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Nitrate Uptake and Partitioning by Corn Root Systems[1]

DIFFERENTIAL EFFECTS OF AMMONIUM AMONG GENOTYPES AND STAGES OF ROOT DEVELOPMENT

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ABSTRACT

The relative effects of ammonium on nitrate uptake and partitioning during induction were compared among decapitated seedlings of three corn (*Zea mays* L.) genotypes at two developmental stages. This study tested the hypothesis that root systems efficient at translocating products of ammonium assimilation away from sites of nitrate uptake or reduction would exhibit less inhibition of nitrate uptake by ammonium compared to root systems with inefficient N translocation efficiency. Inhibition of nitrate uptake by ammonium was relatively slight at day 5 ranging from 0% to 20% among the three genotypes, as compared to greater inhibition, from 20% to 37%, at day 8. Five-day-old roots exhibited negligible xylem translocation capacity in comparison with those grown for 8 days. Thus, although the capability to translocate ammonium assimilates out of the root increased between days 5 and 8, inhibitory effects of ammonium also increased. In the absence of ammonium, nitrate uptake per unit root mass increased between days 5 and 8. This increased activity of the uptake system was proportionally more sensitive to ammonium.

Partitioning of entering nitrate into the reduction process was positively correlated with lateral root development of the inbred root systems at 5 and 8 days. This is supportive of a localization of a major portion of nitrate reduction occurring in root apical regions. Nitrate reduction was the partitioning process most severely inhibited by ammonium in all cases, ranging from 39% to 55% inhibition. In contrast, ammonium-inhibition of nitrate accumulation in the root tissue and translocation via xylem vessels varied with genotype and root age.

Two mechanisms of ammonium-inhibition of nitrate are implicated, one which directly affects nitrate reduction and the uptake system associated with it, and another which may involve potassium as an intermediate regulator of nitrate accumulation in the root tissue and nitrate translocation out of the root tissue.

Inhibition of nitrate uptake by ambient ammonium may involve several mechanisms. It has been suggested that ammonium or product(s) of ammonium assimilation may alter the rate of activation or synthesis of the nitrate uptake system (5, 13) or nitrate reductase (14, 20). Inhibition of the latter could restrict nitrate uptake by inhibiting nitrate reduction and hence limit hydroxyl generation for antiport with nitrate across the plasmalemma (10, 11). In addition, ammonium may limit nitrate uptake by accelerating nitrate efflux (4). Moreover, potassium enhances nitrate uptake (1) and moderates inhibition of nitrate uptake by ammonium, possibly by facilitating the flow of water and ammonium assimilates (potential negative effectors) through the corn root symplasm to the xylem (21). From these earlier findings, it seems reasonable to suggest that plants efficient in translocating negative effectors away from sites of nitrate uptake or reduction would exhibit minimal inhibition of nitrate uptake by ammonium. Conversely, nitrate uptake by root systems exhibiting lesser translocation capacity would be expected to be more severely inhibited by ammonium.

This report presents an evaluation of this hypothesis utilizing inbred corn seedlings previously shown to differ in their capacity for water flow (and nitrate translocation) from the root system as well as in the change in these attributes with root age (18). Corn root systems increase in translocation capacity between 5 and 8 d after germination. The experimental material thus provided a range of translocation capcity for movement of reduced nitrogen out of the root system. Because the inbreds provide a range of lateral root development (18), the experiments also permitted an assessment of the relationship between lateral root proliferation and nitrate reduction anticipated by the greater *in vitro* nitrate reductase activity of root apices than in more mature root tissue (16, 19).

MATERIALS AND METHODS

Plant Culture. Three experimental corn (*Zea mays* L.) inbred lines, developed from 'Jarvis Golden Prolific' and 'Indian Chief' varieties, were germinated in paper towels soaked in 0.1 mM $CaSO_4$ for 4 d in a dark chamber maintained at 30°C and 99% RH. Seedlings lacking lateral roots and having seminal root lengths between 9 and 14 cm were then transferred to a minus NO_3^- nutrient solution containing 0.175 mM K_2SO_4, 0.1 mM $Ca(H_2PO_4)_2$, 0.25 mM $MgSO_4$, 10.7 μM Fe as FeEDTA, 1 mM $CaSO_4$, and trace elements at two-fifths Hoagland strength (8), adjusted to pH 6.0 with NaOH. Solutions were changed daily. After transfer, the seedlings were grown in the dark at 30°C and 99% RH for 1 or 4 d. Shoots were excised 19 h prior to the uptake period, and exudate collectors (plastic pipet tips) were fitted to the remaining mesocotyl and sealed with silicone grease.

Experimental Procedure. Five- and 8-d-old detopped plants were exposed for 8 h to 115 or 260 ml aerated treatment solution, respectively. The treatment solution contained the same nutrients as the pretreatment solutions with the exception of the replacement of K_2SO_4 with 0.35 mM $K^{15}NO_3$ (98 atom % ^{15}N) ± 0.175 mM $(NH_4)_2SO_4$. The treatment solutions were replaced every 2 h. Xylem exudate was collected every 2 h, weighed, and frozen. Each genotype-age combination was replicated four times. After 8 h, roots were placed in cold 0.1 mM $CaSO_4$ for 30 min, and were then weighed and freeze-dried.

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Analytical Procedures. Net nitrate, ammonium, and potassium uptake were estimated by solution depletion over each 2-h interval. Partitioning of the entering nitrate by the end of the 8-h period was calculated from the quantities of ^{15}N in tissue and xylem nitrate, tissue- and xylem-soluble reduced N, and tissue-insoluble N. Estimates of nitrate uptake obtained by summation of the tissue and xylem ^{15}N values were lower than values obtained by the solution depletion method, but differences among genotypes and with plant age were similar.

Nitrate in uptake solutions, xylem exudate, and root tissue was analyzed by a modified procedure of Lowe and Hamilton (12). Potassium was analyzed by flame photometry. Due to low exudation rates exhibited by 5-d plants, xylem exudate and tissue of the same culture were combined for analysis of reduced N. In contrast, exudation rates of 8-d plants were substantial, and tissue analysis was separate from xylem exudate analysis. Soluble reduced N and nitrate in tissue were determined on the methanol-water fraction of methanol-chloroform-water extracts (17). Nitrate in the soluble fraction was volatilized prior to Kjeldahl digestion to minimize contamination during digestion (17). Soluble reduced N in the xylem exudate was analyzed by the same procedure.

The insoluble reduced N fraction included the chloroform-soluble N fraction of the extract and the insoluble residue of the tissue, which were combined prior to digestion. Ammonium in the uptake solutions and in the digests of exudate, tissue-soluble, and tissue-insoluble digests was analyzed by a NaOCl colorimetric procedure (3). The atom % ^{15}N in the various digested fractions was determined by MS following the separation of ammonia from the digest by diffusion and conversion to N_2 (22). The atom % ^{15}N in the nitrate contained in the uptake solutions was determined by conversion to nitric oxide and mass spectrometric analysis (23).

A separate set of plants for each genotype were grown under the conditions described above for the characterization of lateral root proliferation. At 5 and 8 d after germination, the roots were separated and floated in a shallow tray, thereby allowing a separation of lateral roots from each other. Photographs of the roots were taken and were later used for enumeration of lateral roots.

RESULTS

Nitrate Uptake. In the absence of ammonium, nitrate uptake rates per unit root mass increased with time from initial exposure to nitrate, although the magnitudes differed among the three genotypes and at both root ages (Fig. 1). On day 8, inbreds A and B had substantially higher nitrate uptake rates per unit root mass than on day 5, whereas the increase was less marked with inbred C, owing to its relatively high rates in 5-d-old seedlings. With all three inbreds, the rates increased linearly with time on day 5, but on day 8, a tendency to approach a constant rate was evident in all instances.

No inhibition of nitrate uptake by ammonium was apparent during the initial 2-h interval (Fig. 1). Thereafter, inhibition increased with time, but varied with genotype and with root age. At day 5, inhibition in all three genotypes was slight, ranging from 9% to 17% during the 6- to 8-h period. At day 8, greater inhibition was evident, ranging from 26% to 31%.

Nitrate Partitioning. Nitrate translocation rates at day 5 were very low in comparison with rates exhibited at day 8 (Fig. 2). Those at day 8 increased steadily with time, and differences among the genotypes were clearly evident in the quantitites of nitrate translocated as well as in the extent to which this process was inhibited by ammonium. In contrast to the other two genotypes, the inhibition in nitrate uptake by ammonium with genotype B (Fig. 1) was not accompanied by an inhibition in nitrate translocation. Generally, the increase with age in nitrate uptake per unit root mass was accompanied by increases in nitrate translocation and, to a lesser quantitative extent, with nitrate reduction (Table 1).

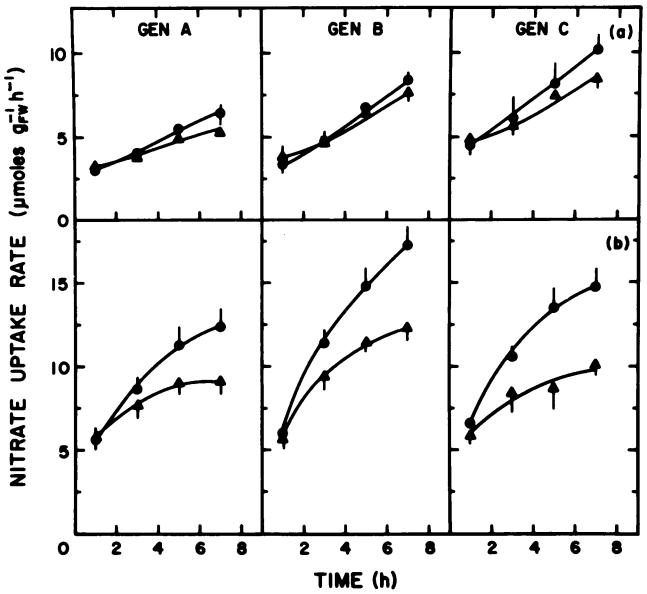

FIG. 1. Rates of nitrate uptake by (a) 5-d and (b) 8-d-old roots (●, $-NH_4^+$; ▲, $+NH_4^+$) of three corn genotypes (inbreds A, B, C). Nitrate uptake during the 2-h intervals was estimated by solution depletion. SE larger than the symbol size are represented by vertical lines.

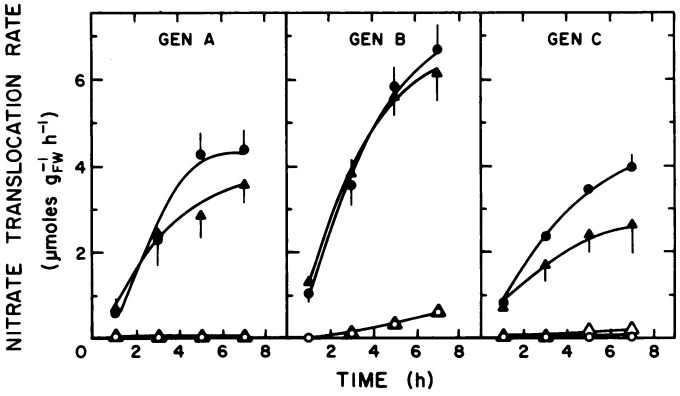

FIG. 2. Rates of nitrate translocation by 5-d (○, △) and 8-d (●, ▲) old roots (○, ●, $-NH_4^+$; △, ▲, NH_4^+) with three corn genotypes. SE larger than the symbol size are represented by vertical lines.

The proportion of the entering nitrate that was reduced during the 8-h period differed among the genotypes and increased between 5 and 8 d in all three (Table I). The ranking among genotypes (C > B > A) in percentage nitrate reduced was not altered by root age or presence of ammonium. Ammonium restricted nitrate reduction more than uptake at each root age. The percentage of entering nitrate that was reduced during the 8-h exposure was positively related to the number of lateral roots per unit mass (Fig. 3).

Reduced ^{15}N Partitioning. In the absence of ammonium at day 5, genotypes differed 3-fold in the quantitites of reduced ^{15}N which were incorporated into insoluble nitrogen forms during the 8-h experimental period (Table II). At day 8, differences among genotypes in the quantitites of reduced ^{15}N which were translocated in the xylem were substantially greater than differences in incorporation into the insoluble and soluble fractions of the tissue. Although the ranking among the genotypes (B > C > A) in translocation of reduced ^{15}N was the same as the ranking

Table I. *Nitrate Uptake and Partitioning by Root Systems of Three Corn Inbred Lines*

Tissue was grown for 5 or 8 d and exposed to 0.35 mM $^{15}NO_3$ (>97% ^{15}N) with and without 0.175 mM $(^{14}NH_4)_2SO_4$ for 8 h. Values represent means of four replicates ± SE.

Genotype	NH_4^+	Xylem NO_3^- (a)	Tissue NO_3^- (b)	Reduced ^{15}N (c)	Total $^{15}NO_3^-$ uptake (a + b + c)
			μmol g^{-1} fresh wt 8 h^{-1}		
			Day 5		
A	−	0.12 ± 0.02 (0.5)[a]	20.8 ± 2.2 (84.8)	3.6 ± 0.4 (14.7)	24.5 ± 2.5
A	+	0.11 ± 0.04 (0.4)	24.9 ± 2.2 (92.9)	1.8 ± 0.4 (6.7)	26.8 ± 2.5
% Δ				−49.7	
B	−	2.2 ± 0.2 (6.6)	22.8 ± 1.2 (68.3)	8.4 ± 0.4 (25.1)	33.4 ± 1.2
B	+	2.5 ± 0.6 (8.8)	20.9 ± 2.3 (73.3)	5.1 ± 0.8 (17.9)	26.8 ± 2.5
% Δ				−39.3	−19.8
C	−	0.2 ± 0.04 (0.5)	27.8 ± 3.0 (70.4)	11.5 ± 1.7 (29.1)	39.5 ± 4.7
C	+	0.8 ± 0.2 (2.4)	25.4 ± 1.0 (77.0)	6.7 ± 0.9 (20.6)	32.9 ± 1.3
% Δ				−41.8	−16.7
			Day 8		
A	−	25.9 ± 2.8 (38.3)	28.3 ± 0.8 (41.8)	13.7 ± 1.5 (20.2)	67.7 ± 4.8
A	+	19.3 ± 2.7 (40.3)	21.7 ± 1.7 (45.3)	6.9 ± 1.4 (14.4)	47.9 ± 4.4
% Δ		−25.5	−23.3	−49.8	−29.2
B	−	34.3 ± 3.0 (44.0)	17.5 ± 1.0 (22.4)	26.2 ± 3.0 (33.6)	78.0 ± 6.0
B	+	34.2 ± 3.0 (55.1)	15.3 ± 0.3 (24.6)	12.6 ± 0.7 (20.3)	62.1 ± 4.0
% Δ			−12.6	−52.2	−20.4
C	−	21.2 ± 1.2 (34.8)	15.0 ± 1.0 (24.6)	24.5 ± 1.1 (40.6)	60.7 ± 1.9
C	+	14.9 ± 2.9 (38.0)	12.3 ± 1.3 (31.4)	11.1 ± 0.8 (28.3)	38.4 ± 4.1
% Δ		−29.7	−29.8	−54.7	−36.7

[a] Numbers in parentheses, percentage of $^{15}NO_3$ uptake.

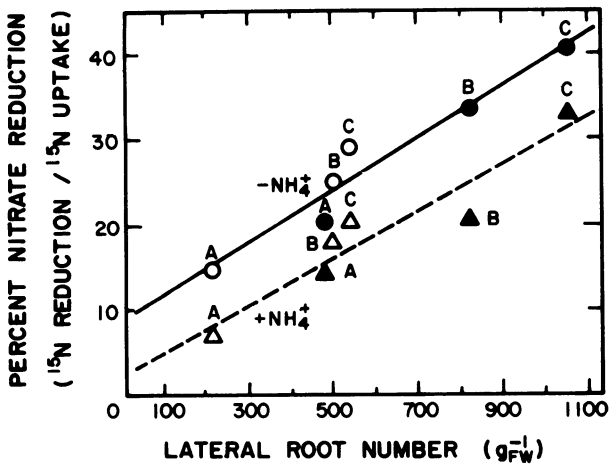

FIG. 3. Relationship between per cent nitrate reduction and lateral root proliferation among three corn genotypes at two root ages (○, △, 5 d; ●, ▲, 8 d) without ambient ammonium (○, ●) ($\hat{y} = 0.15x + 8.9$; $r = 0.97$) and with ambient ammonium (△, ▲) ($\hat{y} = 0.14x + 2.2$; $r = 0.95$).

for nitrate translocation (Table I), the differences among the genotypes were more pronounced (4-fold) for reduced ^{15}N translocation (Table II). Differential partitioning of the reduced ^{15}N is indicated for genotypes B and C which had similar quantities accumulated in the insoluble fraction but differed inversely in the amounts of reduced ^{15}N translocated and in the ^{15}N accumulated in the tissue as soluble reduced ^{15}N. Whereas genotype A reduced only about half as much nitrate as the other two (Table I), a larger proportion of that which was reduced accumulated in the insoluble plus soluble reduced ^{15}N fractions in the tissue, and a substantially smaller proportion was translocated.

Presence of ammonium altered the partitioning of the reduced ^{15}N between soluble and insoluble fractions (Table II). Although its effects were not identical among the genotypes nor at the two root ages, ammonium consistently inhibited ^{15}N incorporation into the insoluble fraction most severely. This restriction was greater on day 8 than on day 5. Translocation of reduced ^{15}N was inhibited substantially on day 8 in genotypes B and C, but not affected in genotype A (Fig. 4a). During the 0 to 2-h interval, reduced ^{15}N content of the xylem fluid was negligible in all cases. Subsequently with genotypes B and C, the percentage inhibition of reduced ^{15}N translocation during each interval after the first 2-h period remained within a relatively narrow range of 39% and 50%.

Ammonium Uptake and Reduced ^{14}N Translocation. In contrast with the apparent induction pattern of nitrate uptake (Fig. 1), ammonium uptake rates were relatively constant during the 8-h exposure to ammonium and nitrate (Fig. 5) although, like nitrate uptake, they were greater at day 8 than at day 5 (Fig. 5). The parallel increase in total (0–8 h) uptake between day 5 and day 8 for the two ions is illustrated by a constancy of the ammonium to nitrate uptake ratio for each genotype at the two dates (Table III). Yet, the ammonium to nitrate uptake ratio of genotype A was greater than those of genotypes B and C.

Translocation of reduced ^{14}N to the xylem fluid of these seedlings includes components from the endosperm and from protein turnover in root cells (6), as well as that originating from ammonium uptake and assimilation in the root tissue. At day 8 in the absence of ambient ammonium, rates of reduced ^{14}N

Table II. *Nitrate-^{15}N Assimilation into Reduced Nitrogen Fractions by Root Systems of Three Corn Inbred Lines*

Tissue was grown for 5 or 8 d and exposed to 0.35 mM $^{15}NO_3$ (>97% ^{15}N) with and without 0.175 mM $(^{14}NH_4)_2SO_4$ or 8 h. Values represent the means of four replicates ± SE.

	Day 5		Day 8		
	Tissue-insoluble ^{15}N	Tissue + xylem-soluble ^{15}N	Tissue-insoluble ^{15}N	Tissue-soluble ^{15}N	Xylem-soluble ^{15}N
	μmol reduced N g^{-1} fresh wt 8 h^{-1}				
Genotype A					
$-NH_4$	1.77 ± 0.25	1.84 ± 0.47	5.99 ± 0.35	4.89 ± 0.54	2.86 ± 0.68
$+NH_4$	0.74 ± 0.14	1.07 ± 0.23	1.52 ± 0.10	2.37 ± 0.36	3.02 ± 0.97
% Δ	−58%	−42%	−75%	−52%	
Genotype B					
$-NH_4$	3.31 ± 0.24	5.04 ± 0.24	8.51 ± 0.59	6.16 ± 0.96	11.57 ± 1.14
$+NH_4$	1.56 ± 0.22	3.53 ± 0.59	2.52 ± 0.22	3.75 ± 0.39	6.24 ± 0.48
% Δ	−53%	−30%	−70%	−39%	−46%
Genotype C					
$-NH_4$	5.90 ± 0.93	5.57 ± 1.07	8.52 ± 1.12	7.93 ± 0.62	8.07 ± 1.53
$+NH_4$	2.30 ± 0.31	4.37 ± 0.59	2.82 ± 0.33	3.74 ± 0.76	4.63 ± 0.13
% Δ	−61%	−21%	−67%	−53%	−43%

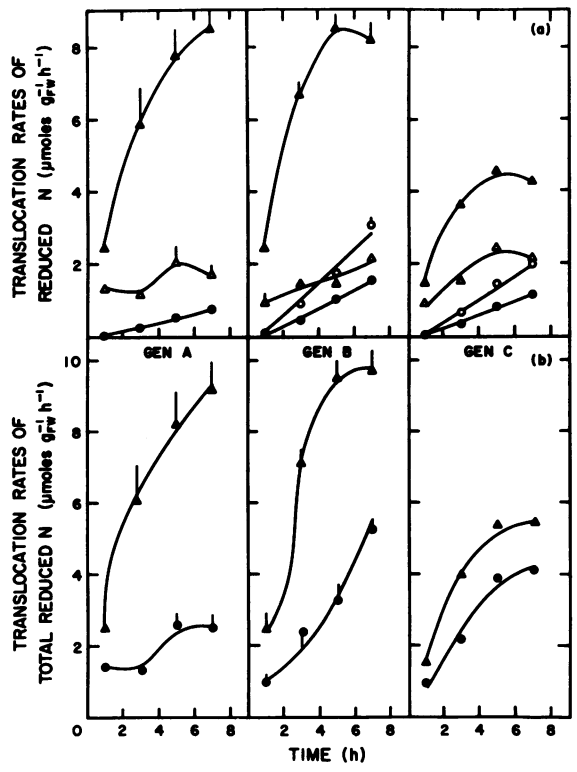

FIG. 4. (a), Translocation rates of reduced nitrogen derived from nitrate (●, $+NH_4^+$; ○, $-NH_4^+$), ambient ammonium and endogenous sources (▲), and endogenous sources in the absence of ambient ammonium (△) by 8-d-old roots of three corn genotypes. (b), Translocation rates of total reduced nitrogen (●, $-NH_4^+$; ▲, $+NH_4^+$) by 8-d-old roots of three corn genotypes. SE larger than the symbol size are represented by vertical lines.

translocation were similar among the three genotypes (Fig. 4a). In contrast, reduced ^{14}N translocation rates were increased by ambient $^{14}NH_4^+$ to a much greater extent in genotypes A and B relative to genotype C (Fig. 4a). This difference was not reflective of a corresponding difference in ammonium uptake (Fig. 5). Assuming that the quantity of reduced ^{14}N in the xylem fluid derived from endogenous sources was unaltered by ambient ammonium, it is apparent that the proportion of ammonium-

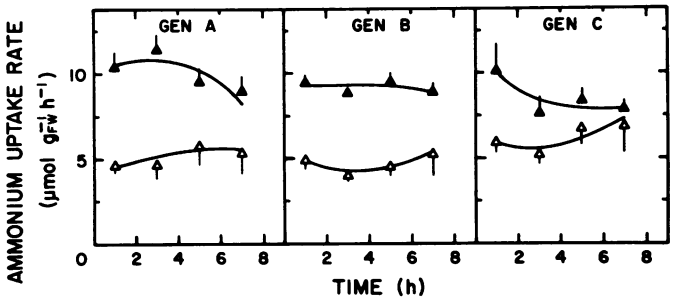

FIG. 5. Rates of ammonium uptake with 5-d (△) and 8-d (▲) old root systems of three corn genotypes. SE larger than the symbol size are represented by vertical lines.

Table III. *Cumulative Ammonium to Nitrate Uptake Ratios among Three Corn Genotypes and Two Root Ages*

Solutions contained 0.35 mM $^{15}NO_3^-$ and 0.175 mM $(^{14}NH_4)_2SO_4$.

Genotype	NH_4^+/NO_3^- Uptake (8 h)	
	5 d	8 d
A	1.21 ± 0.16	1.24 ± 0.06
B	0.84 ± 0.06	0.95 ± 0.07
C	0.94 ± 0.11	1.05 ± 0.14

derived nitrogen that was translocated was considerably less in genotype C than in genotypes A and B (Fig. 6). As a result, translocation of total reduced nitrogen (^{14}N + ^{15}N) in the presence of ammonium was lowest in genotype C, whereas in the absence of ammonium it was lowest in genotype A (Fig. 4b).

Potassium Uptake and Translocation. The pattern and magnitude of inhibition of potassium uptake by ammonium were markedly different from that of nitrate uptake. Whereas ammonium inhibition of nitrate uptake rates by 5-d-old seedlings increased with time from initial exposure to nitrate (Fig. 1), maximal inhibition of potassium uptake generally occurred during the initial 2-h period (Fig. 7). At day 5, complete recovery in the rate of potassium uptake was achieved by the 6- to 8-h interval with all three genotypes. In contrast, at day 8, recovery from inhibition was not complete in any of the genotypes, and substantial inhibition of potassium uptake occurred during the 6 to 8-h interval (from 19% for genotype A to 56% for genotype

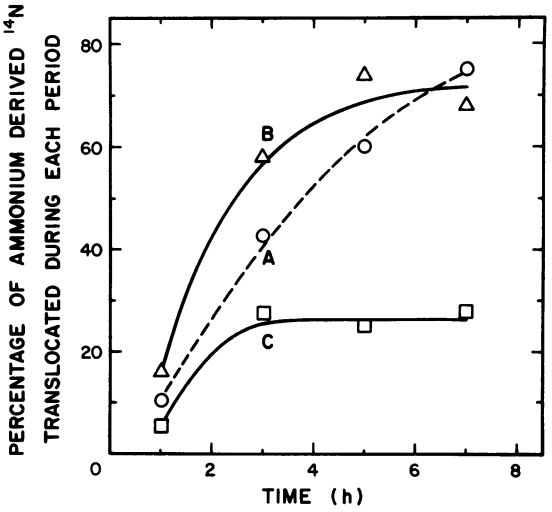

FIG. 6. Percentage of nitrogen absorbed as ammonium that was translocated during each 2-h interval by 8-d-old roots of three corn genotypes.

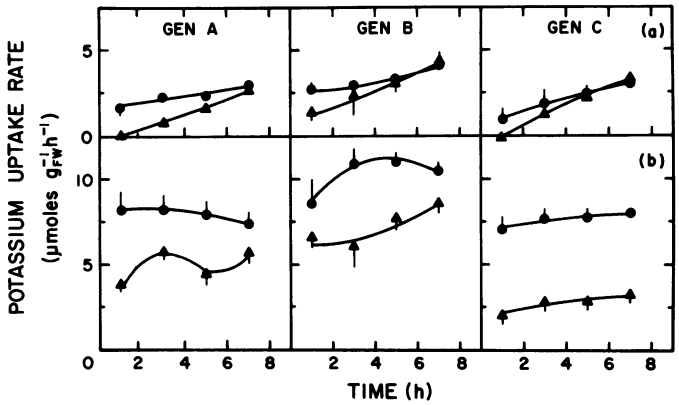

FIG. 7. Rates of potassium uptake (●, $-NH_4^+$; ▲, $+NH_4^+$) of (a) 5-d and (b) 8-d-old root systems of three corn genotypes. SE larger than the symbol size are represented by vertical lines.

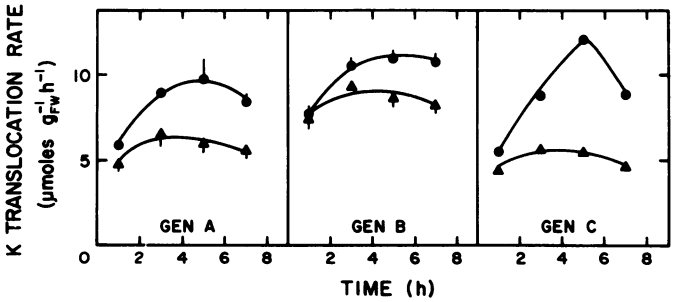

FIG. 8. Rates of potassium translocation (●, $-NH_4^+$; ▲, $+NH_4^+$) of 8-d-old root systems of three corn genotypes. SE larger than the symbol size are represented by vertical lines.

C). The strong degree of inhibition of uptake observed on day 8 for genotype C compared to the other two genotypes was associated with greater inhibition of potassium translocation (Fig. 8). In general, inhibition of potassium translocation by ammonium increased in severity with time through the first 4 to 6 h of exposure to ammonium.

DISCUSSION

Genotypic Differences in Nitrate Uptake and Reduction. Genotypic differences in nitrate uptake were not paralleled by corresponding differences in ammonium uptake (Table III). The contrast between the induction pattern of nitrate uptake *versus* the steady rates of ammonium uptake confirms the results of other investigations (14, 21). Thus, separate controls for the absorption of the two ions seem apparent, which indicates that genotypes may differ in their comparative response to nitrogen form as suggested by Harvey (7), and that these differences may in part be related to root characteristics.

Root morphological characteristics may play an important role of influencing genotypic differences in nitrate uptake and partitioning. The present study reveals a relationship between the partitioning of the entering nitrate to reduction and the number of lateral roots per unit mass when the data for the three genotypes at the two root ages are combined (Fig. 3). Presence of ammonium decreased the percentage reduction but did not significantly alter the slope of the regression. The number of lateral roots per unit mass indicates the number of root apices per unit mass. These data imply that greater *in vivo* nitrate reduction per unit mass may occur in root apical regions, which is indicated by observations that corn root tips maintain higher levels of *in vitro* nitrate reductase activity (16, 19) and free amino acids (19) than more mature root sections. Hence, nitrate partitioning may differ along the root axis, and younger regions of the root may reduce a higher proportion of incoming nitrate than older regions. This concept is also indicated by experiments conducted by MacKown *et al.* (15), where it was proposed that root cells may differ in their relative partitioning of nitrate, depending on their location in the root. The present investigation indicates genotypic propensity for lateral root proliferation may influence nitrate partitioning.

Ammonium Effects on Nitrate Uptake and Reduction. As observed in other investigations (14, 21), the inhibition of nitrate uptake by ammonium became more extensive with increasing time from initial exposure to nitrate (Fig. 1). Inhibition of nitrate uptake by ammonium was more severe in 8-d-old than in 5-d-old root systems (Fig. 1, a *versus* b), although ammonium to nitrate uptake ratios exhibited at the two root ages did not differ significantly (Table III). At day 5, ammonium inhibited nitrate-^{15}N incorporation into reduced fractions and total nitrate uptake without affecting nitrate accumulation (Table I). Thus, the inhibition is not envisioned to occur exclusively at a site of uptake that supplies nitrate for both accumulation and reduction. Instead, it appears that at day 5 ammonium affected nitrate reduction and/or the uptake system directly associated with it. Presence of ambient ammonium has been shown to inhibit nitrate reductase activity in roots estimated *in vitro*, which suggests a suppression of its synthesis during induction (14, 19).

In addition to possible direct effects on the synthesis or activation of nitrate reductase during induction, MacKown *et al.* (14) suggested that accumulated ammonium or end products of ammonium assimilation may limit the formation or activation of the uptake system. Furthermore, the capacity to translocate these potential inhibitors out of the root may influence the extent of inhibition (21). However, the present results generally are not supportive of the latter concept. On day 5, substantial quantities of ammonium and/or subsequent assimilatory products were accumulating in the root tissue, as indicated by significant rates of ammonium uptake (Fig. 5) and a general lack of translocation capacity at day 5 (Fig. 2). Accordingly, inhibition of nitrate uptake by ammonium would be expected to be severe at this stage of growth. However, inhibition of nitrate uptake in the 5-d-old roots was relatively slight (Fig. 1a) in direct contrast with the expectation. This does not preclude the possibility of an inverse relationship at 8 d between the extent of inhibition of nitrate uptake and the translocation of assimilatory products of ammonium (Table I; Fig. 6).

Although nitrate uptake per unit mass increased between 5

and 8 d, the enhanced uptake activity on day 8 was more sensitive to the presence of ammonium. A greater increase in the rate of uptake with time of exposure to nitrate accounted for a major proportion of the enhanced activity at day 8, and it was this accelerated phase of nitrate uptake during the 6- to 8-h period that was most affected by ammonium (Fig. 1). Furthermore, this increased inhibition at day 8 was not accompanied by a concomitant increase in the accumulation of ammonium-derived ^{14}N in the root tissue. The quantity of reduced ^{14}N derived from exogenous ammonium that accumulated in the root tissue did not substantially differ between the two root ages. At day 8, the difference between ammonium derived ^{14}N translocation (Fig. 4a) and ammonium uptake (Fig. 5) represents ^{14}N accumulation in the root, with genotypic values of 43.2 (A), 33.9 (B), and 54.2 (C) μmol g^{-1} fresh weight 8 h^{-1}. These quantities are similar in magnitude to cumulative ammonium uptake at day 5, which in the absence of translocation capacity, approximates the total ^{14}N accumulation in the root, during the uptake period with genotypic values of 40.5 (A), 37.9 (B), and 49.6 (C) μmol g^{-1} fresh weight 8 h^{-1}. Thus, the increase in ammonium uptake between 5- and 8-d-old seedlings (Fig. 5) was closely associated with greater ^{14}N translocation rather than a concomitant increase in ^{14}N accumulation in the root tissue.

At day 8, inhibition of nitrate reduction by ammonium was approximately 50% of control (Table I). Nitrate reduction was the partitioning process most highly sensitive to the presence of ammonium, as has been reported previously (13, 14, 19). In addition, the present results are in accord with these previous investigations in regard to the effects of ammonium on the partitioning of reduced nitrogen. Incorporation of nitrate-derived nitrogen into the insoluble reduced fraction was more severely inhibited (58–61% at day 5, 67–75% at day 8) than was incorporation into soluble reduced nitrogen fractions (Table II).

Potassium as Intermediary Regulator of Ammonium Effects. In support of a potassium-mediated inhibition of nitrate uptake by ammonium, the genotypic rankings of the magnitude of inhibition of potassium uptake and translocation by ammonium on day 8 were similar to the rankings of inhibition of nitrate accumulation and translocation. Ambient ammonium exerted the least effect on genotype B in potassium uptake (−29%), potassium translocation (−16%), nitrate uptake (−20%), nitrate translocation (no effect), and nitrate accumulation (−13%). In contrast, genotype C exhibited severe inhibition of potassium uptake (−69%) and translocation (−42%), nitrate uptake (−37%), translocation (−30%), and accumulation (−30%). Nitrate reduction, however, was inhibited by ammonium to the same degree among all three genotypes (−50 to 52%). Furthermore, the temporal patterns of inhibition, whereby potassium uptake was affected prior to inhibition of nitrate uptake (Figs. 1 and 7), may reflect a role of potassium as an intermediary regulator.

Nitrate accumulation and translocation by corn roots are decreased in the absence of potassium (21). The presence of either exogenous or endogenous potassium serves as a counter-ion for deposition of nitrate into vacuoles and xylem vessels (1). In contrast, although ammonium enters the root symplasm in cationic form, a major portion is immediately assimilated into glutamine, the major organic form of translocated nitrogen in corn xylem fluid (9). The isoionic point of glutamine (pI = 5.65) corresponds to xylem exudate pH range in corn roots (2), and would therefore by expected to be transported as a neutral molecule. Therefore, there is a loss of counter-ion balance for nitrate transport during ammonium inhibition of potassium uptake.

Thus, the incomplete recovery in potassium uptake during exposure to ammonium in the 8-d-old seedlings may be directly responsible for the increase in ammonium inhibition of nitrate uptake and partitioning relative to the response at day 5 (Table I).

CONCLUSIONS

In the virtual absence of translocation capacity, 5-d-old seedlings exhibited lower nitrate uptake, reduction, and translocation in comparison with 8-d-old seedlings when expressed on a root mass basis. Although 5-d-old seedlings lacked the capacity to translocate nitrogen assimilates out of the root tissue, nitrate uptake was only slightly inhibited by ambient ammonium. The increased efficiency in nitrate uptake between days 5 and 8 was associated with an increased sensitivity to the presence of ammonium, despite the development of translocation capacity enabling the movement of reduced nitrogen out of the root tissue. Therefore, the data indicate that the extent of translocation of ammonium assimilates out of the root tissue was not an influential factor in determining the difference in the magnitude of inhibition of nitrate uptake by ammonium between 5 and 8 d.

The development of increased nitrate uptake capability with root age may indicate greater efficiency of an uptake system that increases with morphological differentiation and lateral root development. Partitioning of nitrate into the reduction process was positively correlated with the extent of lateral root differentiation among genotypes and stages of development, suggesting meristematic regions of lateral roots may be the site of a major portion of nitrate reduction in root systems of corn. Genotypic differences in nitrate reduction are explained, in part, by differences in lateral root proliferaiton.

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