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FINAL REPORT

UNITED STATES ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
CONTRACT NO. E-(40-1)-2410

FOR THE PERIOD: 1 AUGUST 1973 - 31 DECEMBER 1976

TITLE: NITRATE ABSORBTION AND STRONTIUM ACCUMULATION

DEPARTMENT OF SOIL SCIENCE
NORTH CAROLINA STATE UNIVERSITY

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W. A. Jackson, Principal Investigator
R. G. Butz, Research Assistant
P. B. Kologiski, Research Technician

II. MANUSCRIPTS AND REPORTS

1. <u>Introduction</u>

The list below includes material associated with work reported since initiation of the present contract in 1973. Some of the publications are of experimental work largely completed under the objectives of the contract prior to that time. The papers presented in early 1977 (see II 3d and e) relied heavily on work conducted under the present contract.

2. <u>Ph.D. Theses Completed</u>

a. Tompkins G. A. September, 1973. Regulation of nitrate uptake in nitrogen-depleted and ammonium-grown wheat seedlings with regard to the causative factors for the inducation phase pattern of initial nitrate uptake. Under the Direction of W. A. Jackson.

b. Ezeta, F. N. January, 1974. Translocation of nitrate and organic nitrogen by dark-grown corn seedlings. Under the Direction of W. A. Jackson.

3. <u>Papers Presented at Scientific Meetings</u>

a. Volk, R. J. and W. A. Jackson. Nitrate uptake by corn seedlings: inhibition by mercury and cadmium. Presented to Division C-2, American Society of Agronomy, Las Vegas, Nevada, November, 1973.

b. Butz, R. G., W. A. Jackson and R. J. Volk. Effects of ammonium on nitrate uptake and reduction by corn roots. Presented to Division C-2, American Society of Agronomy, Knoxville, Tennessee, August, 1975.

c. Jackson, W. A., R. J. Volk and R. G. Butz. The nitrate assimilation pathway in higher plants. Presented to the Symposium on Plant Growth Regulation-Part II. Nitrogen Fixation and Nitrogen Metabolism at The First Chemical Congress of the North American Continent, Mexico City, December, 1976.

d. Jackson, W. A. Efficiency of transport and utilization of nutrients. Presented to the Gordon Research Conference on Agricultural Science, Santa Barbara, California, January, 1977.

e. Jackson, W. A. A critique of nitrate acquisition and assimilation by higher plants. Presented to the Kearney Foundation Workshop Retreat, Lake Arrowhead, California, February, 1977.

4. Manuscripts Published

a. ORO-2410-22. Morgan, M. A., R. J. Volk and W. A. Jackson. 1973. Simultaneous influx and efflux of nitrate during uptake by perennial ryegrass. Plant Physiol. 51:267-272.

b. ORO-2410-23. Jackson, W. A., Donna Flesher and R. H. Hageman. 1973. Nitrate uptake by dark-grown corn seedlings: Some characteristics of apparent induction. Plant Physiol. 51:120-127.

c. ORO-2410-26. Jackson, W. A., R. E. Johnson and R. J. Volk. 1974. Nitrite uptake by nitrogen-depleted wheat seedlings. Physiol. Plant. 32: 37-42.

d. ORO-2410-27 Jackson, W. A., R. E. Johnson and R. J. Volk. 1974. Nitrite uptake patterns in wheat seedlings as influenced by nitrate and ammonium. Physiol. Plant. 32:108-114.

e. ORO-2410-28. Ezeta, F. N. and W. A. Jackson. 1975. Nitrate translocation by detopped corn seedlings. Plant Physiol. 56:148-156.

f. ORO-2410-30. Ashley, Doyle A., W. A. Jackson and R. J. Volk. 1975. Nitrate uptake and assimilation by wheat seedlings during initial exposure to nitrate. Plant Physiol. 55:1102-1106.

g. ORO-2410-32. Jackson, W. A., K. D. Kwik and R. J. Volk. 1976. Nitrate uptake during recovery from nitrogen deficiency. Physiol. Plant. 36:174-181.

h. ORO-2410-34. Morgan, M. A. and W. A. Jackson. 1976. Calcium and magnesium in ryegrass: some differences in accumulation by roots and in translocation to shoots. Plant and Soil. 44:623-637.

i. ORO-2410-35. Jackson, W. A., B. D. Knezek and J. van Schilfgaarde. 1976. Water, Soil and Mineral Input. In: Crop Productivity-Research Imperatives. pp. 201-274. Michigan Agricultural Experiment Station and Charles F. Kettering Foundation.

j. ORO-2410-36. Jackson, W. A., K. D. Kwik, R. J. Volk and R. G. Butz. 1976. Nitrate influx and efflux by intact wheat seedlings. Planta (Berl.) 132:149-156.

5. Manuscripts in Press

a. ORO-2410-37. Butz, R. G. and W. A. Jackson. 1977. A mechanism for nitrate transport and reduction. Phytochemistry.

b. ORO-2410-38. Jackson, W. A. 1977. Nitrate acquisition and assimilation by higher plants: processes in the root system. In "Nitrogen in Relation to Environmental Quality" (D. R. Nielsen, Ed.), Academic Press, New York.

6. Manuscripts in Preparation

a. ORO-2410-3[illegible]. Tompkins, G. A., W. A. Jackson and R. J. Volk. Accelerated nitrate uptake in wheat seedlings: effects of ammonium and nitrate pretreatments and of 6-methylpurine and puromycin. To be submitted to Physiologia Plantarum.

b. ORO-2410-40. Munn, D. A. and W. A. Jackson. Nitrate and ammonium uptake by rooted cuttings of sweet potato (*Ipomoea batatas* Lam). To be submitted to Agronomy Journal.

III. PROGRESS UNDER THE OBJECTIVES

1. Introduction

Two basic objectives were set out in the Research Proposal submitted to ERDA in April, 1973. These were (A) to determine how strontium translocation, and discrimination between strontium and calcium, are influenced by nitrate uptake, assimilation, and translocation, and (B) to characterize the relationships between nitrate uptake, nitrate assimilation and nitrate translocation. Subsidiary objectives were set out in the two Renewal Requests of April, 1974 and 1975. Part of our progress in attaining these objectives is indicated in reprints of published manuscripts, preprints of manuscripts in press or in preparation, and abstracts of papers presented at scientific meetings which accompany this report. Little of the subject matter covered by these documents will be repeated here. Instead, the following sections summarize studies under the contract which have not yet been prepared for publication. In some instances, no further experimentation seems to be required for publication; in others, analyses have not been completed, or further experimentation is necessary to confirm the findings. After manuscripts from these studies have been prepared, they will be forwarded to ERDA. Unless otherwise indicated, all experiments were conducted with corn (DeKalb XL-45) seedlings grown in darkness at 30C for five days. They were decapitated, leaving the seed piece attached, immediately prior to the experimental period. This system permitted uptake to be measured by monitoring the ambient solution while translocation could be monitored simultaneously by analysis of the xylem exudate. Leaving the seed attached resulted in the roots being well nourished from the endosperm throughout the experimental period.

2. The Influence of Nitrate on Discrimination between Strontium and Calcium in Root Systems

Previous experiments with wheat revealed that enhanced uptake of strontium, and translocation of it to shoots, was associated with rapid nitrate uptake. Translocation of previously absorbed strontium, and that absorbed concurrently with nitrate, were both enhanced. Discrimination against strontium relative to calcium occurred during translocation, and this discrimination was greater during the more rapid translocation induced by presence of nitrate. The present experiments, conducted with decapitated dark-grown corn seedlings, revealed the presence of pools in the root tissue which were differentially enriched in Sr-85 relative to Ca-45. Exposure to nitrate modified the proportions of the two ions in each of the pools and the rate of transfer between pools.

Nitrate uptake by the corn seedlings is initially slow and then increases in rate. During the initial slow phase of nitrate uptake, Sr-85 was taken up preferentially relative to Ca-45. However, after the onset of the accelerated rate of nitrate uptake, the rate of Ca-45 uptake increased without a comparable increase in Sr-85 uptake. Thus, there was a change from discrimination in favor of Sr-85 uptake to discrimination against it, the change being associated with the development of accelerated nitrate uptake. In the presence of chloride, there was little indication of discrimination for or against Sr-85 during uptake. Presence of ambient mannitol, which decreased the water flux through the root system, countered the tendency for discrimination against Sr-85 uptake during the accelerated phase of nitrate uptake; this effect resulted from mannitol restricting the increase in Ca-45 uptake and increasing slightly the Sr-85 uptake.

The ratio [Sr-85/Ca-45] in the xylem exudate was less than that of the external solution, the root tissue, or the uptake rates. The data clearly indicate discrimination against Sr-85 in the material translocated in the xylem. Presence of nitrate enhanced the translocation rate of both ions with only a slight effect of increasing the discrimination against Sr-85 relative to the composition of the external medium. A positive linear relationship between deposition of Sr-85 and (Ca-45) in the xylem exudate and water flux through the root system was evident from a combination of ambient chloride, nitrate, and mannitol treatments; extrapolation to zero water flux revealed a positive translocation of both ions. The data suggest two translocation processes, one of which was dependent on water flux.

Differences in the relative distribution of Sr-85 and Ca-45 within the root tissue were revealed by efflux analysis and by the composition of the xylem exudate after transfer to unlabeled solutions. Quantities displaced initially from roots to ambient solution were more enriched in Sr-85 relative to Ca-45 then was the bulk root tissue, and the xylem exudate was less enriched. Presence of nitrate in the unlabeled ambient solution increased the deposition of both Sr-85 and Ca-45 in the xylem exudate. After three hours the discrimination against Sr-85 in the exudate became less pronounced, indicating transfer of a small quantity from a more enriched Sr-85 pool to the translocation pool. The material displaced to the unlabeled medium became progressively enriched with Sr-85, with nitrate treatment resulting in consistently larger Sr-85 enrichment. The data are interpreted as indicating a relatively remote pool in corn roots which becomes highly enriched in Sr-85, relative to the immediately replaceable pool, and a separate translocation pool in which significant discrimination against Sr-85 occurs. Movement from the translocation pool into the xylem is suggested to be enhanced by presence of nitrate, possibly as a consequence of increased water flux. Nitrate also is visualized as enhancing deposition into the remote, highly Sr-85 enriched, pool. Movement out of this pool can occur to the immediately replaceable pool and to the translocation pool with presence of nitrate facilitating movement in both directions.

3. Effects of Ambient Ammonium on the Nitrate Assimilation Pathway of Roots

When the corn seedlings had not been exposed previously to nitrate, presence of ambient ammonium restricted nitrate uptake in four separate experiments. The experiments differed in the pretreatment conditions to which the seedlings were exposed and in the composition of the nutrient solution during the uptake period. In each instance, equimolar ammonium curtailed development of the accelerated phase of nitrate uptake with no detectable influence on the slow initial phase. The effect of ambient ammonium on the other components of the nitrate assimilation pathway were not delineated so clearly. With plants pretreated with ammonium, there was a decrease in the proportion of the absorbed nitrate which was reduced in the concurrent presence of ammonium, with a corresponding increase in the proportion which was translocated. In another experiment, with plants not previously exposed to ammonium, the effect was exerted solely through the restriction in nitrate uptake; the proportion of the absorbed nitrate utilized for reduction and translocation was not measureably affected.

A third experiment employed no ammonium pretreatments, and was conducted with plants previously decapitated. The experiment also involved exposure to a complete nutrient solution during the uptake period rather than the minimal two salt solutions previously used. After about six hours an increase in the proportion of the absorbed nitrate which was translocated, and a decrease in the proportion reduced, were detectable when ambient ammonium was present. A restriction in root nitrate reductase activity (in vitro) was clearly evident within two hours. Failure to detect a significant influence on the quantities reduced as soon as nitrate reductase activity was affected could have been owing to the greater variability in the uptake and reduction measurement than in the nitrate reductase assays. Alternatively, sufficient nitrate reductase activity may have been induced even in the presence of ambient ammonium to sustain the observed rate of reduction occurring at the prevailing uptake rates. In some experiments, ambient ammonium resulted in a slight increase in accumulation of nitrate in the root tissue, but the effect was always small.

Transfer of fully-induced plants to nitrate-free media resulted in a rapid decline in *in vitro* nitrate reductase activity within the first hour in spite of substantial quantities of nitrate being present in the tissue. Presence of ambient ammonium had little influence on this immediate rapid decay. However, it did accelerate slightly the subsequent slower rate of decay, and the rate of depletion of root tissue nitrate was slightly restricted during this time. Overall, the data indicate that the dominant influence of ambient ammonium was to restrict the nitrate uptake rate, with the subsequent reactions of the nitrate assimilation pathway being altered as a result of the lowered input of nitrate into the tissue. An additional influence of ammonium in restricting net synthesis of nitrate reductase activity independently of an influence in restricting uptake also is indicated by the data, but at least initially, this effect appeared to be relatively minor.

4. Effects of Chloride on the Nitrate Assimilation Pathway of Roots

We had observed previously that presence of ambient chloride restricted nitrate translocation but had less of an effect on nitrate uptake. In contrast, presence of ambient nitrate restricted chloride uptake (the effect being noted only after the onset of the accelerated phase of nitrate uptake), but there was little influence on chloride translocation. Hence, the proportion of the absorbed chloride which was translocated was increased significantly. Others have reported that previously accumulated chloride and nitrate in root tissue served equally well to restrict uptake of either ion. Further experiments were therefore conducted to clarify the influence of chloride on the various nitrate assimilation pathway processes. In order to examine further the interaction between these two ions, additional experiments were conducted to determine the influence of endogenous as well as exogenous nitrate on chloride uptake and translocation.

A specific influence of previously accumulated chloride in restricting nitrate translocation was observed in three separate experiments. Nitrate uptake was less restricted than translocation, and nitrate reduction was affected least. The decreases in nitrate translocation occurring with increasing levels of accumulated chloride did not result from equivalent replacement of chloride for nitrate in the xylem fluid. At low levels, increasing rates of chloride translocation had relatively little effect in restricting nitrate translocation. On the other hand, pretreatments resulting in large accumulation of chloride in root tissue caused a greater restriction in nitrate translocation than could be accounted for by the increased chloride translocation rates. The data cannot be explained by competitive effects between the two ions in the xylem deposition process. A relatively specific effect of high endogenous chloride concentrations on nitrate translocation, in addition in its effect on restricting nitrate uptake, is indicated.

Exposure of plants to solutions labeled with chloride-36 resulted in the specific activity of the xylem exudate increasing to 90% of the ambient solution within three hours after which it remained at that level. Chloride translocation rates decreased rapidly upon transfer to chloride-free media. Thus, chloride translocation is closely coupled to concomitant uptake as is also true for nitrate. Pretreatments resulting in moderate levels of root nitrate resulted in an enhancement in chloride translocation, especially during the first few hours after transfer to chloride solutions. Experiments with chloride-36 revealed that translocation of endogenous chloride was stimulated more than that of exogenous chloride. An enhancement in chloride uptake also occurred under these conditions, but it was not as great as the increase in translocation of the entering chloride. In contrast to the stimulation at moderate levels of accumulated nitrate, high root nitrate concentrations resulted in restrictions in subsequent uptake and translocation of chloride. Translocation was not so adversely affected as uptake. Whether restrictions or enhancement of chloride uptake and translocation occurs therefore depends upon the level of previous nitrate nutrition; it cannot be concluded than these processes will invariably be curtailed. Moreover, the relative extent to which each process is affected will differ according to the nitrate status of the plants. The data support the concept that activity of the nitrate assimilation pathway in roots can markedly affect those processes responsible for the uptake, accumulation, and translocation of other inorganic ions.

5. Involvement of Malate in the Nitrate Assimilation Pathway of Root Systems

Differential cation and anion uptake induces changes in cytoplasmic acidity which in turn results in net synthesis (during excess cation uptake) or consumption (during excess anion uptake) of organic acids. Nitrate reduction, which results in hydroxyl ion generation in the cytoplasm, also promotes net synthesis of organic acids. In corn roots, as in most others, changes in malate concentrations largely account for the organic acid responses. In addition to these effects, resulting from the relative activities of the uptake and reduction components of the nitrate assimilation pathway, it has been proposed that malate decarboxylation contributes to nitrate uptake by providing bicarbonate ions for counter-transport. It has also been suggested that high tissue malate may tend to suppress nitrate uptake. Accordingly, a series of experiments were conducted to examine the involvement of malate in the nitrate assimilation pathway. Attention was directed specifically to its participation in xylem deposition, as well as to nitrate uptake and reduction.

Pretreatment with $KHCO_3$ substantially increased root malate concentrations but, aside from a slight suppression during the initial hour, there was little effect upon subsequent nitrate uptake. During exposure to nitrate, the root malate concentration declined in those plants pretreated with $KHCO_3$ whereas, in those pretreated with KCl or $CaCl_2$, the concentration remained constant or increased slightly. During exposure to $CaCl_2$, water flux through the roots, and malate deposition in the xylem both declined. Upon transfer to KNO_3, both malate deposition and water flux increased. Analysis of the tissue and exudate fluid revealed significant net malate synthesis during exposure to nitrate regardless of pretreatment. With all experimental conditions employed, most of the increase in malate was recovered in the xylem exudate of which it constituted a significant proportion of the total anions. During exposure to nitrate, the rising nitrate deposition in the exudate was associated with a decrease in malate deposition in those plants initially containing high root malate, and with decreasing chloride deposition in those plants initially containing high root chloride. Although specific competitive effects did not occur, fairly easy replacement among the three anions apparently can take place during xylem deposition.

Pretreatments with 0.5 and 5m *M* KNO_3 enhanced subsequent malate translocation. This was associated with a stimulation in water flux resulting from the nitrate pretreatments. In addition to its sensitivity in response to nitrate treatments, malate translocation was also influenced greatly by other modifications in nitrogen metabolism. It was depressed rapidly in the presence of ambient ammonium and the effect was evident before nitrate uptake or translocation were restricted. In contrast, malate translocation was enhanced by exposure to nitrite after the plants had recovered from the temporary shock resulting from initial exposure to this ion. With the nitrite treatment, sizeable increases in root tissue malate occurred, and malate was the most dominant anionic component of the xylem fluid. Moreover, there was a very close association between malate and potassium translocation during the shock period as well as during recovery from it. The evidence, in general, indicates a capability of the corn root system to respond sensitively to ambient conditions by net synthesis or consumption of malate, and by deposition of malate in the xylem. There was little evidence from these experiments, however, to support the concept that nitrate uptake was dependent upon malate decarboxylation, or that malate accumulation suppressed nitrate uptake significantly.

6. Translocation of Reduced Nitrogen: The Effect of Nitrate

When the corn seedlings were exposed to KNO_3, there was an increase in the rate of appearance of reduced nitrogen in the xylem fluid and a corresponding increase in the exudation rate relative to the rates which occurred upon exposure to KCl. The effects were consistently observed within three or four hours. The changes with time in the exudation rates and in the reduced nitrogen translocation rates were highly correlated (usually $r \sim 0.90$) in a number of experiments implying that the increased deposition of reduced nitrogen in the xylem associated with nitrate treatment was responsible for the increased water flux. Experiments with ^{15}N-labeled nitrate were conducted to determine the proportion of the reduced nitrogen in the xylem fluid which resulted from reduction of the entering nitrate. In one experiment only 2%, and in another experiment only 12%, of the reduced nitrogen could be accounted for as having originated from the entering nitrate, and both values are too low to account for the stimulation resulting from KNO_3 treatment. The methodology employed in these studies needs to be verified and additional experiments conducted to confirm the results. Nevertheless, the present data indicate that part of the increase in reduced nitrogen translocation upon exposure to nitrate resulted from either (a) enhanced turnover of endogenous proteins in root or endosperm, or (b) enhanced rates of those processes involved in reduced nitrogen deposition in the xylem conduits. That the impact of nitrate on these events is substantial is revealed by calculations from four experiments which show that the sum of the nitrate translocated and the increase in reduced nitrogen translocated ranged from 60 to 90% of the nitrate uptake rate after about five hours. How nitrate specifically exerts its effect in stimulating translocation of reduced nitrogen remains to be determined.

7. Nitrate Reduction in Scutella

We have interpreted our experimental results by assuming that all of the observed reduction of nitrate occurs in the root system. However, sizeable nitrate reductase activity is induced in the scutella of the seedlings when the root system is exposed to nitrate. But very little nitrate accumulation is associated with the increase in nitrate reductase activity. These observations may be interpreted as indicating (a) substantial nitrate reduction occurs in the scutella with the nitrate being reduced nearly as rapidly as it enters the tissue, or (b) relatively little nitrate enters, net synthesis of nitrate reductase is initiated effectively, but little nitrate is reduced. Since the proper interpretation of many of our experiments depends upon which of these two possibilities is correct, attempts were made to determine the capacity of scutella to reduce nitrate.

Exposure of roots to ^{15}N-labeled nitrate resulted in only a very small incorporation of ^{15}N into alcohol-insoluble nitrogenous compounds of the scutella compared to that which occurred in root tissue. This observation did not preclude the possibility of substantial reduction occurring in the scutella with transport of the soluble reduced nitrogen out of it to the xylem. Accordingly, scutella were excised from the plants and exposed directly to ^{15}N-labeled nitrate. Two concentrations, 0.5 m*M* and 15 m*M* were employed. The latter concentration approximates that of the xylary fluid of decapitated seedlings after about five hours exposure to nitrate. Triplicate samples were removed after 4, 8, and 12 hours and analyzed for nitrate and presence of ^{15}N in soluble and insoluble forms. Nitrate uptake from both solutions showed the pattern of apparent induction observed wit' root tissue of corn and numerous other species. Reduction was very effective with percentage reduction increasing progressivley with time to 74% with 0.5 m$\underline{M}$ and to 94% with 15 m$\underline{M}$ $K^{15}NO_3$ at 12 hours. Most of the reduced nitrogen accumulated in the alcohol-soluble fraction. Even after the system had been fully induced, however, the quantity of nitrate reduced per scutella was relatively small. In the 15 m$\underline{M}$ treatment, the reduction rate during the 8 to 12 hour period was 2.2 ug ^{15}N hr^{-1} for a single scutella. This is a minimal value because some of the soluble reduced ^{15}N could have been excreted to the ambient medium and been undetected. However, the quantity reduced by a decapitated seedling is usually more than 10-fold the rate of accumulation of reduced ^{15}N by the excised scutella. Apparently at least 90% of the reduction occurs in the root system, although that nitrate which does reach the scutella is reduced effectively. The results indicate our previous assumption, that most of the observed reduction occurs in the root system, is reasonably valid.

8. Influence of Substances from Other Tissue on the Nitrate Assimilation Pathway of Roots

Removal of endosperm at the time seedlings were first exposed to nitrate resulted in a severe restriction in nitrate reduction during the subsequent 10 hours. Development of the accelerated phase of nitrate uptake was initiated but was not sustained; after six hours the rate was restricted. Translocation of reduced nitrogen was restricted sooner than uptake but translocation of nitrate was not. Seedlings which had been pretreated with nitrate for 20 hours prior to endosperm removal also responded with restricted uptake and reduction. Under these conditions, uptake was restricted by the fourth hour and less than half as much nitrate was reduced during the 10 hours after the endosperm was removed. The data indicate that nitrate reduction was the nitrate assimilation pathway process most severely affected by endosperm removal.

There was a rapid decline in the soluble carbohydrates of the roots following endosperm removal. Following this time, however, there was only a slow further decline even though the roots contained 65% of their initial soluble carbohydrate concentrations at the onset of the slow phase. The data may be interpreted as indicating (a) at least two soluble carbohydrate pools exist in the roots, one of which is readily available for maintenance of nitrate uptake and reduction, or (b) transport of other components, possible hormonal substances, into the roots is also required to sustain these processes.

Excision of the shoots resulted in increased root growth rates upon subsequent exposure to nitrate. Analysis of all plants parts revealed that translocation of carbohydrates to the root system was increased by shoot excision although total mobilization of endosperm carbohydrates was curtailed. Hence this experimental manipulation provided a direct contrast to that of endosperm removal in the supply of materials to the root system. During exposure to nitrate, seedlings with shoots excised reduced twice as much nitrate as those with shoots intact (in darkness). Nitrate uptake and translocation were increased 1.4 and 1.2-fold, respectively, by shoot excision. Accumulation of malate by the seedlings was more than doubled by shoot excision, as was incorporation of ^{15}N from applied nitrate into proteins of the root tissue. The experiments indicate that growth and activity of the nitrate assimilation pathway of root systems are closely associated, and that they both are closely dependent upon a supply of materials from outside the root system.

IV. SUMMARY

Loading and displacement studies with decapitated corn seedlings revealed that the easily replaceable fraction of the root tissue was less enriched in Sr-85 relative to Ca-45 than the slowly replaceable pool. The pool used for translocation to the xylem, however, exhibited discrimination against Sr-85. Presence of ambient nitrate

enhanced Ca-45 uptake relative to Sr-85, and increased deposition into the highly enriched slowly replaceable pool. Nitrate also increased movement of Sr-85 and Ca-45 into the translocation pool, possibly through its effect in increasing the water flux through the root system.

During 6-8 hours following initial exposure to nitrate, the decapitated corn seedling consistently reduced 35-45% of the nitrate which was absorbed. Experiments involving exposure of excised scutella to ^{15}N-nitrate indicated that the majority of reduction occurred in the root system. Parallel increases in nitrate uptake rates and in vitro nitrate reductase activities, the relatively consistent proportion of the entering nitrate which was reduced, and various observations in the literature, were used by R. G. Butz to formulate a model which visualizes nitrate reductase to be a transmembrane tetramer with associated ATPases which spand the plasmalemma and serves a transport as well as a reduction function. Variations in the proportion of entering nitrate which is reduced was postulated to result from a thiol-reversible inhibition by ADP.

During exposure to nitrate or nitrite, corn seedlings synthesized substantial quantities of malate, much of it appearing in the xylem exudate. No evidence was obtained to indicate that nitrate uptake was dependent upon malate decarboxylation.

When corn seedlings previously exposed to nitrate were transferred to nitrate-free media, there was a rapid decline in their root in vitro nitrate reductase activity and in the nitrate translocation rate. Moreover, the quantity of nitrate reduced was relatively small during the period in nitrate-free media in spite of sizeable amounts of tissue nitrate. The data support the concept that much of the accumulated nitrate was present in a storage pool and not readily available as subtrate for reduction or translocation.

Presence of ambient ammonium restricted induction of nitrate uptake activity and induction of in vitro nitrate reductase activity. In some experiments, nitrate reduction was more severely restricted than nitrate uptake, whereas nitrate translocation was less restricted. With rooted cuttings of sweet potato, the restriction in nitrate uptake resulting from a low concentration of ambient ammonium was not accentuated by an increased ammonium concentration with a resulting doubling of ammonium uptake. In this system, nitrate uptake was enhanced by increases in acidity from pH 6.5 to pH 4.5 while ammonium uptake was restricted.

Previously accumulated chloride restricted nitrate translocation more than uptake by the decapitated corn seedlings but the effect on translocation was not exerted through exact replacement of chloride for nitrate during deposition in the xylem. Previously accumulated nitrate at moderate levels resulted in a stimulation in the translocation of chloride; high internal nitrate concentrations restricted both uptake and translocation of chloride.

Anaerobiosis, low or high temperatures, and presence of 6-methylpurine or tungstate restricted nitrate translocation more than uptake. Exposure to nitrate enhanced the deposition of reduced nitrogen in the xylem exudate and concomitantly increased water flux through the roots. Experiments with ^{15}N-nitrate indicated that at least part of the increased flux of reduced nitrogen resulted from enhanced mobilization of endogenous nitrogen of roots or endosperm. Some of the influence of nitrate on translocation of other inorganic ions may be a consequence of the increased water flux which is presumably an osmotic adjustment to the increased deposition of reduced nitrogen in the xylem.

Experiments involving endosperm removal and decapitation indicated a close dependency of nitrate uptake and reduction to transport of sucrose and possibly hormonal substances into the root system. Nitrate reduction was more sensitive to the supply of these materials than was nitrate uptake.

Taken as a whole the studies have indicated that the nitrate assimilation pathway in root systems is extremely responsive to the composition of the soil solution and to the flow of materials from other plant tissues into the root system. The component processes are highly interdependent, but the impact of certain of the factors which regulate the pathway can have a relatively greater influence on one reaction than on others. Further, the activity of the pathway can have an impact on the accumulation and distribution of other inorganic ions by higher plants.