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Nitrate Reduction in Response to CO_2-Limited Photosynthesis[1]

Relationship to Carbohydrate Supply and Nitrate Reductase Activity in Maize Seedlings

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ABSTRACT

The effects of CO_2-limited photosynthesis on $^{15}NO_3^-$ uptake and reduction by maize (*Zea mays*, DeKalb XL-45) seedlings were examined in relation to concurrent effects of CO_2 stress on carbohydrate levels and *in vitro* nitrate reductase activities. During a 10-hour period in CO_2-depleted air (30 microliters of CO_2/per liter), cumulative $^{15}NO_3^-$ uptake and reduction were restricted 22 and 82%, respectively, relative to control seedlings exposed to ambient air containing 450 microliters of CO_2 per liter. The comparable values for roots of decapitated maize seedlings, the shoots of which had previously been subjected to CO_2 stress, were 30 and 42%. The results demonstrate that reduction of entering nitrate by roots as well as shoots was regulated by concurrent photosynthesis. Although *in vitro* nitrate reductase activity of both tissues declined by 60% during a 10-hour period of CO_2 stress, the remaining activity was greatly in excess of that required to catalyze the measured rate of $^{15}NO_3^-$ reduction. Root respiration and soluble carbohydrate levels in root tissue were also decreased by CO_2 stress. Collectively, the results indicate that nitrate uptake and reduction were regulated by the supply of energy and carbon skeletons required to support these processes, rather than by the potential enzymatic capacity to catalyze nitrate reduction, as measured by *in vitro* nitrate reductase activity.

Both the uptake and reduction of nitrate by higher plants can be restricted when concurrent photosynthesis is limited by subambient CO_2 levels (CO_2 stress) (2, 9). In one view, CO_2 stress limits the energy available for one or more of the processes which regulate the utilization of exogenous nitrate: (a) nitrate uptake, (b) reduction of nitrate to ammonium, and (c) synthesis of amino acids and macromolecules from ammonium. The alternative view is that CO_2 stress lowers the level of NR[3] protein (*i.e.* the capacity to catalyze the reaction) thereby limiting nitrate reduction (step b).

Substantial evidence supports the postulate for an energy limitation. For example, high endogenous carbohydrate levels enhanced the rate at which nitrate was reduced, especially in leaf tissue (1, 2, 25). In addition, exogenously supplied sucrose increased nitrate reduction not only in the dark but also in the light (1, 9, 12).

The evidence which supports the alternative possibility of a limitation in the enzymatic capacity for nitrate reduction is less conclusive. Carbon dioxide stress severely restricted the induction of NR in nitrogen-depleted rice leaves (21), and it enhanced the decay of NR in nitrate-grown *Perilla* leaves (11). In contrast, CO_2 stress stimulated the induction (by light and nitrate) of NR in ammonium-grown maize plants (18), but had no effect on induction in excised leaves (26).

Although the evidence linking nitrate reduction to carbohydrate supply appears to be more conclusive than that linking it to NR activity, to our knowledge no direct comparisons have been made. Therefore, the present research was initiated to examine with maize seedlings the regulatory effects of CO_2-limited photosynthesis on $^{15}NO_3^-$ uptake and reduction, and to compare the effects with those on *in vitro* NR activity. Both intact and decapitated seedlings were used in order to determine whether root as well as shoot processes were affected by CO_2 stress. The seedlings were grown at low light intensity which minimized endogenous carbohydrate levels and accentuated the effects of CO_2 stress. In addition, the experiments were conducted under quasi-steady state conditions with respect to nitrate supply, thus minimizing the accumulation of carbohydrate which occurs in N-depleted plants.

MATERIALS AND METHODS

Plant Culture

Maize (*Zea mays* L., DeKalb XL-45) caryopses were incubated in darkness at 30°C and 95% RH for 2 d in germination paper moistened with 0.1 mM $CaSO_4$. On the third day, uniform plants were selected and 'cultures' of six seedlings each were supported in hollow polyethylene stoppers, perforated to allow passage of the primary root. Black polypropylene pellets were used to support the emerging shoots and to limit light penetration into the nutrient solution. Each culture was provided 250 mL of basal nutrient solution supplemented with 3.0 mM KNO_3. The basal solution contained 1.25 mM K_2SO_4, 1.0 mM $CaSO_4$, 1.0 mM $MgSO_4$, 0.25 mM $Ca(H_2PO_4)_2$, 0.13 mM Fe as FeEDTA, 46 μM B, 9 μM Mn, 0.8

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[3] Abbreviations: NR, nitrate reductase; DAP, days after planting; A% ^{15}N, atom percent ^{15}N; PMS, phenazine methosulfate.

μM Zn, 0.3 μM Cu, and 0.1 μM Mo. The pH was adjusted to 6.1 with KOH. All solutions were aerated continuously and were replaced at 6, 8, 9, and 10 DAP. A photosynthetic photon flux density of 250 $\mu mol \cdot m^{-2}\ s^{-1}$ was provided by a mixture of fluorescent and incandescent lamps. The 16-h photoperiod (0600–2200 h) supported the development of a relative growth rate of 0.2 $g\ g^{-1}\ d^{-1}$ between 9 to 10 DAP. All experiments were conducted at 10 DAP, beginning at 0800 h, 2 h into the photoperiod.

Treatment Chambers

Carbon dioxide treatments were obtained using four 27-dm^3 acrylic chambers. The chambers were positioned under the light bank to achieve the same illumination received during growth. Each chamber had a port for entry of the treatment atmosphere, a flow meter, a fan to ensure rapid circulation of the atmosphere, a rubber septum for gas sampling, and space for 16 cultures. The seedling cultures were suspended above the treatment solutions through holes in the base of the chamber, effectively separating the shoot atmosphere from the root atmosphere. Two concentrations of CO_2 were used, ambient (approximately 450 μL/L) and depleted (approximately 30 μL/L). The ambient CO_2 atmosphere was obtained by pumping laboratory air through a water-filled gas washing bottle and into a chamber. The CO_2-depleted atmosphere was obtained by passing laboratory air first through a 1.8 m column of Ascarite[4] (Arthur H. Thomas Co., Philadelphia, PA), then through water and finally into a chamber. The ambient and CO_2-depleted air entered the chambers at 8 to 9 L min^{-1} and exited via holes in the stoppers through which the roots passed, thus providing an effective air seal. Periodic measurement of CO_2 (5) within the chambers indicated that equilibrium concentrations were achieved within 12 min after sealing the chambers, and that they remained relatively constant thereafter at about 30 and 450 μL CO_2/L. All root solutions were aerated with ambient air during the course of each experiment. Temperature and relative humidity within the chambers ranged from 26.5 to 28.0°C and 92 to 96%, respectively, during the experiments.

Experiment A

The initial experiment was conducted with intact seedlings to quantify the progressive effects of CO_2 stress on $^{15}NO_3^-$ uptake and reduction during a 10-h treatment period. After the roots of 56 cultures had been rinsed in 0.1 mM $CaSO_4$, 8 were harvested and the remaining 48 were transferred to CO_2 chambers. The treatment solution consisted of basal nutrient solution containing 3.0 mM $K^{15}NO_3^-$ (98.6 A% ^{15}N). At 2, 6, and 10 h after initiation of the concurrent CO_2 and ^{15}N treatments, eight cultures were harvested from both the ambient CO_2 chambers and the CO_2-depleted chambers. After rinsing the roots in distilled water at 2°C, the seedlings were separated into shoot, root, and seedpiece (endosperm, mesocotyl, and a small portion of the root). The tissues were weighed, frozen on dry ice, lyophilized, ground, and mixed thoroughly. Prior to N fractionation and ^{15}N analysis, the roots and seedpieces were combined, while the shoots were analyzed separately. The experiment included four replicates of each treatment, with two cultures (12 plants) serving as a replicate.

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

Experiment B

In the second experiment, effects of CO_2 stress on $^{15}NO_3^-$ uptake and reduction by intact and decapitated seedlings were compared. After the roots of 30 cultures had been rinsed in 0.1 mM $CaSO_4$, 15 cultures were placed in an ambient CO_2 chamber and 15 in a CO_2-depleted chamber. The roots were exposed to the basal solution containing 3.0 mM unlabeled KNO_3. Following a 6-h treatment period, five cultures were harvested (as in experiment A) from each chamber. The roots of the remaining 20 cultures were placed in 0.1 mM $CaSO_4$ at 24°C for 15 min to remove nitrate from the root free space. Ten cultures were then returned to their respective chambers (five to the ambient CO_2 chamber and five to the CO_2-depleted chamber) for an additional 4-h period, during which the roots were exposed to basal solution containing 3.0 mM $K^{15}NO_3^-$ (99.3 A% ^{15}N). The shoots of the remaining 10 cultures (five from the ambient CO_2 chamber and five from the CO_2-depleted chamber) were excised, and the decapitated roots were exposed for 4 h to the ^{15}N treatment solution. Xylem exudate was collected from these cultures during the 4-h treatment period. The seedlings were harvested and prepared for analysis as in experiment A. The study included five replications of each treatment, with a single culture of six seedlings serving as a replicate.

Nitrogen Fractionation and ^{15}N Analysis

Tissue samples from experiments A and B were extracted with methanol:chloroform:water (13:4:3 by volume) using the method outlined by Pace *et al.* (17). The chloroform fraction was discarded since previous experience had shown that it contained little N or ^{15}N. Methanol was removed from the methanol:water fraction by heat (50°C) and surface aeration. Subsamples of the remaining water fraction were analyzed for nitrate (13) and soluble reduced-N (17). Insoluble-N in the residue from tissue extraction was converted to ammonium by Kjeldahl digestion and quantified by a spectrophotometric method (24).

The five replicate samples of xylem exudate were pooled prior to analysis for nitrate (13) and soluble reduced-N (17).

The ^{15}N enrichment in samples containing nitrate (*i.e.* tissue extracts and xylem exudates) was determined by mass spectrometry after reduction of the nitrate to NO (28). The ammonium in Kjeldahl digests of the soluble reduced-N and insoluble-N fractions was recovered by diffusion, oxidized to N_2 gas with NaOBr using a freeze-layer procedure (27), and analyzed for ^{15}N enrichment by mass spectrometry.

In each of the experiments reported here, $^{15}NO_3^-$ reduction is defined as the sum of the soluble reduced-^{15}N and insoluble-^{15}N fractions. Since the former fraction includes unassimilated $^{15}NH_4^+$ and since little $^{15}NH_4^+$ would be expected to accumulate under the conditions employed, $^{15}NO_3^-$ reduction as

defined here is essentially equivalent to $^{15}NO_3^-$ assimilation into the organic-N fraction.

Experiment C

The in vitro NR activity was assayed in six replicate samples of roots and shoots (seedpieces were discarded) just prior to and after a 10-h exposure of the shoots of intact seedlings to ambient and CO_2-depleted air. The experimental conditions duplicated those of experiment A, except that the 2- and 6-h harvests were omitted.

Based on preliminary extraction trials, the following procedure was selected to achieve maximal NR activity and stability. Tissue samples were ground in an ice-chilled mortar using 6 mL of freshly prepared, cold extraction solution per gram fresh weight of tissue. The extractant contained 1 mM Na_2EDTA, 1 mM cysteine, and 3% (w/v) casein in 100 mM potassium phosphate buffer (pH 7.4). Prior to grinding, 0.35 g of acid-washed sand and 1.4 g moist, insoluble PVP were added to the mortar. The latter improved the recovery and stability of NR from shoot tissue but had no effect on that from root tissue. After grinding for 90 s the extracts were centrifuged for 60 s at 15,600*g*. The supernatant fluid was stored on ice until all samples had been ground. Under these conditions NR activity remained stable for at least 1 h, and the assays were completed within that time.

For NR assay, 0.2 mL of extract were incubated at 30 °C with 0.5 mL of 40 mM KNO_3 and 1.0 mL 0.8 mM NADH. After 15 min the reaction was terminated by adding 0.5 mL 0.2 M Zn Acetate, and the excess NADH was oxidized by adding 0.3 mL of 0.1 mM PMS (22). Nitrite was determined spectrophotometrically (7).

Experiment D

Soluble carbohydrate and starch concentrations were measured in roots and shoots (seedpieces were discarded) of intact seedlings after exposure of the shoots to ambient or CO_2-depleted air for 0, 2, 6, and 10 h. In addition, roots of seedlings that had been decapitated after a 6-h exposure to the two treatment atmospheres and harvested at 10 h were assayed. These conditions duplicated those in experiments A and B, respectively. The treatments were replicated four times, with a single culture of six plants serving as a replicate.

Fresh tissue samples were extracted with hot 80% (v/v) ethanol. Soluble carbohydrate in the extract and starch in the residue were analyzed by enzymatic methods (10).

Experiment E

Root respiration (CO_2 release) was measured periodically during the course of a 10-h exposure of the shoots of intact maize seedlings to ambient or CO_2-depleted air. The latter was obtained by passing ambient air through Ascarite and water (as described previously) and then into clear polyethylene bags that were placed around the shoots. This method resulted in a CO_2 concentration of 20 μl CO_2/L, somewhat lower than was obtained in the chamber experiments, A through D.

The roots were sealed into a closed-system respirometer with a rapid-setting silicone rubber (General Electric Co. RTV-11, tin octoate catalyst). During a 5-min period, CO_2 accumulation in the respirometer was determined at 1-min intervals using infrared spectrophotometry (5). Subsequent regression analysis provided an estimate of the rate of CO_2 release from the roots. Regression coefficients were always greater than 0.98.

The treatments were replicated three times, with a single culture of six seedlings serving as a replicate.

RESULTS

Uptake of $^{15}NO_3^-$ was restricted by CO_2 stress within 6 h of initiating the stress (Fig. 1A). Net translocation of ^{15}N ($^{15}NO_3^-$ plus reduced-^{15}N) to the shoot also was limited by CO_2 stress (Fig. 1B). However, when translocation is expressed as a percentage of uptake (numbers adjacent to symbols in Fig. 1B), no limitation is evident. Reduction of $^{15}NO_3^-$ was restricted earlier and to a greater extent than was $^{15}NO_3^-$ uptake (Fig. 1C). Reduction of $^{15}NO_3^-$ as a percentage of $^{15}NO_3^-$ uptake increased with time in control plants (Fig. 1D), reaching a value of 18% by the 10th h of $^{15}NO_3^-$ exposure. In contrast, $^{15}NO_3^-$ reduction by CO_2-stressed plants was only 9% during the initial 2-h period, following which it declined slightly.

The effects of CO_2 stress on the accumulation of $^{15}NO_3^-$, soluble reduced-^{15}N, and insoluble-^{15}N in roots and shoots are shown in Figure 2. In the shoot, $^{15}NO_3^-$ accumulation was unaffected by CO_2 stress (Fig. 2A), whereas in the root it was restricted (Fig. 2D). The accumulation of soluble reduced-^{15}N in root tissue was restricted within 2 h (Fig. 2E). Subsequently, CO_2 stress severely limited the accumulation of soluble reduced-^{15}N in both shoots (Fig. 2B) and roots (Fig. 2E). Although the accumulation of insoluble ^{15}N was similarly affected (Fig. 2, C and F), the percentage of total reduced-^{15}N that had been incorporated into the insoluble-^{15}N fraction was not altered appreciably by CO_2 stress (numbers adjacent to symbols in Fig. 2, C and F).

The uptake and reduction of $^{15}NO_3^-$ by intact and decapitated seedlings during the last 4 h of a 10-h exposure to ambient or CO_2-depleted air (experiment B) are presented in Table I. Carbon dioxide stress restricted the uptake of $^{15}NO_3^-$ by both intact and decapitated seedlings. As in experiment A, $^{15}NO_3^-$ reduction was restricted to a greater extent than was uptake. Of particular interest is the greater reduction of $^{15}NO_3^-$ by decapitated than intact seedlings, both of which had been subjected CO_2 stress: decapitated roots reduced about twice as much $^{15}NO_3^-$ as whole seedlings, both in absolute amount and when expressed as a percentage of incoming $^{15}NO_3^-$. Finally, the accumulation of $^{15}NO_3^-$ and soluble reduced-^{15}N in the xylem exudate of plants subjected to CO_2 stress prior to decapitation was appreciably less than that of nonstressed, decapitated plants.

The *in vitro* NR activities of roots and shoots of maize seedlings before and after a 10-h exposure to ambient or CO_2-depleted air are presented in Table II. The activities are expressed in μmol NO_2^- plant^{-1} h^{-1} to allow comparison with the measured rates of $^{15}NO_3^-$ reduction presented in Figure I. The *in vitro* NR activity increased 34% in shoots and 15% in roots of control plants during the 10-h light period. Depriva-

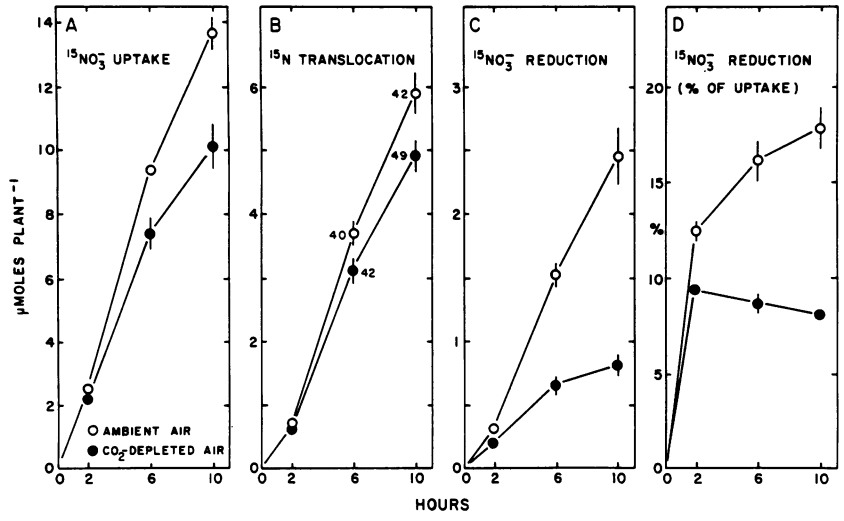

Figure 1. Effect of CO_2-limited photosynthesis on cumulative $^{15}NO_3^-$ uptake (A), net ^{15}N ($^{15}NO_3^-$ plus reduced-^{15}N) translocation to the shoot (B), $^{15}NO_3^-$ reduction (C), and $^{15}NO_3^-$ reduction as a percentage of $^{15}NO_3^-$ uptake (D) by 10-d-old maize seedlings. At time zero, the roots were exposed to 3.0 mM $^{15}NO_3^-$ (98.6 A% ^{15}N) while the illuminated shoots were exposed either to ambient air (≈ 450 μL CO_2/L) or to CO_2-depleted air (≈ 30 μL CO_2/L). The numbers adjacent to the symbols in panel B indicate translocation as a percentage of nitrate uptake. Each symbol is the mean of four replicates ±SE (vertical line). Experiment A.

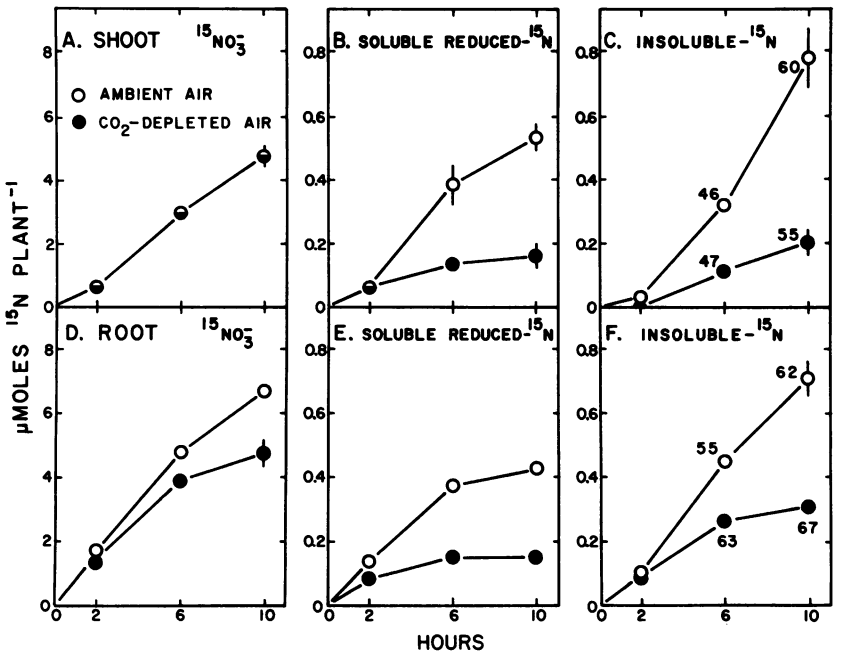

Figure 2. Effect of CO_2-limited photosynthesis on the partitioning of ^{15}N fractions between the shoot and root of 10-d old maize seedlings. At time zero, the roots were exposed to 3.0 mM $^{15}NO_3^-$ (98.6 A% ^{15}N) while the shoots were exposed either to ambient air (≈ 450 μL CO_2/L) or to CO_2-depleted air (≈ 30 μL CO_2/L). The numbers adjacent to the symbols indicate insoluble-^{15}N as a percentage of total reduced-^{15}N in the tissue. Each symbol is the mean of four replicates ±SE (vertical line). Experiment A.

tion of CO_2 during this period decreased NR activity 60% both in roots and in shoots. In spite of this decline, the measured rate of $^{15}NO_3^-$ reduction was much less than the potential capacity for nitrate reduction, as indicated by the average *in vitro* NR activity during the 10-h treatment period. This 'apparent utilization of NR' (NRU as defined in Table II) was only 0.4 to 0.7% for CO_2-stressed seedlings compared to 1.4 to 1.7% for control seedlings. Average tissue fresh weights are presented in Table III, to allow comparison of NR activity and $^{15}NO_3^-$ uptake and assimilation on a per gram fresh weight basis.

Tissue concentrations of starch and soluble carbohydrates measured prior to and during a 10-h exposure of shoots to ambient and CO_2-depleted air are presented in Table IV. Soluble carbohydrate levels in both shoots and roots were diminished by CO_2 stress within 2 h, and thereafter the levels remained considerably below those of control plants. Appreciable starch was detected only in the shoots of control plants.

The respiratory rates of roots during the course of a 10-h exposure of shoots to ambient or CO_2-depleted air are depicted in Figure 3. A significant decrease in root respiration occurred in both treatments, but the decrease occurred earlier and to a greater extent in plants subjected to CO_2 stress.

DISCUSSION

Carbon dioxide stress imposed during a 10-h photosynthetic period restricted $^{15}NO_3^-$ reduction considerably more than $^{15}NO_3^-$ uptake in intact maize seedlings (Fig. 1; Table I). A similar effect was observed in decapitated seedlings, the shoots of which had been subjected to CO_2 stress prior to excision (Table I). Thus, CO_2 stress restricts nitrate reduction in both the shoots and roots of this maize hybrid, although the relative restriction in intact plants cannot be determined exactly because of the possibility of reduced-^{15}N cycling within the plant. Nevertheless, the data from seedlings exposed to ambient air

Table I. *Effect of CO_2-Limited Photosynthesis on $^{15}NO_3^-$ Uptake and Reduction by Intact and Decapitated Maize Seedlings*

At 10 DAP the shoots of illuminated maize seedlings were provided either ambient air ($+CO_2$) or CO_2-depleted air ($-CO_2$) for 10 h. At the sixth hour, the shoots of half the plants were excised, and both the intact and decapitated seedlings were exposed to $^{15}NO_3^-$ (99.3 A%^{15}N), in place of $^{14}NO_3^-$, for the remaining 4 h. Values are means ± SE of five replicates, except for exudate values, which were obtained by pooling the five replicates prior to analysis. Experiment B.

^{15}N Fraction	Tissue[a]	Intact Plant		Decapitated Plant	
		$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$
		nmol plant^{-1} h^{-1}			
$^{15}NO_3^-$	Shoot (exudate)	470 ± 20	470 ± 7	(271)[b]	(157)
	Root	1057 ± 35	900 ± 120	872 ± 60	667 ± 20
Soluble reduced-^{15}N	Shoot (exudate)	67 ± 5	12 ± 2	(9)	(4)
	Root	102 ± 10	15 ± 3	38 ± 8	10 ± 1
Insoluble ^{15}N	Shoot (exudate)	50 ± 2	2 ± 2		
	Root	70 ± 5	22 ± 5	110 ± 27	77 ± 12
$^{15}NO_3^-$ uptake		1816 ± 30	1421 ± 140	1300 ± 55	915 ± 25
$^{15}NO_3^-$ reduction		289 ± 18	51 ± 8	157 ± 27	91 ± 13
Reduction, % of uptake		15.9	3.6	12.1	9.9

[a] Root includes seedpiece. [b] Exudate data are in parentheses.

Table II. *Effect of CO_2 Stress on in Vitro Nitrate Reductase Activity*

NR activity of shoots and roots of 10-d-old, illuminated maize seedlings was measured at 0800 and 1800 h. During the 10-h interval the shoots had been exposed either to ambient air ($+CO_2$) or to CO_2-depleted air ($-CO_2$). The values are means ± SE of 6 replicate tissue samples. Experiment C.

Measurement	$+CO_2$			$-CO_2$		
	Shoot	Root	Plant	Shoot	Root	Plant
	μmol NO_2^- plant^{-1} h^{-1}					
NR, 0800	11.0 ± 0.8	4.0 ± 0.2	15.0 ± 0.8	11.0 ± 0.8	4.0 ± 0.2	15.0 ± 0.8
NR, 1800	14.7 ± 0.9	4.6 ± 0.2	19.3 ± 1.0	5.7 ± 0.3	1.7 ± 0.1	7.4 ± 0.2
NRU[a] %, experiment A	0.4	2.6	1.4	0.3	1.6	0.7
NRU %, experiment B	0.9	4.0	1.7	0.3	1.3	0.4

[a] The apparent utilization of NR (NRU) is defined as the accumulation of reduced-^{15}N in a given tissue (μmol plant^{-1} h^{-1}) expressed as a percentage of the average *in vitro* NR activity in that tissue (μmol NO_2^- plant^{-1} h^{-1}) during the 10-h treatment period.

Table III. *Fresh Weights of Maize Seedlings Used for Experiments A, B, and C*

The shoots and roots of 10-d-old maize seedlings were weighed after the illuminated shoots had been exposed to ambient air ($+CO_2$) or CO_2-depleted air ($-CO_2$) for 10 h. Values are means ± SE.

Experiment	$+CO_2$		$-CO_2$	
	Shoot	Root[a]	Shoot	Root[a]
	g plant^{-1}			
A	1.25 ± 0.01	0.42 ± 0.01	1.16 ± 0.04	0.41 ± 0.02
B	1.40 ± 0.01	0.59 ± 0.01	1.35 ± 0.02	0.60 ± 0.02
C	1.60 ± 0.08	0.54 ± 0.02	1.31 ± 0.07	0.44 ± 0.01

[a] Root does not include seedpiece.

(Table I, $+CO_2$) demonstrate that the decapitated root does have the potential for considerable nitrate reduction, as indicated by the accumulation of reduced-^{15}N at 55% of the rate in intact seedlings. The high rates of $^{15}NO_3^-$ reduction by decapitated roots may reflect an enhanced supply of carbohydrate to the root tissue from the remaining endosperm upon removal of the shoot as a competing sink. This possibility is supported by the higher concentration of soluble carbohydrate in decapitated roots, 2.2%, than in intact roots, 1.7%, of seedlings subjected to CO_2 stress (Table IV, $-CO_2$).

In contrast to the reduction of incoming $^{15}NO_3^-$, little endogenous $^{14}NO_3^-$ was reduced. In experiment A, for example, the initial $^{14}NO_3^-$ levels in shoots and roots were 93.6 ± 4.4 and 41.1 ± 3.2 μmol plant^{-1}, respectively, and little change in these values could be detected during the course of the 10-h experiment (data not presented). Estimates of $^{14}NO_3^-$ reduction are considerably less exact than those of $^{15}NO_3^-$ reduction, because the former requires two separate sets of plants for each determination. Nevertheless, the reduction of endogenous nitrate would have been detectable had it oc-

Table IV. *Effect of CO_2 Stress on Starch and Soluble Carbohydrate Concentrations of Maize Seedlings*

Maize tissues were analyzed just prior to (0800) and at selected intervals during a 10-h exposure of their shoots to ambient air ($+CO_2$) or CO_2-depleted air ($-CO_2$). Values are means of four replicates ± SE. Experiment D.

Time[a]	Tissue[b]	Soluble Carbohydrate		Starch	
		$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$
		% of dry weight			
0800	Shoot	2.38 ± 0.17	2.38 ± 0.17	0.44 ± 0.02	0.44 ± 0.02
	Root	2.45 ± 0.08	2.45 ± 0.08	ND[c]	ND
1000	Shoot	2.00 ± 0.09	1.24 ± 0.22	0.15 ± 0.03	0.03 ± 0.02
	Root	2.10 ± 0.04	1.61 ± 0.08	ND	ND
1400	Shoot	2.75 ± 0.13	1.04 ± 0.03	0.48 ± 0.03	ND
	Root	2.42 ± 0.14	1.40 ± 0.11	ND	ND
1800	Shoot	4.66 ± 0.21	1.49 ± 0.17	1.23 ± 0.21	ND
	Root	3.09 ± 0.08	1.68 ± 0.18	ND	ND
	Root[d]	2.47 ± 0.12	2.21 ± 0.32	ND	ND

[a] Photoperiod 0800 to 2200. [b] Root does not include seedpiece. [c] Not detectable, <0.01% [d] Seedling decapitated at 1400 h.

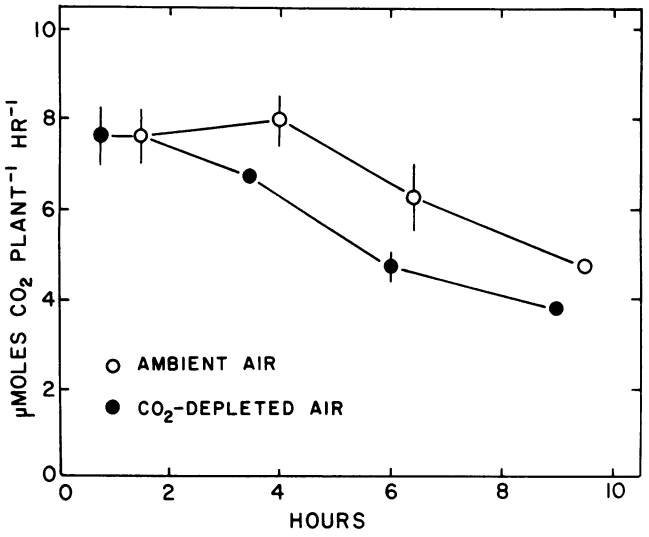

Figure 3. Effect of CO_2-limited photosynthesis on root respiration in 10-d-old maize seedlings. The illuminated shoots were exposed either to ambient air (≈ 450 μL CO_2/L) or to CO_2-depleted air (≈ 20 μL CO_2/L) during the 10-h measurement period. Each symbol is the mean of three replicates ±SE (vertical line). Experiment E.

curred at a rate comparable to the rate of reduction of incoming $^{15}NO_3^-$ (*e.g.* Fig. 1C), either in the presence or absence of CO_2 stress. It thus seems reasonable to conclude that exogenous $^{15}NO_3^-$ was the primary substrate for reduction under the conditions employed.

The apparent absence of endogenous $^{14}NO_3^-$ reduction in the XL-45 hybrid during concurrent $^{15}NO_3^-$ uptake has been noted previously (14). In root tissue, this phenomenon may be related to the predominant localization of NR in epidermal cells and the relative paucity of NR in cortical cells (19). In shoot tissue, nitrate entering by translocation from the root may be more accessible to NR than is endogenous nitrate, most of which is located in vacuoles (6, 15).

It is conceivable that the negative effect of CO_2 stress on the reduction of incoming nitrate (Fig. 1C; Table I) was due to an actual decline in NR protein (11). To examine the effect of CO_2 stress on nitrate reductase, *in vitro* NR activity (an index of the *maximal capacity* for nitrate reduction) was assayed in maize seedlings before and after a 10-h photosynthetic period in ambient air or in CO_2-depleted air (Table II). Although *in vitro* NR activity declined 60% during the period of CO_2 stress, the remaining activity was far in excess of the measured rate of $^{15}NO_3^-$ reduction (Fig. 1). On a whole-plant basis, less than 2% of the potential *in vitro* NR activity was utilized (NRU values, Table II). Since the enzyme was thus present in considerable excess, it appears unlikely that the decline in *in vitro* NR activity restricted nitrate reduction *in situ*. This conclusion is consistent with the observation of Warner and Kleinhofs (30) that NR-deficient mutants of barley, the leaves of which exhibited less than 2% of the usual *in vitro* NR activity, were able to reduce nitrate and to accumulate reduced N at about the same rate as control plants.

A more likely explanation of the observed effects of CO_2 stress is that the supply of energy and carbon skeletons was insufficient to support $^{15}NO_3^-$ reduction at the same level attained by control plants growing in ambient air. Analysis of the seedlings before and after the 10-h treatment period (Table III) revealed that CO_2 stress decreased the soluble carbohydrate concentration in both roots and shoots and prevented the increase which occurred during the latter half of the treatment period in plants exposed to ambient air. These data, and the observation that root respiration was restricted by CO_2 stress (Fig. 3), support the conclusion that the supply of carbohydrate from the shoots to the roots diminished rapidly during the period of CO_2 stress. The soluble carbohydrate concentration of roots had declined by the second hour of CO_2 stress (Table IV), as had the accumulation of products of $^{15}NO_3^-$ reduction (Fig. 2E). Previous studies with this maize hybrid have demonstrated that the addition of exogenous glucose during exposure of decapitated seedlings to $^{15}NO_3^-$ doubled the reduction of incoming nitrate (9). Collectively,

the results indicate that photosynthetic carbohydrate production in shoots, and its delivery to roots, are closely related to the regulation of nitrate reduction in maize seedlings.

The basic concept which emerges from the present study is that it is the carbohydrate supply rather than the enzymatic capacity for nitrate reduction which regulates the uptake and reduction of incoming nitrate by CO_2-stressed maize seedlings. Although total NR activity declined during CO_2 stress, this and other studies indicate that NR activity can be considerably in excess of actual nitrate reduction (Table II; 1, 3, 4, 29). In addition, there is increasing evidence that exogenously supplied sucrose can enhance nitrate uptake (8), NR activity (16, 20, 23), and nitrate reduction (1, 9, 12) under both light and dark conditions. Thus, it is concluded that in the present quasi-steady state experiments the potential for $^{15}NO_3^-$ reduction was limited by the supply of reductant and C-skeletons rather than by the enzymatic capacity to catalyze nitrate reduction.

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