$ uptake. The pattern of alterations in ^{15}N incorporation into reduced N and ^{15}N translocation from the root to the shoot during the experiment largely reflected alterations in uptake and the related supply of NO_3^- substrate (*cf.* Fig. 3A and 3B). The restriction of $^{15}NO_3^-$ uptake was most pronounced during the 30 to 36 h interval, following depletion of the large carbohydrate pool in the shoot. The mechanism responsible for the restriction of net $^{15}NO_3^-$ uptake as energy became progressively less available is obscure. Past diurnal experiments indicate that the restriction could involve both decreased influx and increased efflux (15, 25). The two effects could not be delineated here because of the relatively long $^{15}NO_3^-$ exposure period. Such changes in NO_3^- transport across root cell plasmalemmae likely are associated with decreased ATP availability and generation of the membrane pH gradient driving active NO_3^- uptake (5). Since NO_3^- reduction in the root and shoot also generates cytoplasmic OH^-, which can contribute to the driving force for uptake into root cells (refer to Ref. 15), it is conceivable that the restriction of uptake was related, in part, to the slower rate of $^{15}NO_3^-$ reduction.

Decreases in $^{15}NO_3^-$ uptake consistently exceeded decreases in root respiration. This was most evident in the initial 12 h of darkness, when uptake was 71 to 83% and respiration 85 to 95% of the maximal rates in the light, and in the last three sample intervals when uptake had decreased to 11 to 17% and respiration to 40% of the maximal rates (Figs. 2 and 3A). A similar relative sensitivity between the two processes was noted in experiments where the energy supply was limited by plant exposure to a shortened photoperiod (33) and by stem ringing (4). The physiological basis for the differential responses to declining carbohydrate availability is unknown.

N Translocation

Coincident with the restriction of $^{15}NO_3^-$ uptake from the rhizosphere, limitations in carbohydrate availability also resulted in separate responses in endogenous assimilation processes. The separate responses were most obvious when ^{15}N quantities were expressed as a percent of uptake. Tanslocation of ^{15}N, for example, clearly changed rhythmically throughout the experiment (Fig. 5). The rhythmic response provides direct evidence that carbohydrate availability is not the primary factor limiting translocation of absorbed ^{15}N to the shoot during the dark phase of a normal light/dark cycle. Translocation was lower during the initial 12 h of darkness than in the light, as was also observed in previous experiments with other crop plants (22, 24, 29). However, in the latter half of this 12 h dark period, as $^{15}NO_3^-$ uptake remained relatively stable (*cf.* Fig. 3A), translocation began increasing and approached a level in the following 6 h (h 24–30, Fig. 5) which was similar to that in the light. The adjustment occurred even though carbohydrate concentrations in the plant and root respiration rates were declining sharply. Moreover, subsequent adjustments in ^{15}N translocation were not correlated with carbohydrate availability in the plant tissue.

The consistent reciprocal relationship between ^{15}N translocation and R_s until the last three intervals of the experiment implies a close regulatory linkage between translocation into the xylem and the flux of water through the root and vascular tissues. It seems unlikely, however, that translocation of N is

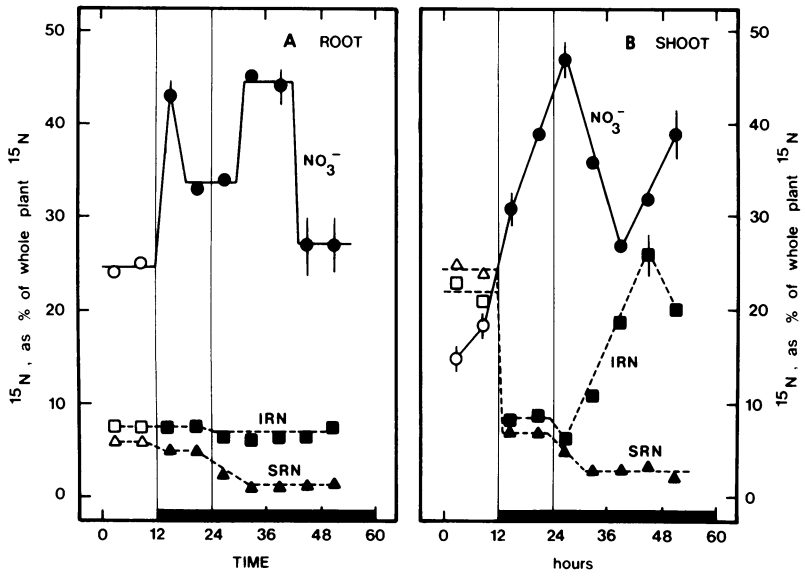

Figure 7. Distribution of ^{15}N among nitrogen fractions in the root and shoot of tobacco plants exposed to $^{15}NO_3^-$ for 6 h intervals during a normal light period and 42 h of continuous darkness. Data are expressed as a percent of the total ^{15}N accumulated by the plants, *i.e.* $^{15}NO_3^-$ uptake (Fig. 3A).

regulated directly by water movement through the root. Nitrate and K^+ translocation from the root into the xylem are closely coupled (28, 30), and translocation of K^+ also has been observed to be restricted during the dark phase of the diurnal cycle (12, 14). In addition, a significant amount of evidence indicates that K^+ transport into the xylem and exudation of xylem fluid can vary rhythmically over extended time periods even in decapitated plants (8, 9, 32, 36). Furthermore, coincident adjustments may occur in properties of the root which control water permeability (7). While cause and effect relationships are to a large extent unclear, a reasonable explanation for the coordinated whole-plant responses is that the rhythmic adjustments in translocation and permeability are controlled by regulatory factors within the root, but are entrained by changes in water relations which, in turn, are governed by stomatal opening and closure. In our experiment, changes in ^{15}N translocation and R_s were not reciprocal during the last three ^{15}N exposure intervals (h 36–54, Fig. 5); the lack of synchronization supports the notion that no obligatory coupling exists between the rhythmic processes in the shoot and root.

Whole-Plant NO_3^- Reduction

The methodology used in this experiment does not allow separate estimation of changes in root and shoot $^{15}NO_3^-$ reduction as carbohydrate availability declined. Nevertheless, it is evident that whole-plant $^{15}NO_3^-$ reduction was consistently limited to a greater extent than $^{15}NO_3^-$ uptake (Fig. 6), and thus reduction was more sensitive to decreasing energy status in the plants. This observation is consistent with those from previous experiments with separated root and shoot tissues (2, 10, 15). Our results indicate, however, that reduction did not always decline in parallel with depletion of internal carbohydrate reserves. After the 24 to 36 h period, the percentage reduction of incoming $^{15}NO_3^-$ increased noticeably even though the carbohydrate concentration in the tissue declined further. Although uptake decreased to minimal rates during that time (*cf.* h 36–54, Fig. 3A), plants clearly retained the capacity to reduce a significant proportion of the absorbed $^{15}NO_3^-$.

Synthesis of Insoluble Reduced-^{15}N

In considering the relationship between carbohydrate availability and $^{15}NO_3^-$ assimilation into reduced ^{15}N, the incorporation of ^{15}N into the insoluble reduced N fraction is of primary importance; it represents *de novo* synthesis of protein and nucleic acids, the metabolically active end-products of the NO_3^- assimilation pathway. As carbohydrate availability and $^{15}NO_3^-$ uptake decreased during the period of darkness, clear differences became apparent in root and shoot assimilation of ^{15}N into insoluble reduced N.

Incorporation of ^{15}N into insoluble reduced N in roots decreased after the light period, closely paralleling decreases in uptake (compare Figs. 3A and 4A). As a consequence, insoluble reduced-^{15}N remained a relatively constant proportion of uptake (Fig. 7A). A similar association consistently occurred in experiments with decapitated corn roots by Morgan *et al.* (21). They observed that ^{15}N incorporation into insoluble reduced N remained a constant proportion of $^{15}NO_3^-$ reduction in the root; and since root reduction is a constant proportion of uptake in that experimental system once the NO_3^- uptake process is fully induced (20), an association between uptake and insoluble reduced N also is implied. The mechanism(s) responsible for the close coupling between uptake of $^{15}NO_3^-$ and concurrent synthesis of ^{15}N-labeled, insoluble reduced N in the root has not been resolved.

Synthesis of insoluble reduced-^{15}N in the shoot was effected differently, being markedly decreased immediately after the light period ended (Fig. 4B). Translocation of ^{15}N from the root to the shoot was restricted during the initial 12 h of darkness (h 12–24, Fig. 3B). Nevertheless, considerable ^{15}N still was available in the shoot, as $^{15}NO_3^-$ was elevated for the initial 18 h of the dark period (h 12–30, Fig. 4B). If it is assumed that the $^{15}NO_3^-$ in the shoot was available to the cytosol of leaf cells, the results indicate that the biochemical capacity for $^{15}NO_3^-$ reduction was limited during this time. After the initial 18 h of darkness, the accumulation of $^{15}NO_3^-$

in the shoot decreased to very low levels, whereas the accumulation of insoluble reduced-^{15}N decreased to a lesser extent (Fig. 4B). This resulted in a marked increase in the proportion of shoot ^{15}N present in the insoluble reduced fraction (data not shown). The results therefore indicate that a limited capacity for the assimilation of ^{15}N into protein and nucleic acids persisted in the shoot even when carbohydrate reserves were severely depleted.

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