Plant Physiol. (1989) 91, 1407–1413
0032-0889/89/91/1407/07/$01.00/0

Received for publication October 13, 1988
and in revised form July 3, 1989

Competitive Al^{3+} Inhibition of Net Mg^{2+} Uptake by Intact *Lolium multiflorum* Roots[1]

I. Kinetics

Zdenko Rengel*[2] and Donald L. Robinson

Department of Agronomy, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803

ABSTRACT

Aluminum impairs uptake of Mg^{2+}, but the mechanisms of this inhibition are not understood. The depletion technique was used to monitor net Mg^{2+} uptake from nutrient solution by intact, 23-day-old plants of ryegrass (*Lolium multiflorum* Lam., cv Gulf and Wilo). Activities of Mg^{2+} and monomeric Al species in nutrient solution were calculated and used as the basis for expressing the results. The kinetics of net Mg^{2+} absorption was resolved into (a) a transpiration-dependent uptake component, (b) a metabolically mediated, discontinuous saturable component that is Al^{3+} sensitive and *p*-chloromercuribenzene sulfonic acid (PCMBS) resistant, and (c) a linear, carbonyl cyanide *m*-chlorophenylhydrazone resistant, Al^{3+} sensitive component that might be a type of facilitated diffusion. Lowering the pH from 6.0 to 4.2 exerted a noncompetitive inhibition of net Mg^{2+} uptake, while aluminum at 6.6 micromolar Al^{3+} activity exerted competitive inhibition of net Mg^{2+} uptake at pH 4.2. The Al^{3+}-induced effect was obvious after 30 minutes. Cultivar-specific ability to retain a higher affinity for Mg^{2+} by postulated transport proteins in the presence of Al^{3+} might be one of the mechanisms of differential Al tolerance among ryegrass cultivars.

Aluminum is the most important yield-limiting factor in many acid soils (11). Inhibition of root growth is a primary effect of Al toxicity (6). A vast body of literature has also been published on decreased nutrient concentrations and total nutrient contents in plants exposed to Al during extended periods of time. Mechanisms of these and other deleterious Al effects are still unclear (for a review see ref. 11).

One of the nutrient elements apparently affected by Al to a great extent is Mg. Exposure to Al results in decreased Mg concentration and total Mg content in plants (5, 12, 31). This may be due to decreased Mg^{2+} absorption brought about by reduced root growth or to a direct Al inhibition of Mg^{2+} uptake. The suggestion was put forward that Al directly affected Mg^{2+} absorption in oats (12), sorghum (15), and ryegrass (31). Kinetic analysis of the relationship between Mg absorption and Al does not appear to have been undertaken previously.

Differential Al tolerance among cultivars may be due to differences in the ability to absorb and retain more Mg when exposed to Al (5). The hypothesis that kinetic parameters of Mg^{2+} uptake will be less affected by Al in Al-tolerant cultivars than in Al-sensitive cultivars remains to be tested.

The objective of this study was to determine the influence of Al on the kinetics of net Mg^{2+} uptake in two ryegrass cultivars differing in Al tolerance.

MATERIALS AND METHODS

Plant Material

Seeds of two ryegrass (*Lolium multiflorum* Lam.) cultivars, Wilo (relatively Al-sensitive) and Gulf (relatively Al-tolerant), were surface sterilized, germinated, and grown in complete nutrient solution in the growth chamber as described elsewhere (31). The pH of nutrient solution was initially adjusted to 6.0 with 0.2 N HCl and was monitored daily. Solution was renewed on d 10 after the start of germination and in 4-d intervals thereafter, which prevented pH change of more than 0.2 units. On d 22, the complete nutrient solution was replaced with the solution from which Mg was omitted. The pH of the Mg-free solution was adjusted to 4.2 with 0.2 N HCl, except for the solution used to nourish plants to be utilized for measuring Mg^{2+} absorption at pH 6.0, in which case pH 6.0 was used. Distilled water was added to the pots daily to replace water lost by evapotranspiration.

Nutrient Solutions for Measuring Net Mg^{2+} Uptake

On d 23, after 24 h of growth in the Mg-free nutrient solution, net Mg^{2+} uptake was measured from the nutrient solutions having Mg^{2+} and Al concentrations and pH varied according to the experimental objectives. Sodium nitrate was supplemented as appropriate to compensate for different concentrations of Mg^{2+} added as $Mg(NO_3)_2 \times 6\ H_2O$. Aluminum was added from a stock solution prepared by adding 1 mmol $Al_2(SO_4)_3 \times 18\ H_2O$ to 1 L of 0.002 N H_2SO_4. When applicable,

[1] Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript No. 88-09-2498 (Hatch Project No. 1579).

[2] Present address: Faculty of Agriculture, Institute of Agroecology, Simunska 25, 41000 Zagreb, Yugoslavia.

CCCP,[3] a metabolic uncoupler, and PCMBS, a nonpermeant sulfhydryl reagent, were added to nutrient solutions in concentrations of 1 and 100 μM, respectively.

Ionic strength and the ionic speciation of the nutrient solutions used for measuring Mg^{2+} absorption were calculated by a modified version (28; and errata in Soil Sci Soc Am J 51: 1680) of the GEOCHEM computer program (33). The program was edited to reflect our choice of equilibrium constants for Mg as well as Al complexes (taken from ref. 20). Mg, Ca, Fe, Mn, Zn, Cu, K, Na, and P in the nutrient solutions were determined by ICPES (Applied Research Laboratories, Sunland, CA), while SO_4^{2-}, NO_3^-, Cl^-, and F^- were measured with an ion chromatograph. Input values for Al were the estimates of monomeric Al concentrations obtained by the aluminon method (2). The absorbance was measured after 30 min at 530 nm in 10-mm path length cuvettes. The estimates of monomeric Al were 16 ± 2 and 69 ± 5 μM (means ± SE) after addition of 18 and 74 μM Al, respectively. Calculated activities of Mg and Al species are presented in Table I. The means of $\{Al^{3+}\}$ were calculated to be 6.6 and 26 μM after addition of 18 and 74 μM Al, respectively. These were reference values for this study. At the end of the 12-h depletion experiment, concentrations of cations, anions, and monomeric Al species were determined again and activities were calculated as described above; the $\{Al^{3+}\}$ in nutrient solutions did not fall below 5.5 and 24 μM for 6.6 and 26 μM initial $\{Al^{3+}\}$, respectively.

Sampling

The depletion technique, modified after Claassen and Barber (4), was used to monitor net Mg^{2+} uptake. Nutrient solution was continuously sampled from the pots by a peristaltic metering pump which removed 0.4 mL min^{-1}. At the same rate, distilled water was pumped into the pots. The sampled solution was fed into a series of tubes in a fraction collector set to change tubes at 30-min intervals. The depletion experiment was initiated at the beginning of the 13-h full light period in the growth chamber. Plants were allowed 30 min to attain dynamic equilibrium with the nutrient solution before sampling started (time 0 of depletion period). The depletion period lasted for 12 h, during which time the pH of nutrient solutions did not change more than 0.05 units.

Following the depletion period, roots were separated from shoots, rinsed with distilled water, gently blotted twice between paper towels, weighed, and subsampled by weight. Preliminary measurements showed that such a subsampling did not significantly affect the accuracy of whole-sample root-length estimation. Root length was measured on one subsample with an area meter (DELTA-T Devices Ltd., Cambridge, U.K.). The whole-sample root length ranged between 132 and 185 m. Root radius was calculated from the root volume which was estimated from the root fresh weight, assuming the density of fresh roots to be 1000 kg m^{-3}. Root surface area was calculated assuming that roots were smooth cylinders. Increases in root length that occurred during the 12-h depletion period were assumed to be negligible compared to the whole root mass.

Evapotranspiration

Water loss due to transpiration was estimated by weighing the pots with plants at the beginning and end of the depletion period; differences between the two values were used to calculate transpiration rates. The same procedure was followed for pots without plants to assess water loss due to evaporation. Transpiration rates per unit of root surface area ranged from 29 to 34 μL m^{-2} s^{-1}.

Sample Analysis and Result Calculations

Samples of the nutrient solution from the depletion experiments were analyzed for Mg^{2+} with ICPES. A computer

[3] Abbrevations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; PCMBS, *p*-chloromercuribenzene sulfonic acid; $\{Al^{3+}\}$ and $\{Mg^{2+}\}$, ionic activities of Al^{3+} and Mg^{2+}, respectively; ICPES, inductively coupled plasma emission spectrometry; I_{max}, maximum net ion influx.

Table I. *Ionic Strength and Activities of Mg^{2+} and Al Species in the Nutrient Solution (pH 4.2) Used for the Depletion Experiment*

Calculations were done by the modified GEOCHEM computer program.

Concentration		Mg^{2+} Activity Coefficient	Activities						Ionic Strength
Al	Mg^{2+}		Mg^{2+}	Al^{3+}	$Al(OH)^{2+}$	$Al(OH)_2^+$	$Al(OH)_3^0$	$AlSO_4^+$	
μM			μM						
0	20	0.71	14.2						8030
0	80	0.71	56.8						8050
0	320	0.70	224						8090
0	1280	0.67	858						8260
18	20	0.71	14.2	6.8	1.1	0.9	0.0	1.1	8090
18	80	0.71	56.8	6.8	1.1	0.9	0.0	1.1	8100
18	320	0.70	224	6.6	1.1	0.8	0.0	1.1	8150
18	1280	0.67	858	6.1	1.0	0.8	0.0	0.9	8300
74	20	0.70	14.0	26.8	4.2	3.4	0.1	6.5	8210
74	80	0.70	56.0	26.6	4.2	3.4	0.1	6.5	8220
74	320	0.69	221	26.1	4.2	3.3	0.1	6.2	8280
74	1280	0.66	845	24.2	3.9	3.0	0.1	5.2	8420

program was written in FORTRAN to adjust measured Mg^{2+} concentrations for time-dependent variables imposed by the depletion technique itself (Mg^{2+} removal by sampling while distilled water was pumped into the pot) as well as for the amount of water transpired, which was assumed to be a linear function of time. Net Mg^{2+} uptake was calculated using the difference between two sequential nutrient solution Mg^{2+} concentrations after the previously described adjustments had been made. Calculated net Mg^{2+} uptake was further adjusted by subtracting the portion of Mg^{2+} uptake that was due to water flux. Following the work of Dalton *et al.* (7) and introducing certain additional assumptions (for details on formula derivations see ref. 30), it can be shown that

$$I_{netX} = I_{tX} + (\sigma - 1)C_{laX}I_W$$

where I_{netX} is net energy-dependent uptake of ion X, I_{tX} is the total flux of ion X, σ is the reflection coefficient, C_{laX} is the concentration of ion X at the root surface, and I_W is the water flux. Using this formula to calculate net Mg^{2+} uptake that was corrected for the water flux-driven Mg^{2+} uptake (mass flow of Mg^{2+}), σ was assumed to be independent of Al and value of 0.7 from Dalton *et al.* (7) was used throughout, while $C_{la}Mg^{2+}$ was assumed to be the same as the Mg^{2+} concentration in the bulk solution that was vigorously stirred by air-bubbles throughout the experiment. In contrast, for calculating the parameters of net Mg^{2+} uptake kinetics (I_{max} and K_m, Table II) and for Figures 1 to 4, Mg^{2+} activities of the depletion solutions were used.

Statistics

Each experiment contained two replicates and was performed twice. To simplify Figures 1 to 4, results of only one replicate obtained after 1, 3, 5, 7, 9, 11, and 12 h following the start of the depletion period, were presented. The parameters of net Mg^{2+} uptake were calculated from the regression lines fitted to the data which had been transformed by the Eadie-Hofstee equation of Michaelis-Menten kinetics. Analysis of covariance (= multisource regression analysis) was conducted to compare I_{max} (Y intercept) and K_m (slope) of the Eadie-Hofstee transformed data obtained for different treatments. Statistical analyses were performed using routines of the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

Magnesium

Due to slow uptake of Mg^{2+} from the solution, Mg^{2+} depletion did not exceed 50% of the starting amount and was much smaller in most treatments (not shown). The resulting depletion curves made it mathematically impossible to directly calculate I_{max} and K_m. Net Mg^{2+} uptake data obtained at four different initial Mg^{2+} concentrations in nutrient solution were thus combined. The difference between net Mg^{2+} uptake corrected or uncorrected for transpiration-dependent Mg^{2+} uptake is shown in Figure 1 for the cv Gulf at pH 6.0. The Michaelis-Menten function ($I = [I_{max} \times \{Mg^{2+}\}]/[K_m + \{Mg^{2+}\}]$, where I represents net Mg^{2+} uptake) was fitted to the mass flow corrected net Mg^{2+} uptake (Fig. 1; see also Figs. 2 and 4). The uncorrected Mg^{2+} uptake did not follow the Michaelis-Menten function (Fig. 1). Net Mg^{2+} uptake data obtained at nutrient solution $\{Mg^{2+}\}$ above 700 μM could not be fitted to the same curves as data obtained with $\{Mg^{2+}\}$ below 230 μM (for an example see Fig. 1). Such a result indicates that Mg^{2+} uptake parameters (I_{max}, K_m) applicable to the uptake process at $\{Mg^{2+}\}$ greater than 700 μM did not apply to uptake at $\{Mg^{2+}\}$ less than 230 μM. Hence, only results obtained at nutrient solution $\{Mg^{2+}\}$ below 230 μM were considered in the following analyses.

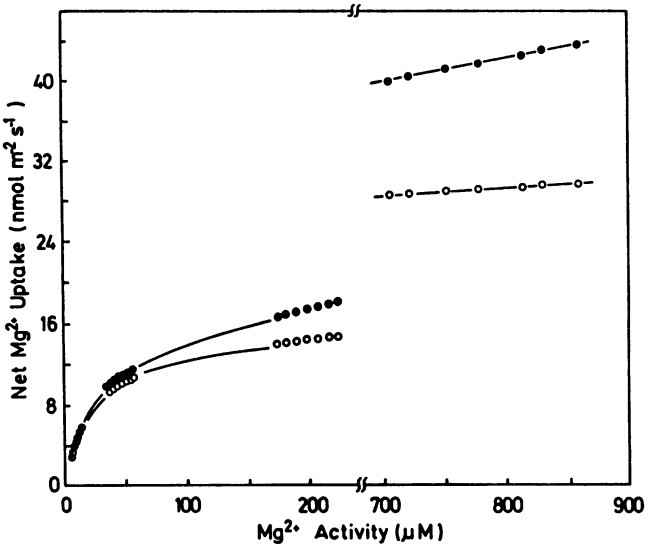

Figure 1. Net Mg^{2+} uptake from the complete nutrient solution (pH 6.0, no Al) by 23-d-old plants of Gulf ryegrass. Data were presented as corrected (○) or uncorrected (●) for transpiration-driven Mg^{2+} uptake. The Michaelis-Menten function was fitted to the corrected data (<230 μM Mg^{2+} activity), while the curve to uncorrected data was fitted by eye.

Table II. *Kinetic Parameters of Mass Flow Corrected Net Mg^{2+} Uptake*

The parameters were calculated from the Eadie-Hofstee equations using the data obtained at $\{Mg^{2+}\}$ <230 μM. The K_m is expressed as activity of Mg^{2+}. The cv Gulf is Al-tolerant relative to the cv Wilo. Means ± SE

pH	$\{Al^{3+}\}$	I_{max}		K_m	
		Gulf	Wilo	Gulf	Wilo
	μM	nmol m^{-2} s^{-1}		μM	
6.0	0	16.6 ± 0.5	15.6 ± 0.5	28.5 ± 4.3	28.1 ± 4.8
4.2	0	9.8 ± 0.3	9.6 ± 0.3	25.2 ± 3.2	24.8 ± 4.2
4.2	6.6	9.5 ± 0.5	9.1 ± 0.4	54.4 ± 8.9	93.3 ± 14.2

pH

Lowering the pH from 6.0 to 4.2 decreased I_{max} of net Mg^{2+} uptake ($P < 0.013$ for both cultivars), while no significant change was observed for K_m ($P < 0.301$ and $P < 0.586$ for the cv Gulf and Wilo, respectively) (Table II; *cf.* Figs. 1 and 2, Fig. 3).

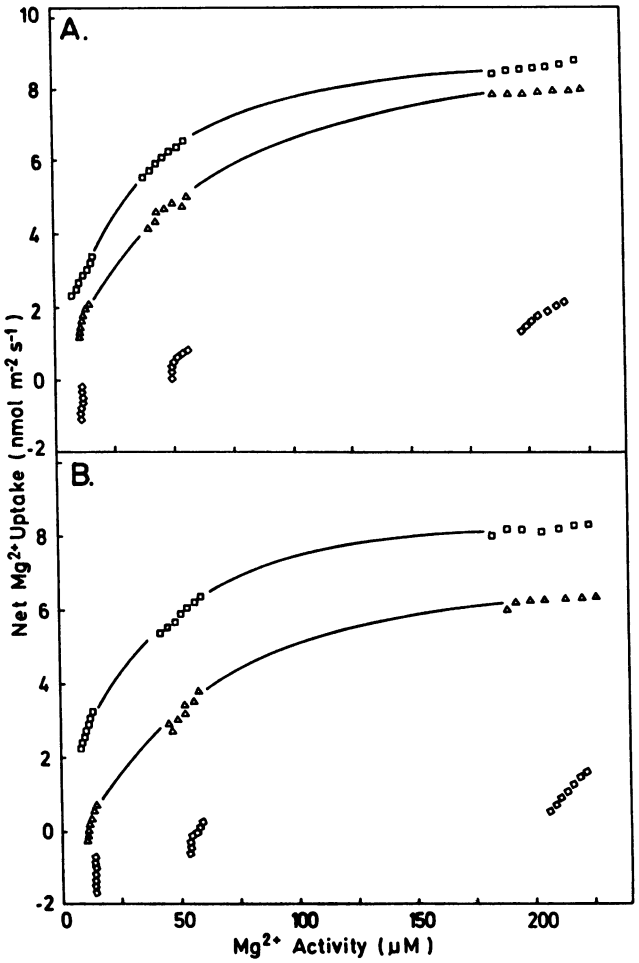

Figure 2. Influence of $\{Al^{3+}\}$ on mass flow corrected net Mg^{2+} uptake by 23-d-old plants of Gulf (A) and Wilo (B) ryegrass from the complete nutrient solution (pH 4.2) having $\{Al^{3+}\}$ 0 (□), 6.6 µM (△) and 26 µM (◇).

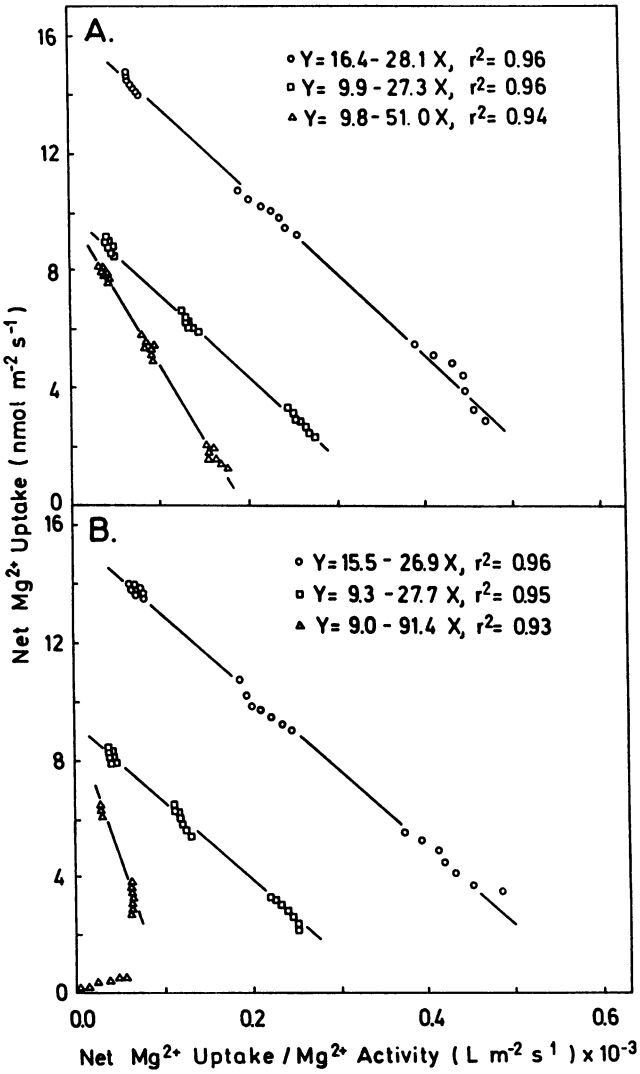

Figure 3. Effects of pH and $\{Al^{3+}\}$ on the parameters (I_{max}, K_m) of mass flow corrected net Mg^{2+} uptake by 23-d-old plants of Gulf (A) and Wilo (B) ryegrass from the complete nutrient solution. The Eadie-Hofstee function (intercept = I_{max}; slope = $-K_m$) was fitted to the data obtained at pH 6.0 and no Al (○), pH 4.2 and no Al (□), {pH 4.2} and $\{Al^{3+}\}$ = 6.6 µM (△).

Aluminum

At pH 4.2, 6.6 µM $\{Al^{3+}\}$ in nutrient solution considerably ($P < 0.0001$) increased K_m (216 and 376% for the cv Gulf and Wilo, respectively), while no significant change was detected for I_{max} of net Mg^{2+} uptake ($P < 0.418$ and $P < 0.203$ for the cv Gulf and Wilo, respectively) (Table II; Fig. 3). Net Mg^{2+} uptake by Wilo roots from the nutrient solution having $\{Mg^{2+}\} < 14$ was severely affected by 6.6 µM $\{Al^{3+}\}$ after 30 min only, while net Mg^{2+} efflux occurred after 9 h. This result was assumed to be a consequence of Al-caused deleterious effects not directly related to the process of Mg^{2+} uptake; these data were therefore omitted from the analysis. In contrast, 26 µM $\{Al^{3+}\}$ in nutrient solution caused net efflux, *i.e.* actual loss, of Mg^{2+} from roots of both tested cultivars at $\{Mg^{2+}\}$ below 14 µM and severely depressed net Mg^{2+} uptake at greater Mg^{2+} activities (Fig. 2). Hence, I_{max} and K_m could not be estimated from the results obtained at 26 µM $\{Al^{3+}\}$ in nutrient solution.

Cultivars

At pH 6.0 as well as at pH 4.2 and no Al added, there was no difference in net Mg^{2+} uptake between the two cultivars (Table II). In contrast, 6.6 µM $\{Al^{3+}\}$ produced a larger K_m ($P < 0.0001$) in the Al-sensitive cv Wilo than in the more Al-tolerant cv Gulf (Table II). I_{max} values were the same for both cultivars ($P < 0.118$).

CCCP and PCMBS

For the cv Wilo, the addition of PCMBS in the nutrient solution having $\{Al^{3+}\}$ 0 or 6.6 µM at pH 4.2 (Fig. 4) slightly decreased I_{max} and K_m of net Mg^{2+} uptake (I_{max} 8.5 and 8.4, K_m 25.3 and 83.1 for $\{Al^{3+}\}$ activities 0 and 6.6 µM, respectively; *cf.* with respective values obtained with no PCMBS supplied, Table II), but no significant differences were detected (from $P < 0.089$ to $P < 0.912$). In contrast, the addition of CCCP to nutrient solutions lacking Al significantly decreased net Mg^{2+} uptake (Y), which became linear in the

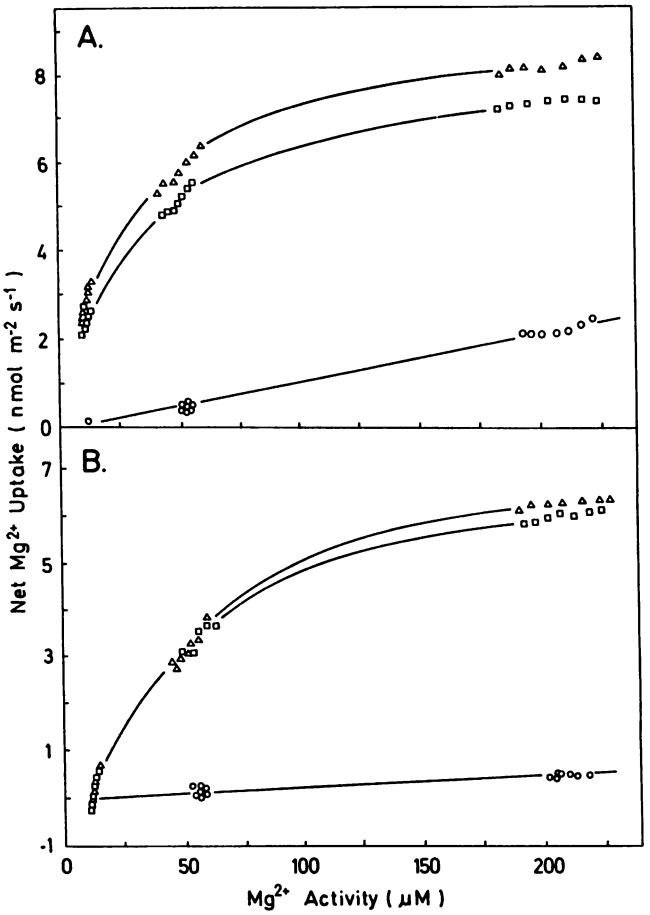

Figure 4. Influence of PCMBS and CCCP on the mass flow corrected net Mg^{2+} uptake by 23-d-old plants of Wilo ryegrass from the complete nutrient solution (pH 4.2). Nutrient solution had $\{Al^{3+}\}$ 0 (A) or 6.6 μM (B). The measurements were made at no inhibitor supplied (control, △), 100 μM PCMBS (□) and 1 μM CCCP (○).

range of $\{Mg^{2+}\}$ (X) tested ($Y = -0.06 + 0.01X$, $r^2 = 0.98$; for the units of measure see Fig. 4). The detrimental effect of CCCP on net Mg^{2+} uptake was even larger in nutrient solutions having 6.6 μM $\{Al^{3+}\}$ (Fig. 4). However, the linear relationship between net Mg^{2+} uptake and nutrient solution $\{Mg^{2+}\}$ was still significant ($Y = -0.05 + 0.002X$, $r^2 = 0.92$; for the units of measure see Fig. 4).

DISCUSSION

The depletion technique, as originally used to monitor K^+ influx (4), did not allow plants to approach steady state absorption since their nutrient environment was drastically changed over the short time. McLachlan *et al.* (23) showed that only in the first phase of a depletion period concentration of the nutrient in question is not limiting, and that only then plant processes control the nutrient uptake parameters (I_{max}, K_m) to be determined. Depletion of Mg^{2+} from the nutrient solution in the present study was rather limited in extent (not shown), and plant characteristics presumably exerted a large influence on net Mg^{2+} uptake.

It has recently been shown that using activities instead of concentrations in ion uptake studies may change the interpretation of results (9), especially where uptake of divalent and trivalent ions is monitored. In the present study, K_m of net Mg^{2+} uptake was significantly smaller when calculated from Mg^{2+} activities rather than from Mg^{2+} concentrations (not shown).

Since intact plants were used in the present study, observed values of net Mg^{2+} uptake represent average values for the whole root system. Uptake of Mg^{2+} may be quite variable along the root axis.

It has been shown that Mg^{2+} might move largely in the root free space in transpiration-supported solution flow (10). Therefore, the Mg^{2+} uptake data presented in this paper has been corrected for mass flow of Mg^{2+}. Net Mg^{2+} uptake (Fig. 1) appeared to show characteristics of discontinuous, saturable carrier-mediated transport, as was also shown earlier for net Mg^{2+} uptake (14, 19, 21) as well as for Mg^{2+} influx (13).

Although it has been well documented that -SH groups are often involved at the binding sites of both enzymes and transport proteins (8), the sulfhydryl reagent, PCMBS, did not significantly alter net Mg^{2+} uptake (Fig. 4). The apparent discrepancy observed here may be due to lack of -SH groups at the bindings sites of Mg transport proteins or due to recovery of these proteins during the course of the relatively long (12 h) depletion period.

The metabolic uncoupler, CCCP, did not completely inhibit net Mg^{2+} uptake (already corrected for mass flow of Mg^{2+}, Fig. 4). Experiments with KCN added to the nutrient solution of the depletion experiment gave the results that were essentially the same as those obtained with CCCP (data not shown). Energy dependence of Mg^{2+} uptake has been shown earlier in work with excised roots (19, 21). In addition, a nonactive component of Mg^{2+} uptake that was not transpiration dependent is suggested in the present study (Fig. 4). The linear, nonactive component of Mg^{2+} absorption does not necessarily need to be free diffusion. In contrast, a carrier-mediated character of this component of Mg^{2+} absorption is suggested (*cf.* ref. 18 for K^+ absorption), which is also in accordance with the hypothesis (24) that carrier-mediated uptake may become less energy dependent and more similar to facilitated diffusion as the external ion concentration is raised. The existence of a discontinuous, saturable component plus a linear component of ion uptake has been seriously questioned in favor of multiphasic ion uptake isotherms (25), but the present study does not allow such a distinction.

Apparently, Al^{3+} adversely affected the linear component of Mg^{2+} uptake (Fig. 4). This result might have been a consequence of greatly decreased amounts of Mg^{2+} cations bound in the root free space of ryegrass plants grown in Al-containing nutrient solution (32). Altered Mg^{2+} concentrations in the vicinity of postulated membrane transport proteins may affect Mg^{2+} transport across the membrane.

Lowering the pH of the nutrient solution did not change K_m but decreased I_{max} of net Mg^{2+} uptake, indicating noncompetitive inhibition. A decrease in Mg^{2+} uptake due to low solution pH has been previously observed (13, 19, 21).

Among all mononuclear Al complexes, Al^{3+} appeared to be the most powerful inhibitor of root growth (27). In the present study, the Al^{3+} was the prevalent Al species and is

most likely to have influenced Mg^{2+} uptake. It should, however, be emphasized that computational speciation of such a complex Al-containing solution is plagued with many uncertainties. From the estimates of monomeric Al concentrations (see "Materials and Methods"), it appears that the formation of solid-phase Al-P products has not been significant although it may theoretically occur under conditions tested (26 and references therein).

Compared to nutrient solution with no Al added, 6.6 μM $\{Al^{3+}\}$ considerably increased K_m while leaving I_{max} of net Mg^{2+} uptake unchanged (Table II), suggesting competitive inhibition. Increased K_m indicates the postulated transport proteins in the membrane have a lower affinity for Mg^{2+}. This might be due to (a) competition between Al cations (presumbly Al^{3+}) and Mg^{2+} for the binding sites on the transport protein molecules, or (b) competition between ATP-Mg^{2+} and 'ATP-Al' complexes (3, 29) for the active sites on ATPase, thus impairing the release of energy needed to create a negative electrochemical potential across the membrane. However, it was recently shown (16) that wheat roots subjected to Al stress maintained negative electrochemical membrane potential by retaining intact proton pump and, supposedly, ATPases. In the present study the metabolic uncoupler, CCCP, exerted greater deleterious effects on Mg^{2+} absorption than did 6.6 μM $\{Al^{3+}\}$ but smaller effects compared with 26 μM $\{Al^{3+}\}$ (Fig. 4B). This result suggests that Al^{3+} at 6.6 μM activity does not operate exclusively through the ATPase inhibition, but that binding of Al^{3+} to Mg^{2+} specific sites on transport proteins might have also taken place. Substitution of Al^{3+} for Mg^{2+} is based on similarity in effective ionic radii and has also been proposed for several animal *in vitro* systems (for the review see ref. 22). Alternatively, Al^{3+} at 6.6 μM activity may not be as efficient an inhibitor of energy-generating cell system as is CCCP. Aluminum at 26 μM $\{Al^{3+}\}$ in nutrient solution caused net loss of Mg^{2+} from roots (Fig. 2) thus showing greater deleterious effects than CCCP. This might have been due to Al-caused membrane damage. Greater $\{Mg^{2+}\}$ in nutrient solution diminished the deleterious effects of Al (Fig. 2). Increasing Mg^{2+} concentration has also ameliorated Al-induced impairment of wheat root growth (17). Therefore, the classical inhibition analysis of Al^{3+} effects on the process of Mg^{2+} uptake appears very hard to achieve because of the complexity of Al-caused deleterious effects unrelated to Mg^{2+} uptake and because of concentration (activity)-dependent Mg^{2+} protection of membranes and alleviation of Al^{3+} toxicity symptoms.

The deleterious effects of Al on Mg^{2+} absorption were more obvious for the cv Wilo than for the cv Gulf (Table II). Earlier experiments showed that the cv Wilo was much more sensitive to Al stress than the cv Gulf (31). Many mechanisms of differential Al tolerance among different crop cultivars have generally been proposed (for the review see ref. 11). The present study shows that ability of ryegrass cultivars to retain higher affinity for Mg^{2+} by transport proteins (lower K_m) in the presence of Al^{3+} might be one of the mechanisms of Al tolerance.

The Al-mediated difference in the apparent affinity of a Mg transporter between ryegrass cultivars tested in nutrient solution (Table II) does not appear to be large enough to account for possible differences in Mg^{2+} uptake from acid, Al-toxic soils because the Mg^{2+} concentrations in soil solutions generally range from 0.5 to 2 mM for leached, acid soils (1), thus exceeding the K_m values reported here (Table II). However, when the same two ryegrass cultivars were grown in acid (pH 3.8 in H_2O), Al-toxic Stough soil having $\{Mg^{2+}\}$ in soil solution 0.5 mM, the cv Gulf had greater shoot Mg concentration than the Al-sensitive cv Wilo although the statistical significance of this difference was not convincing ($P < 0.063$) (30). Subsequent attempts to mathematically model Mg^{2+} uptake by Gulf and Wilo ryegrass from Stough soil using kinetic parameters measured in nutrient solution failed to yield a good agreement with experimentally measured Mg accumulation from the soil (30). The complete understanding of the differences between ion uptake kinetics in nutrient solution compared with much more complicated soil system is beyond the present knowledge, especially if Al toxicity affecting a multitude of various biochemical and physiological processes is superimposed.

It has been shown that root meristem cell division, as the earliest symptom of Al toxicity, stopped after 6 h following addition of Al (6). The detrimental effect of Al^{3+} on Mg^{2+} uptake observed in the present study was much faster (<30 min) which might mean that Al^{3+} exerted deleterious effects on Mg^{2+} uptake at the site of the plasma membrane (where postulated transport proteins are situated) without necessarily entering the cell (see also ref. 34).

There is a growing body of evidence that the primary mechanism of Al^{3+}-induced toxicity in animal *in vitro* systems is mediated through the Al^{3+}/Mg^{2+} substitution at critical enzyme and regulatory sites in the cell (22). Further research to test this hypothesis in plants is warranted.

LITERATURE CITED

1. **Barber SA** (1984) Soil Nutrient Bioavailability: A Mechanistic Approach. Wiley-Interscience, New York
2. **Blamey FPC, Edwards DG, Asher CJ** (1983) Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. Soil Sci **136:** 197–207
3. **Bock JL** (1980) The binding of metal ions to ATP: a proton and phosphorus NMR investigation of diamagnetic meta-ATP complexes. J Inorg Biochem **12:** 119–130
4. **Claassen N, Barber SA** (1974) A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. Plant Physiol **54:** 564–568
5. **Clark RB** (1977) Effect of aluminum on growth and mineral elements of Al-tolerant and Al-intolerant corn. Plant Soil **47:** 653–662
6. **Clarkson DT** (1965) The effect of aluminium and some trivalent metal cations on cell division in the root apices of *Allium cepa*. Ann Bot **29:** 309–351
7. **Dalton FN, Raats PAC, Gardner WR** (1975) Simultaneous uptake of water and solutes by plant roots. Agron J **67:** 334–339
8. **Dixon M, Webb EC** (1979) Enzymes, Ed 3. Academic Press, New York
9. **Evangelou VP, Wagner GJ** (1987) Effects of ion activity and sugar polyalcohol osmotica on ion uptake. J Exp Bot **38:** 1637–1651
10. **Ferguson IB, Clarkson DT** (1976) Simultaneous uptake and translocation of magnesium and calcium in barley (*Hordeum vulgare* L.) roots. Planta **128:** 267–269
11. **Foy CD** (1988) Plant adaptation to acid, aluminum-toxic soils. Commun Soil Sci Plant Anal **19:** 959–987

12. **Grimme H** (1983) Aluminium induced magnesium deficiency in oats. Z Pflanzenernaehr Bodenk **146:** 666–676
13. **Hannaway DB, Leggett JE, Bush LP, Shuler PE** (1984) Magnesium (Mg) and rubidium (Rb) absorption by tall fescue. J Plant Nutr **7:** 1127–1147
14. **Joseph RA, van Hai T** (1976) Kinetics of potassium and magnesium uptake by intact soybean roots. Physiol Plant **36:** 233–235
15. **Keltjens WG** (1988) Short-term effects of Al on nutrient uptake, H^+ efflux, root respiration and nitrate reductase activity of two sorghum genotypes differing in Al-susceptibility. Commun Soil Sci Plant Anal **19:** 1155–1163
16. **Kinraide TB** (1988) Proton extrusion by wheat roots exhibiting severe aluminum toxicity symptoms. Plant Physiol **88:** 418–423
17. **Kinraide TB, Parker DR** (1987) Cation amelioration of aluminum toxicity in wheat. Plant Physiol **83:** 546–551
18. **Kochian LV, Lucas WJ** (1982) Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear component. Plant Physiol **70:** 1723–1731
19. **Leggett JE, Gilbert WA** (1969) Magnesium uptake by soybeans. Plant Physiol **44:** 1182–1186
20. **Lindsay WL** (1979) Chemical Equilibria in Soils. Wiley-Interscience, New York
21. **Maas EV, Ogata G** (1971) Absorption of magnesium and chloride by excised corn roots. Plant Physiol **47:** 357–360
22. **MacDonald TL, Martin RB** (1988) Aluminum ion in biological systems. Trends Biol Sci **13:** 15–19
23. **McLachlan KD, Yan-Hua K, Muller WJ** (1987) An assessment of the depletion technique for comparative measurement of phosphorus uptake in plants. Aust J Agric Res **38:** 263–277
24. **Nissen P** (1980) Multiphasic uptake of potassium by barley roots of low and high potassium content: Separate sites for uptake and transitions. Physiol Plant **48:** 193–200
25. **Nissen P** (1987) Multiple or multiphasic uptake mechanisms in plants? Plant Cell Environ **10:** 475–485
26. **Nordstrum DK, May HM** (1989) Aqueous equilibrium data for mononuclear aluminum species. *In* G Sposito, ed, The Environmental Chemistry of Aluminum. CRC Press, Boca Raton, FL, in press
27. **Parker DR, Kinraide TB, Zelazny LW** (1988) Aluminum speciation and phytotoxicity in dilute hydroxy-aluminum solutions. Soil Sci Soc Am J **52:** 438–444
28. **Parker DR, Zelazny LW, Kinraide TB** (1987) Improvements to the program GEOCHEM. Soil Sci Soc Am J **51:** 488–491
29. **Pfeffer PE, Tu S-I, Gerasimowicz WV, Cavanaugh JR** (1986) *In vivo* ^{31}P NMR studies of corn root tissue and its uptake of toxic metals. Plant Physiol **80:** 77–84
30. **Rengel Z** (1988) Effects of aluminum on magnesium uptake by ryegrass *Lolium multiflorum* Lam. PhD thesis. Louisiana State University, Baton Rouge
31. **Rengel Z, Robinson DL** (1989) Aluminum effects on growth and macronutrient uptake in annual ryegrass. Agron J **81:** 208–215
32. **Rengel Z, Robinson DL** (1989) Aluminum and plant age effects on adsorption of cations in the Donnan free space of ryegrass roots. Plant Soil **116:** 223–227
33. **Sposito G, Mattigod SV** (1980) GEOCHEM: A computer program for the calculation of chemical equilibria in soil solution and other natural water systems. Kearney Foundation of Soil Science, University of California, Riverside
34. **Zhao X-J, Sucoff F, Stadelmann EJ** (1987) Al^{3+} and Ca^{2+} alteration of membrane permeability of *Quercus rubra* root cortex cells. Plant Physiol **83:** 159–162