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# Nitrate Influx and Efflux by Intact Wheat Seedlings: Effects of Prior Nitrate Nutrition*

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**Summary.** Wheat (*Triticum vulgare* L., cv. Blueboy) seedlings, grown with 0.25, 1.0 and 15 mM nitrate in complete nutrient solutions, were transferred 10 days after germination to 1.0 mM $K^{15}NO_3$ (~99 A% $^{15}N$) plus 0.1 mM $CaSO_4$ at pH 6.0. The solutions were replaced periodically over a 6-h period (5 mW $cm^{-2}$; 23°). Changes in the [$^{15}$N]- and [$^{14}$N]nitrate in the solution were determined by nitrate reductase and mass-spectrometric procedures and potassium by flame photometry. Influx of [$^{15}$N]nitrate was depressed in plants grown at 1.0 mM nitrate relative to those grown at 0.25 mM, but there was no appreciably difference in [$^{14}$N]nitrate efflux. Prior growth at 15 mM further restricted [$^{15}$N]nitrate influx which, together with a substantial increase in [$^{14}$N]nitrate efflux, resulted in no net nitrate uptake during the course of the experiment. Efflux of [$^{14}$N]nitrate occurred to solutions containing no nitrate but it was significantly enhanced upon exposure to [$^{15}$N]nitrate in the external solution. Influx of [$^{15}$N]nitrate was more restricted at 5°, relative to 23°, than was [$^{14}$N]nitrate efflux. The nitrate concentrations of the root tissue immediately before exposure to the $K^{15}NO_3$ solutions did not give a precise indication of the subsequent [$^{15}$N]nitrate influx rates nor of the [$^{14}$N]nitrate efflux rates. Net $K^+$ uptake was related to the magnitude of the net nitrate uptake, not to the initial $K^+$ concentration in the roots. The data are interpreted as indicating that [$^{15}$N]nitrate influx and [$^{14}$N]nitrate efflux are largely independent processes, subject to different controls, and that net nitrate uptake provides the driving force for net potassium uptake.

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## Introduction

The net rate of nitrate uptake by intact plants is the resultant of an influx and an efflux process across the plasmalemma of root cells (Morgan et al., 1973). That efflux can be a significant component of the uptake rate is indicated by nitrate efflux to the ambient solution when plants, previously grown with nitrate, were placed in a nitrate-free medium (Minotti and Jackson, 1970), and by efflux of previously absorbed [$^{14}$N]nitrate when plants were placed in solutions containing highly enriched [$^{15}$N]nitrate (Morgan et al., 1973). Efflux of previously absorbed [$^{15}$N]nitrate to [$^{14}$N]nitrate solutions has also been demonstrated (Morgan et al., 1973; Ashley et al., 1975). Influx of nitrate takes place against a steep electrochemical gradient (Higinbotham et al., 1967; Higinbotham, 1973). It is dependent on aerobic metabolism and may require continual protein synthesis (Jackson et al., 1973; Ezeta and Jackson, 1975).

In an intact, vigorously-growing plant, substantial net nitrate uptake must occur in order to sustain the required rates of translocation of nitrate (or its reduction products) to the shoots for them to function effectively. However, little is known about the manner in which the influx and efflux rates are controlled. Among those factors which may be involved are the nitrate, or the nitrate plus chloride, concentration of root cells (Cram, 1973; Smith, 1973), the carbohydrate concentration in these cells (Pitman et al., 1971; Minotti and Jackson, 1970), the rate of photosynthate translocation into the roots (Jackson et al., 1976), and the effects of growth regulators (Pitman and Cram, 1973; Collins and Kerrigan, 1974; Pitman et al., 1974). The present investigation was initiated to examine the extent to which prior exposure to different nitrate concentrations modified the subsequent nitrate influx and efflux rates in wheat.

## Materials and Methods

### *Growth of Plants*

Wheat (*Triticum vulgare* L. cv. Blueboy) seed, supplied by R.W. McMillen, North Carolina Foundation Seed Producers, Raleigh, N.C., were germinated in darkness for 3 days in 0.1 mM $CaSO_4$. The roots of six seedlings (one culture) were then threaded through stainless-steel screen held in the bottom of No. 7 hollow polyethylene stoppers. Thirty such cultures were placed in 15-l tanks containing solutions at pH 6.0 containing 1.0 mM $MgSO_4$, 0.5 mM $Ca(H_2PO_4)_2$, 0.1 mM KCl, and $NaNO_3$ at 0.25 to 15 mM (see figure headings for each experiment). The solutions also contained trace elements at 1/5 those of Hoagland's solution (Hoagland and Arnon, 1950) and FeEDTA at 1 mg/l Fe. The seedlings were grown for an additional 7 days at $21 \pm 1°$ during the 16-h light period (5 mW $cm^{-2}$) and $16 \pm 1°$ during the 8-h dark period. The solutions were aerated continuously, adjusted after 24 h to pH 6.0 with dilute $H_2SO_4$ or NaOH, and replaced every 48 h.

### *Experimental Period*

On the 10th day after germination, 3 h after the start of the light period, cultures were removed from the growth tanks and their roots rinsed thoroughly in distilled water. The experimental treatments usually consisted of 1.0 mM $K^{15}NO_3$ (~99 A% $^{15}N$) plus 0.1 mM $CaSO_4$, pH 6.0, although other $K^+$ salts were employed in some instances (e.g. Figs. 3, 4). Roots of individual cultures were placed in 50 ml of the appropriate uptake solution for either 30 or 60 min. At the end of this time, they were lifted out of the solution and the solution allowed to drain freely from the roots back into the original container. The roots were then placed in another 50 ml of the identical solution. The sequence was continued through 3–6 h. Light intensity was 5 mW $cm^{-2}$ and temperature was 23° (unless indicated otherwise) during the uptake period. The solutions were aerated constantly by a stream of air scrubbed through $H_2O$. Four or six cultures were employed per treatment, each culture constituting a replication. Data presented herein are the means of all replications for a given experiment. After the desired uptake period, the cultures were removed, roots rinsed thoroughly in distilled water, roots and shoots excised just below and above the seed, blotted dry, weighed, frozen and lyophilized. Six to eight cultures were also harvested from the growth tanks prior to initiating the uptake period for a measure of the initial chemical status of the plants.

### *Analytical Procedures*

The uptake solutions were analyzed for total nitrate by a non-automated modification of the procedure of Lowe and Mamilton (1967) and for atom percent (A%) $^{15}N$ by mass spectrometric procedures (Rittenberg, 1948) after reducing the nitrate to ammonium with Devarda's alloy. Quantities of [$^{15}N$]nitrate and [$^{14}N$]nitrate in the 50 ml of the original solution, and in the solutions after the 30–60-min exposure periods, were then determined. In some experiments, the solutions were analyzed for potassium by flame photometric procedures; and net potassium uptake was calculated from depletion of the solution.

The aqueous portion of a methanol:chloroform:water (13:4:3, v/v) extract of the plant material was used to determine nitrate by a non-automated modification of the Lowe and Hamilton (1967) procedure, chloride with an automated chloride titrator (Buchler Instruments, Fort Lee, N.J., USA), malate by malic dehydrogenase (Hohorst, 1965), and amino acids with fluorescamine using glycine as a standard (Stein et al., 1973). Separate portions of plant material were ashed at 480°. After suitable dilution, potassium and sodium were determined flame-photometrically and calcium and magnesium were determined by atomic absorption techniques by standard methods employed in the Analytical Laboratory, Soil Science Department, N.C. State University. Total organic acids were determined by the method of Hiatt and Hendricks (1967) and soluble carbohydrates by a phenol procedure (Dubois et al., 1956).

### *Concepts*

The decrease (in μmoles) of [$^{15}N$]nitrate of the ambient solution (corrected for transpirational water loss) is referred to as [$^{15}N$]nitrate influx. Similarly, the increase (in μmoles) of [$^{14}N$]nitrate in the ambient solution is referred to as [$^{14}N$]nitrate efflux. Analysis of the ambient solutions for ninhydrin-reactive substances (by the method of Yemm and Cocking, 1955) showed only insignificant amounts to be present relative to [$^{14}N$]nitrate efflux (compare Morgan et al., 1973). Net nitrate uptake is the difference between [$^{15}N$]nitrate influx and [$^{14}N$]nitrate efflux. The influx and efflux data are therefore the absolute quantities of each isotopic species which move in a given direction. No assumptions are made regarding changes in internal or external specific activity (A%) as are involved in flux studies using radioactive isotopes in which the labeled species is an extremely small proportion of the total ionic species. The short periods between replacements of the ambient solution

resulted in only small changes in the ambient [$^{15}$N]nitrate and [$^{14}$N]nitrate concentrations. The relative proportions of the two isotopic species in the root cell cytoplasm was of course constantly changing during the experimental period. The impact of this change cannot be directly determined without a means of precisely separating the cyctoplasmic from the vacuolar concentrations. An indirect estimate is afforded by the constantly decreasing rates of [$^{14}$N]nitrate efflux (Figs. 2B, 3, 5B) observed in presence of ambient [$^{15}$N]nitrate. The fact that [$^{15}$N]nitrate influx remained essentially linear after an initial absorption shoulder (Figs. 1A, 5A) implies that its rate was not affected by accumulation of [$^{15}$N]nitrate in the root tissue during these experimental conditions.

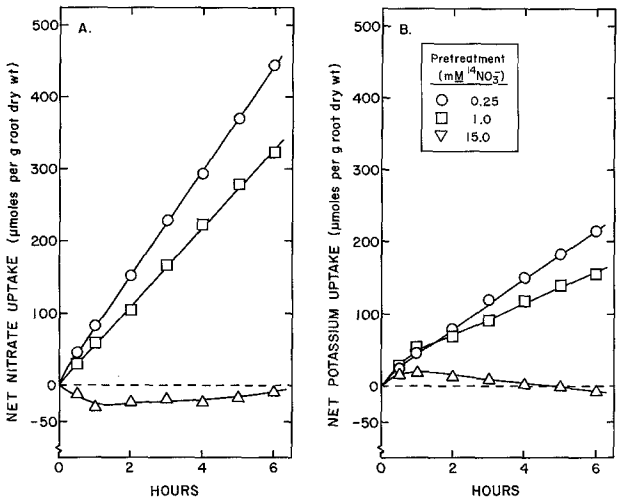


**Fig. 1A and B.** Net nitrate uptake (**A**), and net potassium uptake (**B**) from 1 mM $K^{15}NO_3$ (99 A% $^{15}$N)+0.1 mM $CaSO_4$, pH 6.0, by 10 day old wheat seedlings previously grown with 0.25 mM, 1.0 mM, and 15 mM [$^{14}$N]nitrate. Light intensity, 5 mW cm$^{-2}$, temperature 23°. Initial root nitrate and potassium concentrations are given in Table 1. Experiment I

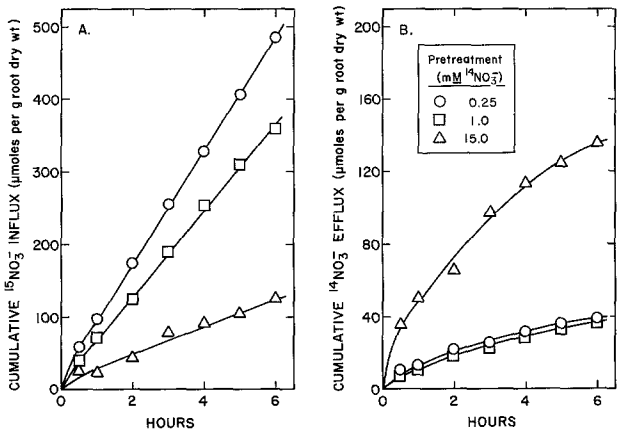


**Fig. 2A and B.** Influx of [$^{15}$N]nitrate (**A**) and efflux of [$^{14}$N]nitrate (**B**) for plants the net nitrate and potassium uptake of which are shown in Figure 1. Note that the ordinate for B is expanded 2.5fold relative to A. Experiment I

## Results

Prior growth at 1.0 mM nitrate, compared to 0.25 mM, decreased net nitrate uptake (Fig. 1A) from 73 to 54 µmole g$^{-1}$ h$^{-1}$ and net $K^+$ uptake (Fig. 1B) from 37 to 21 µmole g$^{-1}$ h$^{-1}$ during the 1–6 h period of exposure to 1.0 mM $K^{15}NO_3$ (99 A% $^{15}$N)+0.1 mM $CaSO_4$, pH 6.0. When previously grown with 15 mM nitrate the roots initially exhibited a net loss of nitrate to the ambient solution following which net uptake was essentially zero (Fig. 1A). A significant net potassium uptake occurred during the the first 0.5 h by plants previously grown with 15 mM nitrate, but this was followed by a slow net loss (Fig. 1B).

There was no significant difference in the dry weight of the roots or shoots at initiation of the uptake period although 0.25 mM nitrate pretreatment resulted in a significantly higher proportion of the total dry weight being present in the roots (Table 1). Initial nitrate concentrations in the root tissue increased with each increase in pretreatment nitrate concentration (Table 1); but the increase for 15 mM over 1.0 mM was relatively small. The decrease in net $K^+$ uptake with increasing nitrate pretreatment (Fig. 1B) occurred in spite of progressively lower initial $K^+$ concentrations in the root tissue (Table 1). Sodium concentrations did increase progressively with the increasing $NaNO_3$ concentrations used during pretreatment (Table 1) but the sum of the root $K^+$ and $Na^+$ concentrations were about constant. The data indicate that net $K^+$ uptake was associated with net nitrate uptake and was not dependent on the initial $K^+$ (or $K^+ + Na^+$) concentration of the root tissue.

Influx of [$^{15}$N]nitrate with plants exposed to 0.25 mM nitrate pretreatment was 78 µmole g$^{-1}$ h$^{-1}$ during the 1–6-h period (Fig. 2A). This was decreased to 59 µmole g$^{-1}$ h$^{-1}$ by prior growth at 1.0 mM but no significant difference in [$^{14}$N]nitrate efflux was evident (Fig. 2B). The lack of net nitrate uptake resulting from prior growth at 15 mM nitrate (Fig. 1A) was associated with a marked increase in [$^{14}$N]nitrate efflux (Fig. 2B) and a further decrease in [$^{15}$N]nitrate influx (Fig. 2A). These pronounced changes in both flux rates occurred in spite of the fact that the initial nitrate concentration in the root tissue was only 13% greater than that of plants previously grown with 1 mM nitrate (Table 1). It is significant that a sizeable flux in both directions occurred in the absence of net nitrate uptake.

**Table 1.** Weights and chemical composition of 10-day old wheat plants, grown at 0.25, 1 and 15 mM nitrate, immediately prior to exposure to $K^{15}NO_3$. Experiment I

| $NaNO_3$ (mM) | Dry weight (mg culture$^{-1}$) | $W_R$%[a] (%) | $NO_3^-$[b] | $Cl^-$[b] | Malate[b] | Total organic acids[b] | Soluble carbo-hydrate[c] | Amino acids[d] | $K^+$[b] | $Na^+$[b] | $Ca^{2+}$[b] | $Mg^{2+}$[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. Roots* | | | | | | | | | | | | |
| 0.25 | 76 | 34.0 | 857 | 207 | 83 | 130 | 91 | 42 | 1,342 | 173 | 36 | 95 |
| 1.0 | 65 | 30.7 | 1,200 | 91 | 22 | 119 | 110 | 44 | 1,215 | 206 | 41 | 128 |
| 15.0 | 67 | 29.9 | 1,360 | 60 | 18 | 108 | 106 | 48 | 1,072 | 426 | 36 | 143 |
| Significance[e] | NS | *** | *** | *** | *** | NS | NS | NS | ** | *** | NS | *** |
| LSD 0.05[f] | | 1.4 | 71 | 19 | 18 | | | | 111 | 49 | | 5 |
| *B. Shoots* | | | | | | | | | | | | |
| 0.25 | 147 | | 590 | 215 | | 263 | 121 | 30 | 3,636 | 73 | 53 | 82 |
| 1.0 | 146 | | 906 | 126 | | 245 | 104 | 27 | 3,558 | 67 | 49 | 81 |
| 15.0 | 157 | | 903 | 68 | | 224 | 90 | 28 | 2,578 | 152 | 42 | 74 |
| Significance[e] | NS | | *** | *** | | NS | NS | NS | NS | ** | ** | NS |
| LSD 0.05[f] | | | 119 | 23 | | | | | | 32 | 4 | |

[a] $W_R\% = (\text{root wt.}/\text{total wt.}) \times 100$
[b] μeq $g^{-1}$ dry wt.
[c] glucose equivalents, μmole $g^{-1}$ dry wt.
[d] glycine equivalents, μmole $g^{-1}$ dry wt.
[e] (* $p \leqq 0.05$), ** $p \leqq 0.01$, *** $p \leqq 0.001$, NS = not significant
[f] Least significant difference for $p \leqq 0.05$

**Table 2.** Changes in tissue nitrate of 10-day-old wheat plants, previously grown with 15 mM nitrate, during a 6-h exposure to various solutions. Experiment II. All values are μmole $NO_3^-$ $g^{-1}$ root dry wt.

| Treatment[a] | Tissue quantities | | | Change 0–6 h | | | Net uptake | Reduction[b] |
|---|---|---|---|---|---|---|---|---|
| | Roots | Tops | Total | Roots | Tops | Total | | |
| Initial | 1,292 | 3,048 | 4,340 | | | | | |
| KCl | 1,111 | 2,622 | 3,733 | −181 | −426 | −607 | −62 | 545 |
| $H_2O$ | 1,084 | 2,582 | 3,666 | −208 | −465 | −674 | −49 | 625 |
| $KHCO_3$ | 1,145 | 2,712 | 3,857 | −147 | −336 | −483 | −89 | 394 |
| $K^{15}No_3$ | 1,165 | 2,701 | 3,866 | −127 | −347 | −474 | 182 | 656 |
| Significance[c] | ** | * | ** | | | | | |
| LSD 0.05[d] | 91 | 297 | 328 | | | | | |

[a] All potassium salts were 1.0 mM and each solution contained 0.1 mM $CaSO_4$, pH 6.0
[b] Reduction is the change in total plant nitrate not accounted for by net uptake
[c] * $p \leqq 0.05$, ** $p \leqq 0.01$
[d] Least significanct difference for $p \leqq 0.05$

In Experiment II, plants grown at 15 mM nitrate were either placed in $H_2O$, 1.0 mM KCl, 1.0 mM $K^{15}NO_3$, or 1.0 mM $KHCO_3$, all with 0.1 mM $CaSO_4$. In addition, some cultures were transferred to 1.0 mM $K^{15}NO_3$ after 3 h in 1.0 mM KCl. Cumulative efflux of [$^{14}$N]nitrate is shown in Figure 3. Presence of the salts increased the [$^{14}$N]nitrate efflux over the control which contained only 0.1 mM $CaSO_4$. With KCl, the increase was largely due to displacement during the first 0.5 h. With $KHCO_3$ and $K^{15}NO_3$, both the initial [$^{14}$N]nitrate efflux and the subsequent [$^{14}$N]nitrate efflux rate were increased relative to the control. Transfer to $K^{15}NO_3$ after a 3 h exposure to KCl resulted in an increase in [$^{14}$N]nitrate efflux (Fig. 3). Sustained [$^{14}$N]nitrate efflux clearly was enhanced in presence of ambient [$^{15}$N]nitrate.

Throughout the 6-h experimental period, there was a continual net $K^+$ efflux from the roots to the external solutions in the $H_2O$, KCl and $KHCO_3$ treatments (Fig. 4). Net $Cl^-$ uptake from KCl was negligible (data not presented). Net nitrate uptake from $K^{15}NO_3$ occurred in this experiment (Table 2) and, in the presence of external nitrate, net $K^+$ efflux was abolished (Fig. 4). Transfer at 3 h from KCl to

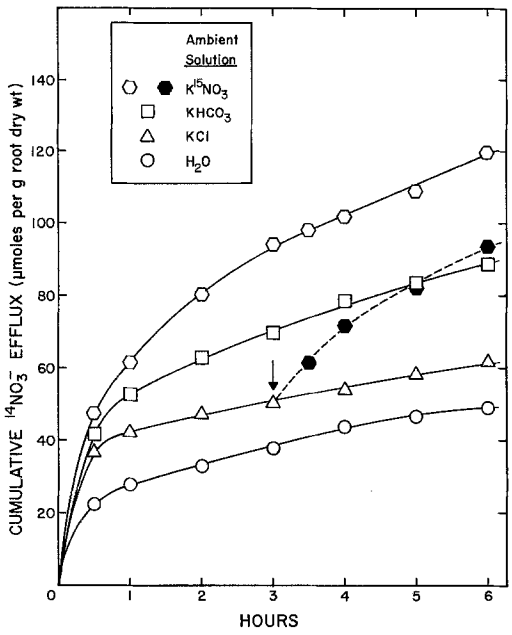


**Fig. 3.** Efflux of [$^{14}$N]nitrate to $H_2O$ and to 1 mM solutions of $K^{15}NO_3$, $KHCO_3$, or KCl (each containing 0.1 mM $CaSO_4$ pH 6.0), by plants previously grown with 15 mM [$^{14}$N]nitrate. Other conditions as in Figure 1. At the arrow, cultures were transferred from KCl to $K^{15}NO_3$. The initial root nitrate concentration was 1,293 μmole $g^{-1}$. Experiment II

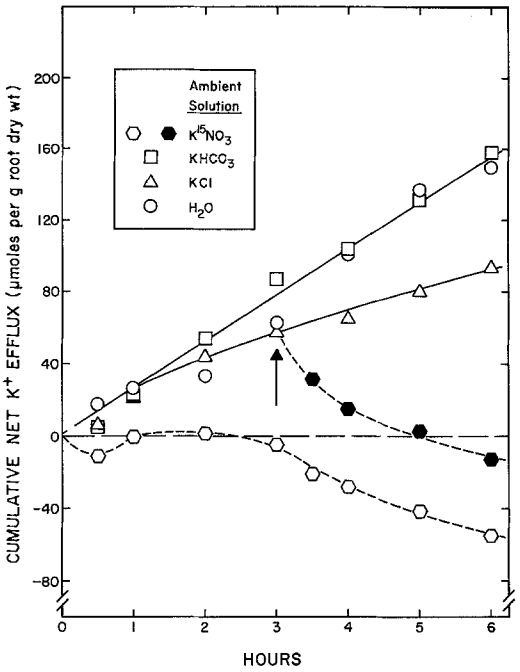


**Fig. 4.** Net potassium efflux for the treatments shown in Figure 3. The initial root potassium concentration was 931 μmole $g^{-1}$. Experiment II

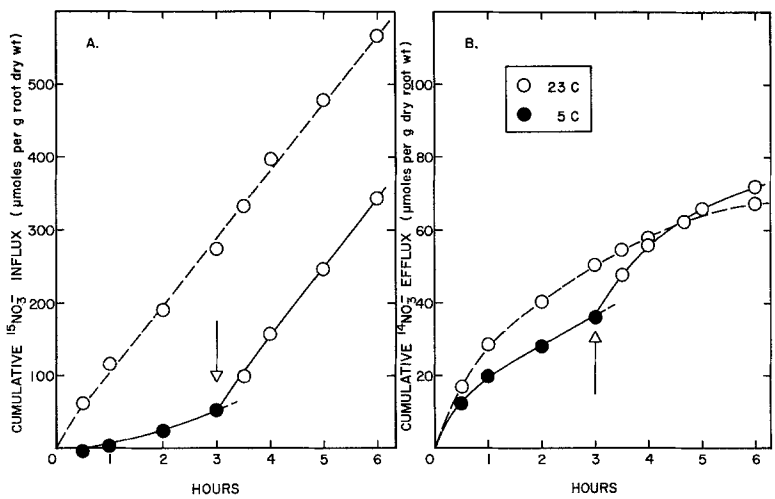


**Fig. 5A and B.** Influx of [$^{15}$N]nitrate (**A**) and efflux of [$^{14}$N]nitrate (**B**) at 23° and 5° during exposure to 1.0 mM $K^{14}NO_3$+0.1 mM $CaSO_4$, pH 6.0, by plants previously grown with 5.0 mM [$^{14}$N]nitrate. Other conditions as in Figure 1. At the arrow plants at 5° were transferred to 23°. The initial root nitrate concentration was 869 μmole $g^{-1}$. Experiment III

$K^{15}NO_3$ resulted, within 0.5 h, in a change from net $K^+$ movement from the roots to net $K^+$ movement into the roots.

Significant nitrate reduction occurred in all treatments during the 6-h exposure period (Table 2). Root nitrate concentrations for plants exposed to $H_2O$, KCl, or $KHCO_3$ decreased significantly ($p \leqq 0.01$) and by a greater amount than could be accounted for by efflux, indicating effective nitrate translocation from or reduction in the roots. A significant decrease in root nitrate also occurred in the $K^{15}NO_3$ treatment; translocation from roots and/or reduction by roots thus exceeded net uptake during the experimental period.

Influx of [$^{15}$N]nitrate was severely restricted by 5° relative to 23° (Fig. 5A). After exposure to 5° for 3 h, transfer to 23° resulted in a rapid increase in [$^{15}$N]nitrate influx to the rate obtaining with the

plants exposed to 23° throughout. Efflux of [$^{14}$N]nitrate was also slower at 5° than at 23° (Fig. 5B), but the difference was not as marked as [$^{15}$N]nitrate influx. The [$^{14}$N]nitrate efflux data can be accomodated in a process mediated by diffusion but the [$^{15}$N]nitrate influx data cannot.

## Discussion

### *Nitrate Influx and Efflux*

Restrictions in [$^{15}$N]nitrate influx (Fig. 2A) and net nitrate uptake (Fig. 1A) were associated with progressive increases in root nitrate concentrations (Table 1) resulting from prior growth at 0.25, 1.0 and 15 mM nitrate. But the difference in [$^{15}$N]nitrate influx between plants grown at 1 mM and 15 mM was inordinately large (59 vs. 20 μmole $g^{-1}$ $h^{-1}$) compared to the difference in initial root nitrate concentrations (1,200 vs. 1,360 μmole $g^{-1}$ respectively).

Total organic acids were not affected by the three nitrate concentrations during growth (Table 1). The initial $Cl^-$ and malate concentrations in the roots were quite low relative to the concentration of nitrate (Table 1), and they were both decreased by increasing ambient nitrate during the growth period. However, the sum of these anionic components in the roots were no more directly related to the [$^{15}$N]nitrate influx rates than were the initial nitrate concentrations. It is of interest that net $Cl^-$ uptake by these plants was negligible in spite of their relatively low initial $Cl^-$ concentrations (Table 1). Roots of the plants in Experiment II (Table 2) were also low in $Cl^-$ (60 μmole $g^{-1}$) and organic acids (212 μeq $g^{-1}$) relative to nitrate (1,260 μmole $g^{-1}$). When exposed to 1 mM KCl, there was no significant net $Cl^-$ uptake during the 6-h period, indicating either that (a) the high endogenous nitrate, rather than endogenous $Cl^-$, was restricting net $Cl^-$ uptake (compare Cram, 1973; Smith, 1973), or (b) an entirely separate restricting influence, not directly associated with endogenous anion levels, was involved.

Soluble carbohydrates in the root tissue of these plants were quite low and no relationship between the initial soluble carbohydrates and the magnitude of [$^{15}$N]nitrate influx or [$^{14}$N]nitrate efflux is evident (Table 1, Fig. 2). It has been suggested previously (Minotti and Jackson, 1970; Jackson et al., 1976) that the rate of carbohydrate translocation to the roots of intact plants is crucial in regulating net nitrate uptake. This suggestion is supported by the close relationship between downward sucrose translocation rates and the respiratory activity of the root (Hatrick and Bowling, 1973; compare Pitman, 1972). Downward translocation of organic acids from the shoots and subsequent decarboxylation to provide OH and $HCO_3$ ions for counter-transport with nitrate may also be involved (Dijkshoorn, 1971; Ben-Zioni et al., 1971). Additionally, regulation of the influx rates through hormonal substances translocated from the shoots cannot be disregaded (Pitman, 1972; Collins and Kerrigan, 1974; Pitman et al., 1974).

Initial [$^{14}$N]nitrate concentrations did not give an exact indication of the [$^{14}$N]nitrate efflux rates. Similar patterns of [$^{14}$N]nitrate efflux resulted from prior growth at 0.25 mM and 1.0 mM nitrate (Fig. 2B) although the roots initially contained 857 and 1,200 μmole $g^{-1}$, respectively (Table 1). The relatively small increase to 1,360 μmole $g^{-1}$ in root nitrate resulting from prior growth at 15 mM nitrate was associated with a marked increase in [$^{14}$N]nitrate efflux. It follows that growing the plants at different nitrate concentrations must have resulted in (a) differential permeability of the root-cell plasmalemmae to nitrate, this difference not being precisely reflected by the endogenous nitrate concentrations in the roots (nor in the shoots), or (b) differential compartmentation of the root nitrate such that total concentrations did not accurately reflect the concentration available to the efflux channels. The present data do not permit a separation of these two possibilities.

The stimulation of [$^{14}$N]nitrate efflux when plants were simultaneously absorbing [$^{15}$N]nitrate (Fig. 3) and the sizeable [$^{14}$N] efflux at 5° (Fig. 5B) are in accord with a previous suggestion (Morgan et al., 1973) that appearance of endogenous nitrate in the ambient solution results from a passive leakage phenomenon. This concept includes the possibility of effluxed ions being recirculated (within outer unstirred layers) to active absorption sites on the external plasmalemma surfaces such that a significant but unknown proportion of these ions moving out through the plasmalemma would not be detected in the ambient solution. Saturation of the active absorption sites by ions from the ambient solution would result in diffusion gradients of the effluxing ions which favor their movement to the external solution. Hence the magnitude of the detectable [$^{14}$N]nitrate efflux would be dependent upon (a) the size of the cytoplasmic [$^{14}$N]nitrate pool relative to that of the ambient [$^{14}$N]nitrate (the latter maintained close to zero in the present case), (b) the permeability of the plasmalemma to nitrate ions, (c) the activity of the active absorption mechanism on the external surfaces of the plasmalemma, and (d) the extent to which the absorption sites are dominated by the ambient [$^{15}$N]nitrate ions. In this view, therefore, the nitrate influx and nitrate efflux processes are largely independent.

*Nitrate and Potassium Uptake*

The present data (Table 1, Fig. 1B) as well as previous results (Jackson et al., 1974b; Jackson et al., 1976), indicate that the $K^+$ uptake in intact wheat plants is largely conditioned by nitrate uptake. In the present experiments, the initially high $K^+$ concentrations in the roots resulted in a net $K^+$ efflux when the were placed in $H_2O$, in 1 mM KCl, or in 1 mM $KHCO_3$ (Fig. 4). Chloride uptake from KCl was negligible. However, transferring the plants to 1 mM $K^{15}NO_3$ after 3 h in KCl resulted in a significant net nitrate uptake which was accompanied by a corresponding onset of net $K^+$ uptake (Fig. 4). Prior growth in 15 mM $NaNO_3$ resulted in no net nitrate uptake and a slow efflux of $K^+$ (Fig. 2). This occurred despite the fact that the initial root $K^+$ concentration (Table 1) was lower than that of plants grown with lower nitrate concentrations, in which sizeable rates of both nitrate and $K^+$ uptake obtained (Fig. 1). With nitrogen-depleted wheat plants, the development of an accelerated rate of net nitrate or nitrite uptake upon first exposure to $KNO_3$ generally was accompanied by an increase in net $K^+$ uptake (Minotti et al., 1968; Jackson et al., 1974a). This increase was dependent both in time and magnitude on the increase in net nitrogen-anion uptake (Jackson et al., 1974b). Moreover, addition of $NaNO_3$ to $KNO_2$, which increased total net anion uptake, markedly stimulated net $K^+$ uptake (Jackson et al., 1974b). Finally, when nitrogen-depleted wheat was exposed to complete solutions containing nitrate, the changing patterns of nitrate and $K^+$ uptake were similar (although the rates differed) over a 7-day period during which the plants were recovering from the nitrogen-depleted state (Jackson et al., 1976). In these investigations with wheat seedlings, it therefore appears that it is the anion uptake which drives the $K^+$ uptake (compare Johansen and Loneragan, 1975) although it is not possible to tell whether the influence is on $K^+$ influx or $K^+$ efflux.

During initial exposure of nitrogen-depleted wheat to nitrate, substantial net nitrate uptake occurred in the absence of ambient $K^+$ (Ashley et al., 1975; Minotti et al., 1968, 1969) and considerable nitrate uptake from $Ca^{2+}$ and $Mg^{2+}$ nitrate salts has been observed in ryegrass plants well nourished with nitrate (Morgan et al., 1972). Moreover, sizeable nitrate uptake, with negligible $K^+$ uptake, was observed with nitrogen-depleted wheat plants previously grown at high $K^+$ concentrations (Jackson et al., 1974b). Accordingly, net uptake of the nitrogen anions is not dependent upon concomitant net uptake of $K^+$ (compare Smith, 1973). Thus, there is a decided responsiveness of $K^+$ uptake to nitrate uptake, nitrate uptake can occur without concomitant $K^+$ uptake, and under certain circumstances, an additional component of $K^+$ uptake, not dependent upon nitrate uptake, also is possible.

The observations can be accomodated in a general way by the attractive hypotheses of Hodges (1973) or Smith (1973). Both visualize separate cation and anion transport systems, together with a process which results in charge separation at the plasmalemma (H ions extruded outward, OH ions extruded inward). The anion transport system is then driven by the outward movement of the internally generated hydroxyl or bicarbonate ions. Cooperativity or relative independence of the two systems are both possible, the anion transport system being capable of independent action provided a supply of $OH^-$ or $HCO_3^-$ is continually generated in the cytoplasm. Net $K^+/NO_3^-$ uptake ratios less than 1.0 (Fig. 1; compare Jackson et al., 1976) imply that additional $OH^-$ or $HCO_3^-$ ions must be generated internally. Either decarboxylation of organic acids (Hodges, 1973; Smith, 1973; Raven and Smith, 1974) or nitrate reduction (Kirkby and Mengel, 1967; Dijkshoorn, 1962) would be capable of generating these anions. In high-nitrate wheat, indirect evidence indicates that relatively little nitrate reduction occurs in the root system (Minotti and Jackson, 1970) and a similar conclusion is evident for barley seedlings exposed to $KNO_3$ (Blevins et al., 1974). Under these conditions, nitrate reduction in the roots would not be sufficient to sustain the nitrate uptake rates. Although the initial organic-acid concentrations in the root tissue were quite low in the present experiment (Table 1), it is conceivable that organic acids were supplied either directly or indirectly from the shoot and that their decarboxylation maintained the excess nitrate uptake (Dijkshoorn, 1971; Ben-Zioni et al., 1971).

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