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Effect of Soil pH on Growth and Cation Deposition in the Root Tip of *Zea mays* L.

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Abstract

The effects of sandy soil pH on the distribution of growth velocities and on cation concentrations and deposition rates in root growth zones of *Zea mays* L. seedlings were investigated. The pH values of the rooting medium varied between 4.2 and 8.6 in sand culture (70% saturated) without external supply of nutrients. At all pH values, densities (in µmoles per g fresh weight) of potassium, magnesium, and calcium increased toward the root tip. Lower pH in the medium increased calcium tissue density fivefold and magnesium density 1.7-fold, whereas the density of potassium, the overall elongation rate, and the growth velocity distribution did not show any significant pH dependence. Throughout the growth zone the deposition rates of the divalent cations, as calculated on the basis of the continuity equation, increased with lower pH. The data are consistent with the hypothesis that the effects of pH on the cation deposition rates are due to the increase in the divalent cation concentration of the soil solution at low pH and that the abundant uronic acid residues of the young walls of the meristem provide a reservoir of storage capacity for Ca and Mg under conditions of low nutrient availability.

Key words: pH; Root growth; *Zea mays;* Calcium; Magnesium; Potassium

Introduction

Acid environments may affect root growth by direct interaction with growth processes or indirectly by impairment of nutrient relations (Marschner 1995). At the same time, growing roots affect the pH of the rhizosphere significantly by growth processes and nutrient uptake (Jaillard and others 1996; Kim and others 1999; Marschner and others 1986; Nye 1981; Ruiz and Arvieu 1990). Acidity can limit root growth in soil (Yan and others 1992) and nutrient culture (Lang and Kaiser 1994). Proton concentration can alter cell expansion directly according to the acid growth theory (Rayle and Cleland 1992) by changing cell wall properties (Virk and Cleland 1988). Despite a huge literature in favor of the acid growth theory, it has been criticized for the lack of evidence for a role in intact tissues (Kutschera and Schopfer 1985; Schopfer 1989). In contrast to above ground organs covered by a waxy surface that can cause experimental artifacts (Rayle and Cleland 1992), the root apoplast pH is somewhat affected by the external pH (Felle 1998). For roots, even cytoplasmic pH can be altered (Gerendas and others 1990; Reid and others 1985). Therefore, root growth may be influenced directly by variation in pH.

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Furthermore, pH conditions may impair uptake and the internal distribution of mineral nutrients (for example, Adams 1981; Mengel and Geurtzen 1988). Toxic ions, especially aluminum, are mobilized at low pH from ion exchange sites of soil (Kochian 1995) and impede root growth. If growth is affected, there will be an indirect impact on nutrient fluxes as new surface must be produced to mine nutrient uptake in unexplored soil. In the shorter term, there may be a feedback effect because reduced available surface area for nutrient uptake will reduce growth by reducing the supply of osmotica (Colmer and Bloom 1998; Frensch and others 1996; Silk and others 1986).

Analysis of nutrient relations in growing tissues needs to take into account the highly dynamic nature of growth zones (Schurr 1997). Mechanistic understanding of the interaction of growth processes and nutrient relations requires quantitative analysis of growth and nutrient distribution (Silk and others 1986): Root expansion rates can be changed by alterations of the length of the expansion zone and/or the intensity of expansion (Green 1976). Nutrient relations in the growth zone of roots can be addressed quantitatively on the basis of deposition rates that require data on growth distribution. In contrast to mature tissues, growing tissue has concentration profiles that are produced by the dual processes of nutrient import and cell expansion. For example, a uniform distribution of concentrations along the growing zone of a root indicates local nutrient deposition rates are balancing the local expansion rates.

In this article, the possibility of interaction between pH effects on growth and nutrient relations was addressed by analyzing simultaneously the impact of bulk medium pH on (i) the spatial distribution of growth and (ii) the profiles of ion concentrations and deposition rates in growing root tips at different pH in the soil medium.

Materials and Methods

Plant Cultivation

Seeds of *Zea mays* L. (cv. FR27RHM × FRM017RHM) were surface sterilized for 30 s in 3% H_2O_2 and washed three times (each 10–20 s) in twice distilled water. Afterward, they were imbibed in the distilled water for 9 h (same time each day) and germinated in vermiculite well moistened with 0.1 mM $CaCl_2$ for 48 h at 26°C. Seedlings with straight, long roots were selected for transplanting to Plexiglas boxes filled with moist, autoclaved sand (154 mL distilled water per kilogram sand) and covered with plastic wrap. Twenty seedlings were planted in predrilled holes (diameter, 1 mm; length, 10 cm) situated 2.5 cm apart along the panes of the three Plexiglas boxes and covered completely with sand. Growth was at 26°C, and seedlings were kept in the dark except for measuring and marking. Defined pH-values of 4.2, 5.4, 6.2, 7, and 8.6 were achieved in the sand by adding different amounts of HCl to the water before mixing it with the sand. The final pH of the sand was determined by preparing a 1:2 slurry of sand and water, incubating for 30 min at room temperature, shaking the slurry at 0 and 15 min, and measuring the supernatant pH with an Orion research 211 meter. Because this study is part of a larger project to examine interactions between growth zones and their rhizospheres (Kim and others 1999), the naturally low buffering capacity of the soil substrate was left unaltered in the experimental protocol.

Determination of pH of Rhizosphere Soil

Individual seedlings of *Zea mays* L. were transplanted into minirhizotrons made from 100 × 50 mm plastic Petri dishes (Falcon). The lid and bottom of each dish had a notch melted into the edge to allow the plants to grow into the substrate. The bottom half of each plate was filled with sand similar to that used in ion determination studies. A metal guidewire (diameter, 0.8 mm) was placed through the notch in the plate to give the roots a straight path for growth. A piece of nylon mesh, 136 µm pore size (Small Parts Inc.), cut to fit the circumference of the plate bottom, was placed over the wire. Additional sand was placed on top of the mesh, and the cover was taped in place to provide a sandwich arrangement so that the root was surrounded by substrate. The dishes were then set in a vertical position and the guidewire removed. A corn seedling (radicle length approximately 20 mm) was inserted into the hole left by the wire. Plates with plants, substrate, and mesh were arranged vertically and placed in a dark growth chamber at 26°C for 24 h. For measurement of rhizosphere pH, rhizotrons were taken from the growth chamber; the lids and mesh removed; and the plate with the seedling was placed vertically on the lab bench next to a micromanipulator (Olympus, Japan), to which a flat membrane pH microelectrode (MI-406, Microelectrodes, Inc.) was attached. A separate flat membrane reference microelectrode (MI-402, Microelectrodes, Inc.) was placed near the point of contact of the pH electrode. The seedling was quickly removed from the soil, and pH readings were taken 0.2-mm deep in the sand directly behind the root at the locations 4 mm and 12 mm basal to the root tip.

Growth Analysis

The tip growth velocity was followed by marking the position of the tip at the outside of the box initially and measuring the distance between this point and the actual position of the root tip with a ruler 3, 14.5, 18, and 20.5 h after transplanting. At 20.5 h the roots were between 30 and 80 mm long. The growth distribution within the apical 12 mm of the root was determined with a marking experiment. Eighteen hours after transplanting, 6 of the 20 roots (selected for average tip growth velocity) were marked at 1-mm increments with India Ink. Photographs of the marked roots were taken every 15 min between 19.25 and 20.5 h after transplanting. The images were digitized (2048 × 3072 pixels); and distances between corresponding marks were measured with a software package (Sigma Scan Pro, Jandel Scientific, Corte Madera, CA). One-millimeter grids positioned between sand and Plexiglas wall next to each measured root served for calibration. Because the image size was approximately 4 × 6 cm, one pixel corresponded to 20 µm.

Growth velocities v(x) [mm/h] of marks were calculated with the following difference formula:

$$\nu(x_a) = \frac{x_f - x_i}{1.25\ h} \tag{1}$$

where x_i is the initial distance of an ink mark from the root tip (photograph taken at 19.25 h); x_f is the final distance of an ink mark from the root tip (photograph taken at 20.5 h), and x_a is the average distance from the root tip during the time interval ($x_a = (x_i + x_f)/2$). The measurement interval of 1.25 h was necessary for analysis of the small velocities near the root tip.

The growth velocity data was fitted with a flexible logistic function (Morris and Silk 1992):

$$\nu(x) = \frac{\nu_0 \cdot \nu_f}{\left[\nu_0^n + (\nu_f^n - \nu_0^n) \cdot e^{-k(x-x_0)}\right]^{1/n}} \tag{2}$$

where v_0 is the velocity found at an arbitrarily chosen x_0 (selections: $v_0 = 0.01$ mm/h; $x_0 = 0.1$ mm); v_f is the velocity observed at the base of the growth zone (12 mm); parameters k and n were obtained from fitting of the data (Sigma Plot; Jandel Scientific; Corte Madera, CA).

Differentiation of Eq. (2) with respect to position gives an analytic expression for the relative elemental growth rate $r(x)$:

$$r(x) = \frac{\partial \nu}{\partial x} = \frac{k \cdot \nu(x) \cdot (\nu_f^n - \nu(x)^n)}{n \cdot \nu_f} \tag{3}$$

Fresh Weight Distribution and Mineral Composition

For the determination of the distribution of fresh weight and concentrations of K, Mg, and Ca along the growth zone, primary roots were harvested at 14.5 and 20.5 h after transplanting. Between 8 and 10 seedlings, selected for average root tip growth velocity, were taken from the Plexiglas boxes. Their roots were excised; adhering sand and liquid was removed with "Kimwipes" (Kimberly-Clark; Roswell, GA); and the roots were placed on moist, but not wet, paper towels. A major part of the root cap was excised and discarded by making a transverse cut with a sharp razor blade 0.5 mm from the tip of the root cap at the junction of the root cap and apex. The remaining roots were then sectioned into 1-mm serial segments, starting at the root apex and ending at 12 mm. The scale was marked on a millimeter grid, on which all roots were placed parallel to each other for the sectioning. The pooled segments were placed in a preweighed vial, weighed, and stored at −20°C until further chemical procedures.

All samples were oven-dried together at 65°C for 2 days. Ten milliliters 0.5 M HCl was added and the vials were shaken for 2 days at room temperature (Hunt 1982). Three hundred microliters of a CsLa-mixture (50 ppm Cs; 100 ppm La) were added to each sample to allow quantitative analysis. Ca and Mg were determined by flame atomic absorption spectroscopy; K by emission spectroscopy (Perkin Elmer 2380). Sand samples at the different pHs were mixed with small volumes of water and allowed to stand for 30 min; then the soil solution was vacuum extracted and also tested for Ca and Mg by flame atomic absorption spectroscopy and for K by emission spectroscopy. A series of standard solutions containing the three elements was used for calibration.

Deposition Rates

The deposition rate of a specific substance in growing tissue is given by the continuity equation (for example, Silk and others 1986):

$$D(x) = \frac{\partial C(x)}{\partial t} + \frac{\partial (C(x) \cdot \nu(x))}{\partial x} \tag{4}$$

where D represents the local deposition rate in nmoles mm^{-1} root length h^{-1}, C is the local density of the substance, expressed here as nmoles (of potassium, magnesium, or calcium) per mm root length, t is time, x is the distance from the root tip, and $v(x)$ is the local growth velocity according to Eq. (1) and (2), respectively. To express results on a

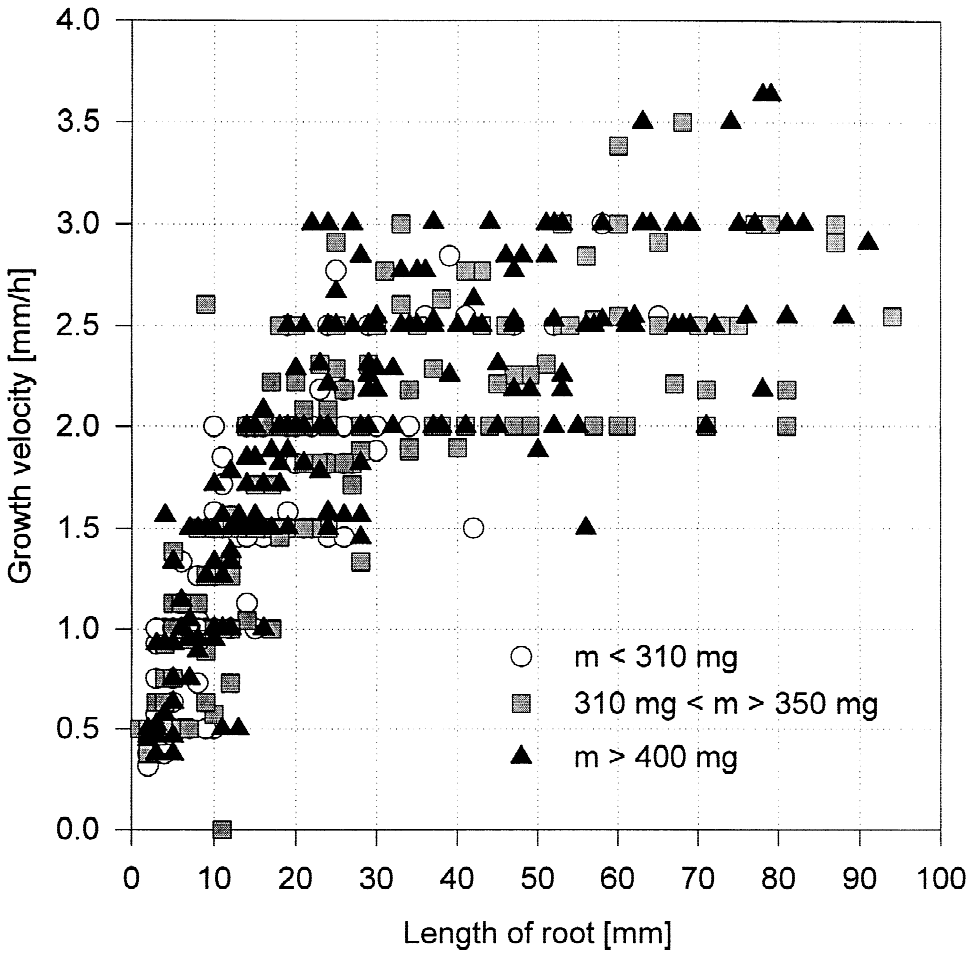

Figure 1. Relationship between growth velocity (V_{tip}) of the root and overall length of the root in *Zea mays* seedlings. V_{tip} was measured every 2 h over a 24-h period with an accuracy of 0.5 mm. Three populations were selected according to the seed dry weights (m < 310 mg, 310 mg < m < 400 mg, m > 400 mg).

fresh weight basis, *D(x)* can be divided by the fresh weight of the segment.

Results

Growth

Tip growth velocity was approximately constant after the roots had reached a length of 20–30 mm (Figure 1). Smaller roots achieved this constant root tip growth velocity asymptotically. No significant differences in root elongation velocity were present, even when the seed dry mass differed by more than 25%. Root tip growth velocities (V_{tip}, equivalent to overall root elongation rates, Silk 1992) of about 2 mm h^{-1} were observed. Within the investigated pH range, the proton concentration of the medium did not affect overall root growth rate (Figure 2). Elongation rates were measured two or three times for each population separately. Variation of growth velocities within the populations was considerable (see error bars in Figure 2), but not caused by diurnal variation, initial seed mass, and overall size of the root because roots larger than 30 mm were used (Figure 1). The distribution of growth velocities along the root growth zone was not affected by pH either (Figure 3). However, the spatial distribution of root growth rate varied among plants with different root elongation rates. Slow root growth was due to a lower maximal relative elemental growth rate (REGR) (0.35 h^{-1} in fast-growing roots; 0.25 h^{-1} in slow-growing roots) accompanied by a slight drift of the maximum of the REGR toward the root apex compared with fast-growing root tips.

Rhizosphere pH

Microprobe measurements of the pH in the sand just behind the growing root revealed that, over the large range of imposed pH, the rhizosphere pH did not differ much from that of the bulk soil (Table 1). This was not unexpected because the moist soil used in these experiments would have high ion diffusivity and low pH gradients. At 4 mm from the root tip (the region of greatest relative elemental growth rate) there was a slight increase in rhizosphere pH relative to the bulk soil pH for soils initially below pH

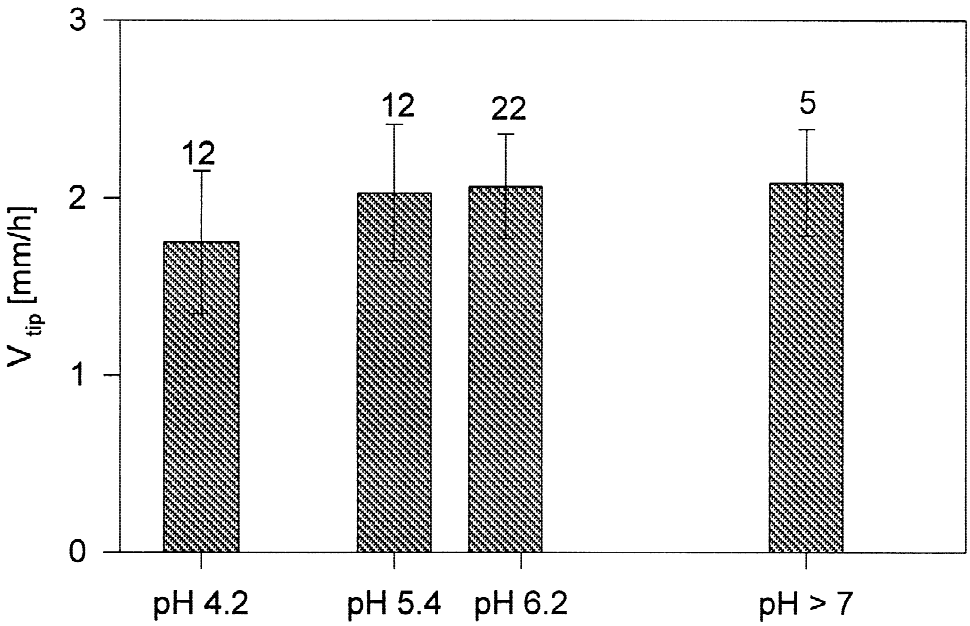

Figure 2. Growth velocities (V_{tip}) of roots of *Zea mays* grown at pH 4.2, 5.4, 6.2. and pH >7 (combined from populations gown at pH 7 [n = 1] and pH 8.6 [n = 2]). Values are means of two to three independent measurements of V_{tip}.

6. For soils with bulk pH greater than 6, there was a slight decrease in rhizosphere pH. These effects are consistent with reports in the literature (Nichol 2000; Peters and Felle 1999). However, the differences between rhizosphere pH and bulk soil pH were small compared with the range of imposed pH, and we can safely conclude that in the moist sand the rhizosphere pH, like the bulk soil pH, spanned three pH units in the experiments conducted to determine cation content of the root tissue. We note, however, that the pH of the plasma membrane may not vary much with bulk pH or even with rhizosphere pH. Recent evidence shows the maize root may maintain an internal apoplastic pH buffered to a rather narrow range of values over a wide range of external pH, even in the presence of a strongly buffered solution (Felle 1998); and theoretical considerations indicate the pH of the cellular compartments cannot be affected by simple transmembrane proton transport (Gerendas and Schurr 1999). Experiments with isolated membranes or patch clamping are needed to characterize the intrinsic dependence of the membrane-bound transport processes on pH.

Cation Content of Soil Solution

The pH of the bulk soil was associated with large differences in cation content of the soil solution (Table 2). The Ca and Mg concentration of the soil solution increased approximately 100-fold as the pH of the soil decreased from pH 7 to pH 4. The K content increased approximately 8.5-fold over the same range of decrease of bulk soil pH. This is not surprising because increased proton concentration would displace other cations from the negative charges on the particles of the sand.

Distribution of Fresh Weight and Cation Densities along the Root

The fresh weight distribution along the growth zone was identical to the one reported by Silk and others (1986). The fresh weight per mm of root length increased in the first 2–3 mm and remained uniform thereafter. Different medium pH values had no effect on absolute fresh weight or on distribution of fresh weight along the growth zone (data not shown).

The cation content in the apical 12 mm of the seminal root was divided by the fresh weight of the growth zone to obtain average tissue cation concentrations at different pH (Figure 4). Potassium content (95 µmol g^{-1} fresh weight, Figure 4A) was not affected by the external pH. In contrast, magnesium and calcium content in this root zone decreased substantially with increasing pH. The magnesium concentration had an average value of 11 µmol g^{-1} fresh weight at pH 4 and reached 6.5 µmol g^{-1} fresh weight at pH >7 (Figure 4B); the calcium concentration decreased from 4.5 µmol g^{-1} fresh weight at pH 4 to less than 1 µmol g^{-1} fresh weight at pH >7 (Figure 4C).

Cations were not uniformly distributed along the axis of the apical 12 mm of the root. Concentrations of all cations (on a fresh weight basis) declined considerably with distance from the root tip at all pH

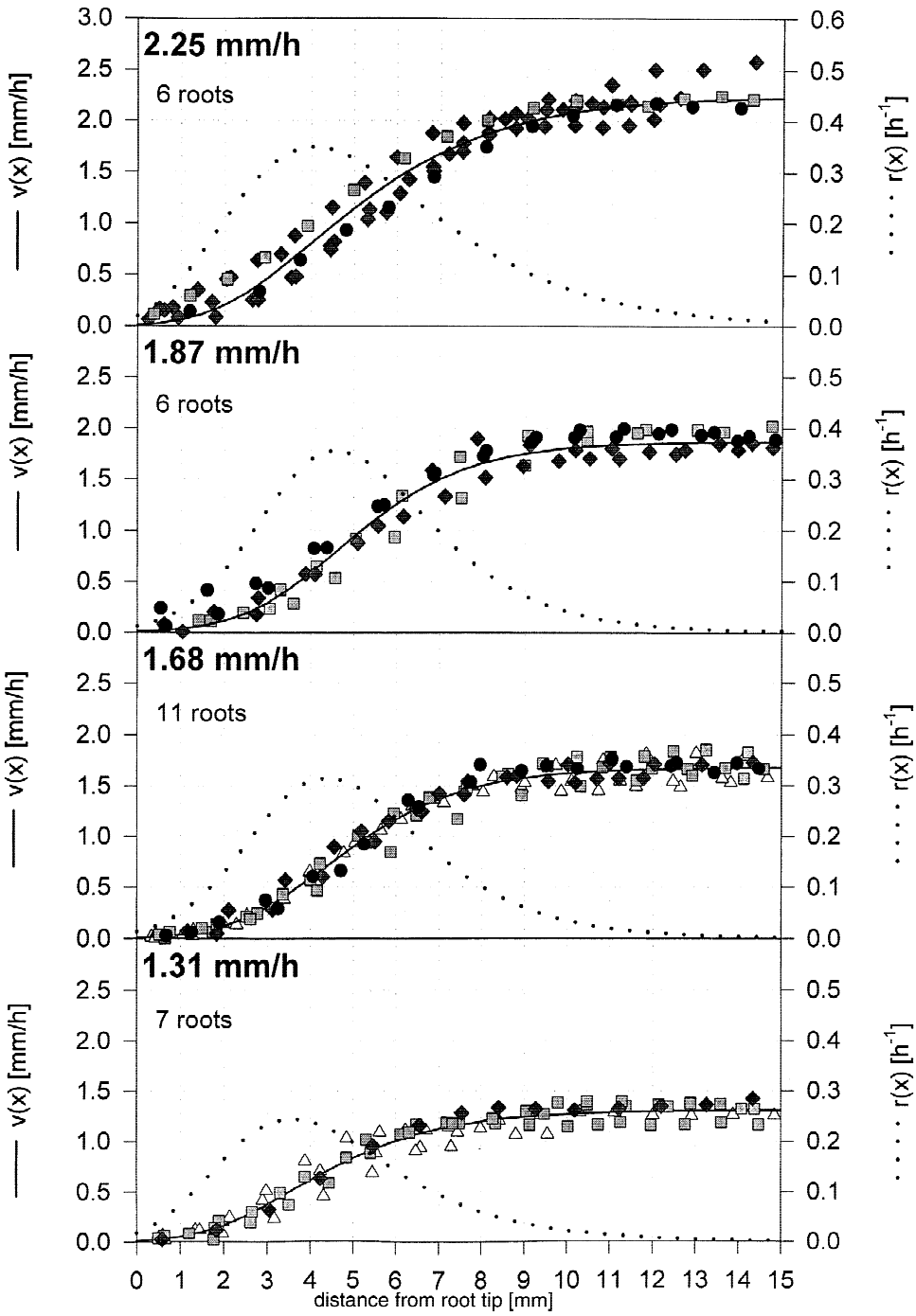

Figure 3. Velocity distributions along the root axis of roots from *Zea mays* seedling growing at different V_{tip}. The pH of the growth medium is indicated by different symbols: triangles denote growth at pH 4.2; squares, pH 5.4; diamonds, pH 6.2; and dark circles, pH >7. The regression line *(solid line)* corresponds to the velocity *v (x)* fit according to Eq. 3. The dashed lines depict the distributions of the relative elemental growth rates (*r(x)*) along the root axis of the populations growing at different V_{tip}.

treatments (Figure 5). The distribution function of potassium was not affected by external pH, and the root tip always exhibited a potassium concentration 2.5-times higher than the minimum (Figure 5, top panel). Minimal magnesium concentrations were also present at the basal end of the root segment, but the concentration at the tip was at least five times higher than at the basal end of the root segment (Figure 5, middle panel). The decrease in magnesium concentration with increasing pH could not be attributed to a specific region of the root but was observed equally over the entire region. Minimal calcium concentrations were present at the end of the expansion zone and increased slightly in the adjacent basal region of the root (Figure 5, lower panel). With increasing pH, calcium concentrations declined at the tip and the base of the growth zone, but the decline at the base was even higher. There-

Table 1. The pH of the Bulk Soil Substrate and in the Rhizosphere, 0.2 mm from the Surface of the Growth Zone, at the Location of Maximum Expansion (4 mm from the tip) and Just Beyond the Growth Zone (12 mm from the tip)

Dist. from Tip	Bulk soil pH	Rhizosphere pH		N
		4 mm	12 mm	
Avg.	3.72	3.91	3.75	
St. Dev.	0.08	0.05	0.07	7
Avg.	4.69	4.72	4.7	
St. Dev.	0.04	0.15	0.13	9
Avg.	6.01	5.83	5.85	
St. Dev.	0.07	0.14	0.17	8
Avg.	7.63	7.57	7.69	
St. Dev.	0.15	0.16	0.2	7

N *denotes the number of replicates used to find the average and standard deviation.*

Table 2. Effect of Soil pH on Cation Concentrations of the Soil Solution

Soil pH	Ca µM	Mg µM	K µM
7.08	0.18	0.09	0.06
6.51	0.39	0.22	0.07
4.95	3.52	2.66	0.25
4.01	19.96	8.97	0.52

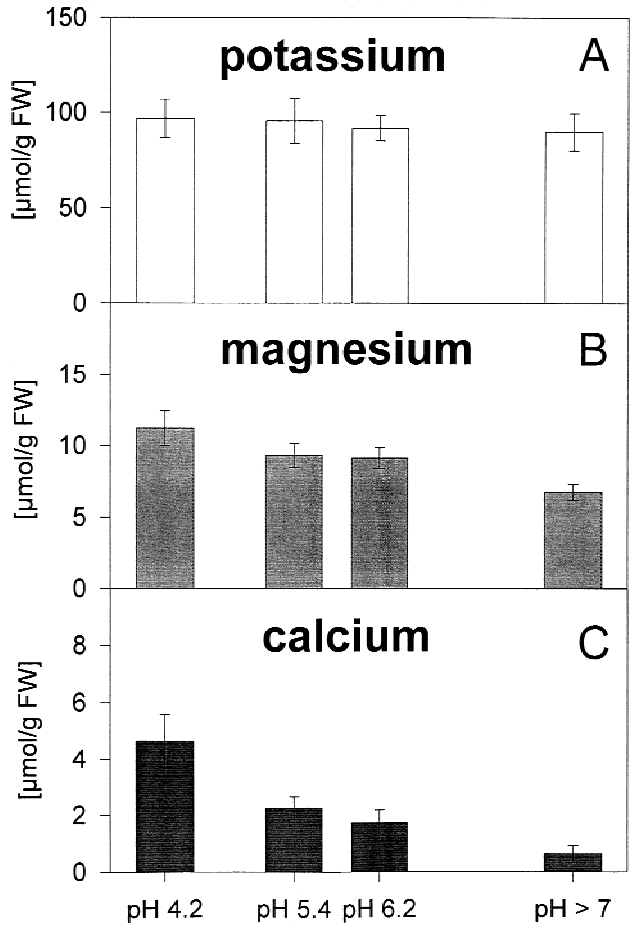

Figure 4. Mean cation amounts per gram fresh weight in root segments (0–12 mm after the tip) of *Zea mays* grown at pH 4.2, 5.2, 6.2, and pH >7 (combined from populations grown at pH 7 and pH 8.6).

fore, in low pH conditions the root tip had three times more Ca than the base of the growth zone, whereas at high pH the tip concentration was higher than that of the basal regions by a factor of 7.

On a time scale of hours, the distribution of cation concentrations was constant with time because samples taken 6 h after the first harvest within a population were not significantly different (data not shown). Thus the temporal term of Eq. (4) could be neglected. In addition, cation concentrations were independent of root tip elongation rates (data not shown).

Deposition Rates

Deposition rates for potassium, magnesium, and calcium were calculated according to Eq. (4). Positive deposition rates were found throughout the growing zone (Figure 6). Behind the expansion zone, the deposition rates of all cations reached slightly negative values indicating net loss/export of cations from these regions, probably to support the growth zone. The deposition rates and their distribution for cations and biomass (fresh weight) calculated in these experiments are in agreement with previous reports for maize roots (Silk and others 1986) and cotton roots (Zhong and Läuchli 1994) and support the earlier conclusions that the growth zone is a strong sink for cations (especially potassium and magnesium). The distribution of the deposition rates was quite different among the analyzed cations. The maximum potassium deposition rates of 30 nmol mm^{-1} h^{-1} occurred 3 mm behind the root tip, close to the maximum of root extension rate, and decreased strongly thereafter. For Mg, the maximal deposition rate of 1.5–2.5 nmol mm^{-1} h^{-1} was present in the most apical part of the root. For calcium, no distinct spatial maximum of deposition rate was present.

Because root growth velocities were not affected by the external pH, the deposition rates show responses analogous to the cation concentrations: de-

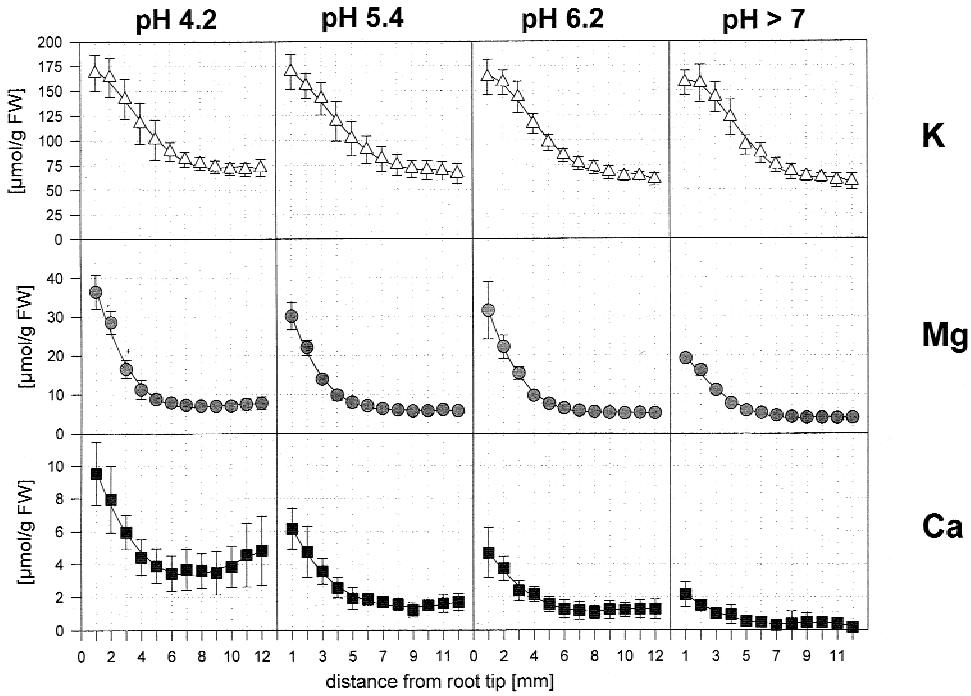

Figure 5. Distribution of amounts of potassium, magnesium, and calcium per gram FW along the roots of *Zea mays* seedlings grown at different pH (pH 4.2, $n = 6$; pH 5.4, $n = 6$; pH 6.2, $n = 10$; pH >7, $n = 3$). Regression lines are 6th order polynomials used to calculate deposition rates of Figure 6.

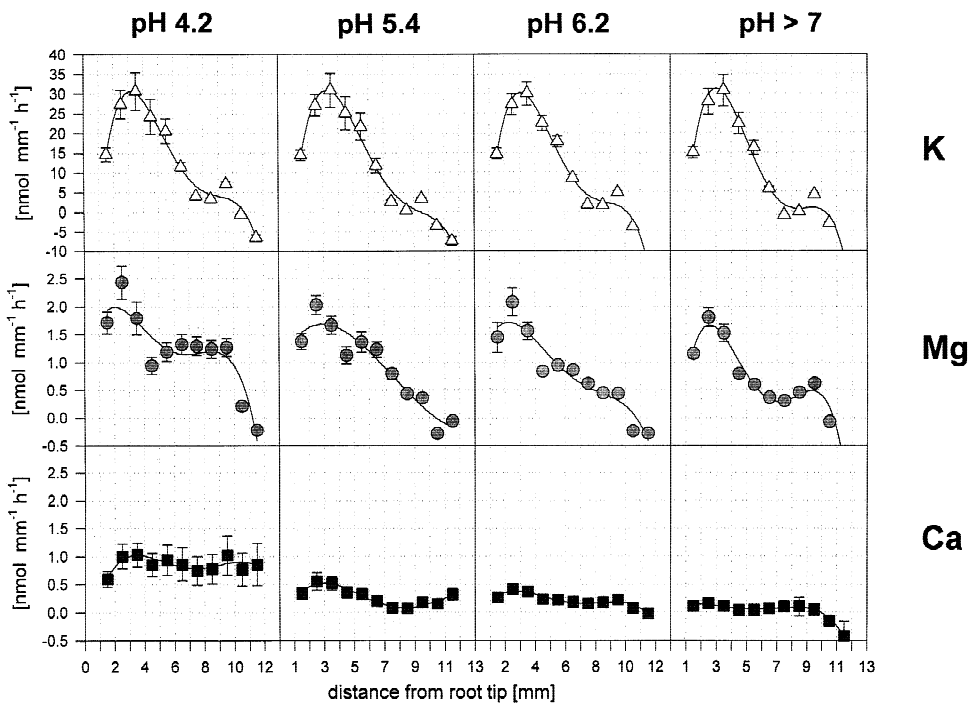

Figure 6. Distribution of deposition rates of potassium, magnesium, and calcium (per root segment and hour) along roots of *Zea mays* grown at different pH (pH 4.2, $n = 6$; pH 5.4, $n = 6$; pH 6.2, $n = 10$; pH >7, $n = 3$).

clining values for Mg and Ca throughout the whole growth zone with increasing pH, whereas potassium deposition rate was unaffected by pH. Magnesium deposition decreased with increasing pH, especially toward the base of the expansion zone. For calcium deposition rates a clear decline with soil pH was observed. The mean deposition rate fell from 1 nmol mm^{-1} h^{-1} (at soil pH 4.2) to 0.1 nmol mm^{-1} h^{-1} (at soil pH > 7).

Discussion

Lack of Effect of Soil pH or Calcium Content on the Growth Rate Pattern

Growth rates and their distribution along the root tip were comparable to previously reported data for maize at 26°C (for example, Barlow and Rathfelder 1985, Felle 1998, Fortin and Poff 1991, Silk and oth-

ers 1989). The observed maximal growth rates of 0.35 h^{-1} about 4 mm behind the root tip, where the average cell length is 90 µm (Silk and others 1989), imply a maximal cell extension rate of 30 µm/h for the cells in this region. This cell growth rate is comparable to the extension rates of elongating root hairs (Bibikova and others 1997: 60 µm/h; Jones and others 1995: 30 µm/h; Herrmann and Felle 1995: 100 µm/h). Root elongation rates increased during the initial phase of root establishment until the root reached a size of 20–30 mm. The distribution of growth rates along these small roots was not analyzed in detail, but with a steady-state growth zone being approximately 12 mm long, it is likely that the growth zone has not reached its full organization and steady-state conditions at a smaller root size. Preliminary data show that the expansion zone in roots smaller than 20 mm is shorter than in the roots used in this study (A. Walter unpublished).

At different pH of the rooting medium, root growth rates were not significantly different. While this manuscript was in preparation, similar results were reported for fast-growing maize roots in buffered nutrient solution (Peters and Felle 1999). Discrepancies with other findings (see introduction) may be due to differences in sensitivity of species to acid conditions. Variation of the pH of the rooting medium has consistently been reported to affect root growth rate in detached tissues (Edwards and Scott 1974; Virk and Cleland 1988) and attached root tips (Yan and others 1992) and has been taken as evidence in favor of the acid growth hypothesis (Rayle and Cleland 1992). However, when roots are subjected to a constant pH for some time, they adapt—at least in maize (Yan and others 1998 and in this article). The observation that even the distribution of growth rates along the growth zone is not different (Peters and Felle 1999; our Figure 3) indicates that the adaptation does not alter the basic organization of root growth. Growth stimulation by acid pH values has repeatedly been shown to be transient (Edwards and Scott 1974; Rayle and Cleland 1992) or was observed only at very low pH (Schopfer 1989; Yan and others 1998). In this context the relevance of the acid growth theory for controlling steady-state growth has to be questioned, especially in roots and other plant tissues not covered by a cuticle (Ridge and others 1991). Obviously, the growing tissue adapts to the low pH, as long as the pH does not actually harm the integrity of the cells. Wall loosening linked to wall acidification might be of special importance in short-term variation of growth or in the location of growth processes during, for example, tropisms (Barlow and Rathfelder 1985; Björkman and Cleland 1991), but growth can—in general—only be sustained, if, for example, constant delivery of cell wall material is guaranteed (Schurr 1997).

The calcium concentration in the cell wall has significant impact on the plastic wall extensibility (PEx: Virk and Cleland 1988). However, the rapid increase in PEx was only observed when it exceeded a threshold of approximately 200 µg Ca g^{-1} dry weight, a value not reached even at the root tip under low pH-conditions. Below this concentration, calcium concentration could be increased up to seven-fold without significant impact on the plastic extensibility of cell wall (Virk and Cleland 1988). This would explain why growth rate does not vary with the calcium increase associated with the low pH of our system.

Ion Concentration Patterns in the Growth Zone at Different External pH

Through the growth zone, ion concentrations decreased strongly away from the root tip for all measured cations (Figure 5). This observation is in agreement with findings in several species (Scott and others 1967; Jeschke and Stelter 1976; Silk and others 1986; Zhong and Läuchli 1994). Uptake and translocation of magnesium and calcium has been shown to be distributed in a similar manner (Ferguson and Clarkson 1976). Similar ion distributions with maxima in highly meristematic regions have been found in growing zones of leaves (Bernstein and others 1995; Hu and Schmidhalter 1998; Meiri and others 1992). Universally, calcium tissue densities are less in rapidly elongating regions than in meristems. However, calcium may accumulate to higher tissue concentrations in nongrowing tissue. For instance, in older regions of monocot leaves, calcium tissue density is quite high (op. cit.). Whereas vacuoles in the growing region of leaves contain only minor calcium concentrations, in older regions of leaves, calcium accumulates significantly in the vacuoles (Fricke and others 1994).

The most apical zone of the growing root is almost exclusively composed of meristematic and thus less-vacuolated cells. The concentrations found in this zone for potassium are in agreement with concentrations expected in the cytoplasm of higher plant cells (Marschner 1995). Only a minor percentage of potassium is located in the apoplast, and the bound fraction is even smaller (Mühling 1998). In contrast, concentrations of magnesium and calcium in our roots exceed the expected concentrations in the cytoplasm by a factor of 2 and 1000, respectively (cf. Marschner 1995), suggesting that magnesium and calcium are located in the apoplast. In roots a considerable amount of calcium in the apoplast is bound to the cell wall (Sentenac and Grignon 1981). Con-

centration of free calcium as measured in situ by ion selective electrodes in the cell wall is small (Björkman and Cleland 1991), but precipitation of Ca in the apoplast as oxalate (Marschner 1995) or calcite (Kinzel 1989) may occur. In growing regions the high production of CO_2 caused by high respiratory activity (Taiz and Zeiger 1998) could favor formation of $CaCO_3$ in the plant cell wall. Thus, the $[Ca^{2+}]$ must not exceed a physicochemical limit value (ion activity product $CaCO_3 = 4.8 * 10^{-9}$; saturation index; Durand and Bellon 1994), depending on the ionic strength, temperature, and pH. In low-pH environments, higher calcium concentrations may occur before the precipitation begins.

The binding of calcium in the cell wall is not very tight, as indicated by the short half-time for exchange with isotopes (Clarkson 1984, Cramer and others 1987, Kuhn and others submitted). The cation load of the cell wall is given by the fixed anionic groups in the cell wall that have to be permanently loaded with counterions to maintain electrical neutrality. Therefore, the ion load in the cell wall is exchangeable but not removable at a given wall composition. The major fixed anionic groups relevant for calcium binding belong to the pectin network in the cell wall. The increase in Ca and Mg concentrations at the root tip is likely to be due to a higher amount of pectins in meristematic cells. Uronic acids are most abundant at the root tip in *Zea mays* (Silk and others 1984) and are the predominant binding sites for calcium in these cell walls (Jarvis 1982). The decline of divalent cation concentrations may be linked to the "dilution" by nonpectic cell wall materials and chemical modification of the pectin network during cell wall maturation (McCann and Roberts 1994). Certainly, changes in the relative contribution of the cell wall to the overall fresh weight of a root segment should play a big role in the observed developmental decline of divalent cation density in the root growth zone. This hypothesis is supported by the quantitative agreement between the spatial decline in uronic acids (reported in Silk and others 1984) and the pattern of divalent cations in the growth zone reported here.

The preceding arguments suggest that the calcium and magnesium distribution patterns along the axis of the growth zone result from the developmental chemistry of the wall. Another possibility is that in young cells, calcium is stored in the endoplasmic reticulum (ER) of dividing cells, which are known to have high calcium and magnesium requirements for mitosis. Trewavas (1999) has pointed out that both the cell wall and intracellular compartments such as ER and mitochondria can be important repositories for calcium. Experiments should be conducted to test these possibilities because the developmental gradient in divalent cation density has been found in most of the growth zones examined to date.

At low pH, the calcium concentration per gram fresh weight was higher than under high-pH conditions at all sites along the root growth zone. This is difficult to understand in terms of the pH effect alone. Acidification of the apoplast should mobilize cations bound to uronic acid moieties of the cell wall and reduce the Ca content (Sentenac and Grignon 1991). However, the lower pH in the soil solution also causes displacement of Ca from the sand particles in our experiments (Table 1). Because calcium binding in the wall is extremely concentration-dependent for concentrations less than 20 µM Ca (Sentenac and Grignon 1991), Figure 5 and Table 1 are consistent with the hypothesis that in sandy soil decreasing pH leads to increasing Ca and Mg in the soil solution. Because of the high calcium affinity of the uronic acid residues, the higher cation concentrations in the medium could lead to higher concentrations of Ca and Mg in the uronic acid fractions of the wall, even with declining pH. This hypothesis can be tested by using defined nutrient media with calcium chelators and performing exchange experiments with externally supplied cations.

The difference in the response to variation of the external pH of potassium on the one hand and calcium and magnesium on the other hand is most easy to explain in terms of the contrasting distribution of the ions between cytoplasm and apoplast. Homeostatic mechanisms are thought to regulate the cytosolic K levels, and a number of genes for K uptake under different conditions have been discovered in recent years (see discussion of deposition rates). By contrast, the concentrations of magnesium and calcium in the apoplast are subject to considerable variations with pH. At low nutrient availability, the protonated uronic acid binding sites can provide a reservoir for storage of Ca and Mg. This may be advantageous to the dividing cells because it facilitates the intracellular uptake of calcium released from the soil at low pH.

Cation Deposition Rates in the Growth Zone at Different External pH

We note that the "deposition rates" of this study are net local rates averaged over tens of cells and tens of minutes. The rapid (seconds to minutes) transport processes that maintain the calcium homeostasis of the cytosol (for example see, Trewavas 1999) and produce observed oscillations in proton and calcium flux across the growth zone (Shabala and Newman 1998) are smoothed and averaged in our calculations.

Our observed independence of K deposition rate on pH is consistent with classical studies of root uptake from buffered solutions (Kochian and others 1989). The homeostatic mechanisms that regulate cytoplasmic potassium concentrations are beginning to be understood in molecular terms; at least three gene families are known to be involved. A consensus is emerging that potassium uptake is regulated by a number of inducible genetic mechanisms, depending on environmental conditions. Spalding and others (1999) have found two (passive) inward-rectifying K channel proteins that cause uptake into *Arabidopsis* root tips at low (10 µM) K concentrations. The AKT1 transporter mechanism is somewhat independent of pH, whereas a non-AKT1 protein is strongly stimulated by protons. Another gene family, the AtKUP genes, is known to cause potassium uptake into *Arabidopsis* roots (Kim and others 1998), but the pH dependence has not been characterized. A high-affinity active transport system in wheat, coded by the HKT1 gene, is not proton coupled (Rubio and others 1995) and thus may be a good candidate for regulating K uptake in our system.

Magnesium deposition rates peaked also in the meristem but decreased much less with position than potassium deposition rates. Calcium deposition rates were rather uniform within the growing zone. Because the mineral ion concentration in the rooting medium was very low, the main source of the nutrients may have been the seed. However, there seems to be a requirement for some external calcium to support seedling root growth even when significant amounts of calcium are present in the endosperm. Our results (Figure 5) are consistent with the notion that calcium reaches the apoplast from the soil solution, whereas some of the magnesium and potassium may arrive from the phloem. Further work should be done to test the effect of pH on mobilization of nutrients from older parts of the plant.

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