

Energy requirements for maintenance of ion concentrations in roots

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Maintenance of ion gradients across plant membranes is considered to be an important process requiring respiratory energy in plant tissues. In order to test this hypothesis, roots of intact plants of potato (*Solanum tuberosum* L. cv. Alcmaria and cv. Pimpernel) were incubated in a closed circulation system. Electrical conductivity of the solution surrounding these roots was continuously monitored to determine total ion efflux into demineralized water. Anion efflux rate from the symplast was 35 neq (g dry weight)⁻¹ s⁻¹. In combination with literature data on the specific costs of ion transport, this efflux rate yields the respiration rate associated with re-uptake balancing efflux (i.e. maintenance of cellular ion concentrations). The results suggest that energy costs associated with re-uptake of ions may account for up to 25–50% of the total respiratory costs involved in ion influx.

Key words – ATP costs, efflux, electrical conductivity, electrolyte leakage, energy costs, influx, ion uptake, maintenance, potato, respiration, roots, *Solanum tuberosum*.

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Introduction

Up to 50% of the photosynthates produced daily are respired (Lambers 1985). Depending on plant growth rate and age, 20–60% of the respiratory energy may be used for maintenance processes (Amthor 1984, Van der Werf et al. 1988). Knowledge about these maintenance processes will improve our understanding of plant growth and crop yield. A major part of the maintenance energy costs is supposed to be associated with (1) the maintenance of ion gradients across membranes and (2) protein turnover (Penning de Vries 1975). The energy demands of these processes in vivo are not well known and estimates are scarce (Amthor 1984). Recently, De Visser et al. (1992) showed that the specific energy costs of protein biosynthesis and turnover may well be twice as high as calculated before by Penning de Vries (1975). However, little work has been reported on the energy costs of maintenance of ion gradients across

membranes (i.e. maintenance of cellular concentrations) in whole plants, and it is the purpose of the present paper to approach this aspect.

The plant maintains a constant membrane potential and a constant composition of the cytosol (Lauer et al. 1989, Tomos and Wyn Jones 1988). Here we address the question whether maintaining ion concentrations under steady-state conditions in roots is quantitatively important for their total energy requirements. The energy requirements for maintenance of ion concentrations are calculated as the product of the specific costs of ion uptake as derived from literature data and the fraction of active transport that compensates for efflux. This fraction of transport is estimated as being quantitatively equivalent to the rate of total ion efflux from intact roots in demineralized water, as measured by monitoring the electrical conductivity of the medium. However, efflux not involving energy-dependent re-uptake, like efflux from the free space and efflux of some cations

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(e.g. cellular potassium) from the symplast, must be excluded in these calculations. Therefore, the efflux kinetics from the Donnan phase and the nature of the cations in the efflux medium and root extracts have been experimentally determined.

Abbreviations – Dp, Donnan phase; EC, electrical conductivity; eq, mol equivalent charge; RGR, relative growth rate; WFS, water free space.

Materials and methods

Plant material

Potato plants (*Solanum tuberosum* L. cv. Alcmaria and cv. Pimpernel) were propagated by in vitro culture, and grown in a circulating aerated Hoagland solution [macro nutrients at 1/2 strength according to Hoagland and Snijder (1933) and micro nutrients at 1/2 strength according to Lewis and Powers (1941), with 90 μM iron as Fe(III)-EDTA]. Entangling of the roots of neighbouring plants was prevented. A uniform supply of nutrients was provided for each individual plant by rapid circulation from a central storage vessel. The light conditions were: 12 h 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD provided by fluorescent tubes (Philips TLD 36W/54) and incandescent lamps (Pope 60 W) in a ratio 12:1. Incandescent light (Pope 60 W) was supplied for another 6 h after the 12-h light period, to prevent tuber induction. Temperature was $18 \pm 2^\circ\text{C}$, and relative air humidity 70%. In all experiments vegetative plants were used at various ages ranging from 18 to 48 days.

Electrical conductivity measurements

Before transfer to the experimental set-up, roots of intact plants were gently rinsed in two separate volumes (500 ml) of demineralized water for less than a min in order to remove adhering nutrient solution. The roots were then incubated in a circulation system filled with demineralized water (Fig. 1) to monitor net charge efflux by electrical conductivity (EC). The EC was measured using a WTW LF530 conductivity meter and WTW LTA01 electrode, and a WTW TFK530 sensor for automatic temperature compensation (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) and expressed in $\mu\text{S cm}^{-1}$. Efflux was calculated from the slope of the change of EC against time at various moments after start of the incubation. At the end of some of the experiments (2 to 29 h after start of incubation; $n = 5$), the medium was sampled to obtain an estimate of the relative contribution of the different cations to the efflux.

Our objective was to prevent re-uptake during efflux by maintaining the external concentrations low compared to the k_m of the uptake system. The concentration in the circulation system (Fig. 1) is determined by the efflux rate integrated over the experimental time ($\int \Phi_{\text{eq}}^{\text{io}} dt$, on a dry weight basis) multiplied by root dry

weight and divided by the volume of the system (V). This volume (300 ml) was a compromise between (1) the ideal infinite volume (a zero source for uptake) and (2) a small volume/root-weight ratio to enable detection of changes in EC. Efficient mixing is required to minimize development of boundary layers close to the roots. Since the EC electrode is sensitive to electromagnetic fields, the medium was mixed by circulation with a pump (Iwaki magnet pump model md-6, max capacity 8 to 9 l min^{-1} ; Iwaki CO., Ltd., Tokyo, Japan). As indicated by the rapid changes in EC after injection of salt solutions, the mixing rate was adequate for the time resolution required (equilibration time less than 1 min). All other conditions were similar to the growth conditions.

The circulation system could be changed from a 300 ml closed system (valve 8 open, valves 9 closed) to an open system (valve 8 closed, valves 9 open), in which the medium was continuously replaced (Fig. 1). Preliminary measurements showed no significant differences in efflux between the two systems, indicating that the closed system behaves like a zero source of ions for re-uptake over the experimental period. Further experiments were performed with the closed system.

Calculation of ion efflux from electrical conductivity measurements

The efflux measured as the change of EC of the medium (ΔEC , $\text{mS m}^{-1} \text{s}^{-1}$) was transformed to a charge equivalent basis. Ion efflux of roots ($\Phi_{\text{eq}}^{\text{io}}$, $\text{eq (g DW)}^{-1} \text{s}^{-1}$; eq is one mol charge equivalents) is calculated according to:

$$\Phi_{\text{eq}}^{\text{io}} = (\Delta\text{EC} \times V) / (\Lambda_a \times \text{DW}) \quad (1)$$

where V is the volume of the measuring system (m^3), Λ_a is the average conductance of equivalent charge ($\text{mS eq}^{-1} \text{m}^2$) and DW is the dry weight of the root (g).

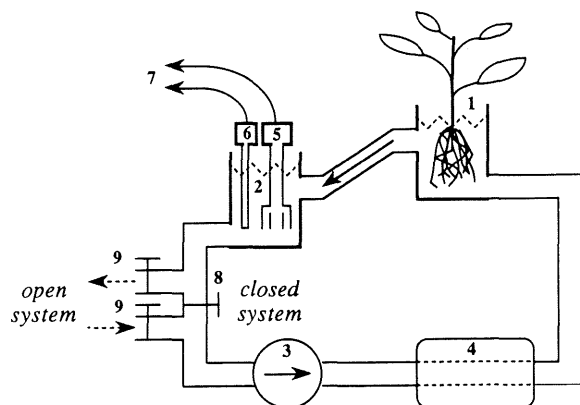


Fig. 1. Incubation system for monitoring electrolyte efflux from intact roots. 1, Plant cuvette; 2, electrode cuvette; 3, pump; 4, temperature control; 5, EC electrode; 6, thermocouple; 7, EC meter; 8 and 9, valves.

Although the relationship between conductance (Λ) and concentration is complex (Chang 1981), it is linear for strong electrolytes at the low concentrations used here. This was verified by calibration with 0 to 1 mM KNO_3 and KCl ($r^2 > 0.99$). The molar conductances as calculated from single ions (after Anonymous 1978), agreed well with our calibrations. Because of the small differences between ion species in Λ (after Anonymous 1978), the overall conductivity of a mixture of ions as expressed on electronic charge basis is hardly affected by the chemical nature of the ions (except for protons and hydroxyl anions). Therefore, the average conductance of equivalent charge ($\Lambda_a = 6.42 \text{ mS eq}^{-1} \text{ m}^2$) was calculated as the average of the average equivalent conductances of K^+ and $1/2 \text{ Ca}^{2+}$ (6.05) and the average of NO_3^- , Cl^- and $1/2 \text{ SO}_4^{2-}$ (6.79; after Anonymous 1978) at the same temperature as used for automatic correction of the output values by the WTW LF 530 (20°C).

Kinetics of loss from the Donnan phase

To enable correction for total efflux from the free space (i.e. loss from the Donnan phase [Dp] and WFS), data on the kinetics of the slowest component of this efflux (i.e. the efflux from the Dp) were used. Cell wall calcium, a major component of the Dp, is reversibly exchangeable against magnesium (Sentenac and Grignon 1981), which is mainly located in the symplast (Hanson 1984). Therefore, we measured the reversible exchange of calcium for magnesium and used these data to calculate the half-life of the Dp ($t_{1/2}$) according to:

$$t_{1/2} = (-t \ln 2) / (\ln V_t - \ln V_{t_0}) \quad (2)$$

where t is the time from the start of the exchange, V_t is the rate of loss of ions from the free space at time t and V_{t_0} is the initial rate of loss, both proportional to the ion concentration in the free space. The reversible exchange was determined in vessels with a relatively small volume (100 ml), to obtain levels of ions detectable by atomic absorption spectrometry within 10 min. The medium was stirred by aeration, and could be rapidly changed without disturbing the roots, via a tap in the bottom of the vessel.

In the first experiment (Dp-1), two plants were used. With the first plant the exchange/efflux rates of calcium and magnesium were determined during incubations for one h each of the roots into successively (1) MgCl_2 , (2) CaCl_2 , (3) MgCl_2 , (4) CaCl_2 and (5) demineralized water. Salt concentrations were 50 mM. The second plant was used to measure the efflux of calcium and magnesium into (6) demineralized water for one h.

In the second Dp experiment (Dp-2), the efflux/exchange rates of calcium and magnesium were measured on 4 plants (two of each cultivar). The media were changed in the following order: incubations for one h each in successively (1) demineralized water, (2) MgCl_2 , (3) CaCl_2 , followed by incubations of 30 min each in (4)

MgCl_2 , (5) CaCl_2 and (6) demineralized water. Salt concentrations were 23 mM.

During all incubations in both experiments Dp-1 and Dp-2, we replaced the medium every 10 min, and kept it for chemical analyses. To remove adhering medium, roots were briefly washed twice in the type of medium to be used for the next treatment.

Calculation of respiratory costs of re-uptake

Efflux [$\Phi_{\text{eq}}^{\text{io}}$; eq (g DW) $^{-1}$ s $^{-1}$] is expressed on a molar basis [Φ_j^{io} ; mol ion j (g DW) $^{-1}$ s $^{-1}$] by dividing half of $\Phi_{\text{eq}}^{\text{io}}$ by the ion valency of the average cat- or anion j (z_j). Due to electroneutrality the ratio anion/cation equivalents is 1, and thus half of $\Phi_{\text{eq}}^{\text{io}}$ is for cations and half for anions.

$$\Phi_j^{\text{io}} = 0.5 \times \Phi_{\text{eq}}^{\text{io}} / z_j \quad (3)$$

From this rate (after correction for the free space) and various literature data, the respiratory energy cost for re-uptake balancing efflux for either cat- or anions was calculated according to:

$$m_{c-j} = \Phi_j^{\text{io}} \times M_j \times (H/I_j) \times (H/P)^{-1} \quad (4)$$

where m_{c-j} is the energy cost for maintaining concentrations for ion j [mol ATP (g DW) $^{-1}$ s $^{-1}$], Φ_j^{io} is the efflux of ion j [mol ion j (g DW) $^{-1}$ s $^{-1}$], M_j is the number of active membrane passages of ion j , H/I_j is the ratio between the protons and ion j [mol H^+ (mol ion j) $^{-1}$] and H/P is the stoichiometry of the proton-ATPase [mol H^+ (mol ATP) $^{-1}$]. By dividing m_{c-j} by the P/O_2 -ratio of the oxidative phosphorylation [mol ATP (mol O_2) $^{-1}$], the respiratory costs on an oxygen basis are obtained [mol O_2 (g DW) $^{-1}$]. Equation 4 describes the calculation method for secondary active anion transport. In case of re-uptake by primary active transport, the product $(H/I_j) \times (H/P)^{-1}$ should be replaced by the stoichiometry of the ion j -ATPase [P/I_j , mol ATP (mol ion j) $^{-1}$].

In our calculations we assumed one membrane passage to be involved in re-uptake balancing efflux. Transport (efflux and influx) of potassium does not require metabolic energy (Hedrich and Schroeder 1989) because the K^+ concentration is generally near the Nernst equilibrium. The proton:ATP stoichiometry of the proton-ATPase (H/P) is 2 (as measured for the tonoplast; Bennett and Spanswick 1984, Guern et al. 1989). Since the major anion absorbed is nitrate (Marschner 1989), we used the anion-valency ($z_i = 1$) and proton/ion ratio ($H/I_j = 2$) of nitrate in our calculations. Under the present growth conditions, the potato plants had a relative growth rate (RGR) of 0.15 g (g DW) $^{-1}$ day $^{-1}$ (Bouma et al. 1992). We assumed an average alternative pathway activity of 30% [P/O -ratio of 2.4; after Tab. 3 in Poorter

et al. (1991) for plants with a RGR less than $0.2 \text{ g (g DW)}^{-1} \text{ day}^{-1}$].

Chemical analyses of root extracts, media from exchange experiments, and media from efflux measurements

In order to relate leaking electrolytes to the cellular ionic contents, two roots of control plants were extracted by boiling for 5 min in 200 ml demineralized water. Before extraction, the roots were washed in demineralized water. Both extracts and samples of the efflux media of the EC measurements were analyzed for K^+ , $\text{Fe}^{2+/3+}$, Mg^{2+} , Ca^{2+} and Na^+ , whereas the samples of Dp-1 and Dp-2 were analyzed for Mg^{2+} and Ca^{2+} . All cation analyses were done by atomic absorption spectrometry (VARIAN SpectraAA-10, Varian Techtron Pty. Ltd., Springvale, Australia). All data were corrected for the mineral content of demineralized water samples stored under identical conditions as the efflux media and extracts.

Statistics

The 95%-probability intervals for efflux (EC measurements) and exchange (experiment Dp-2) rates were calculated using Student's *t*-values.

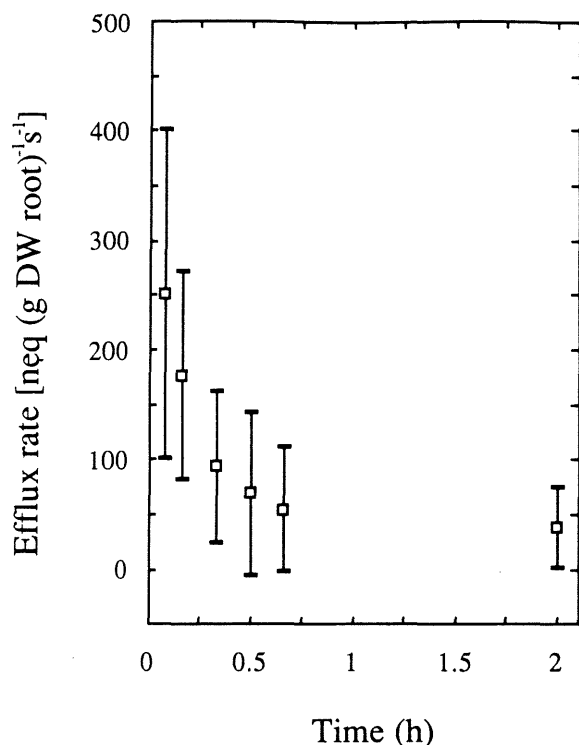


Fig. 2. Efflux of charge equivalents [$\text{neq (g DW)}^{-1} \text{ s}^{-1}$] from potato roots incubated in demineralized water. Bars indicate 95%-probability intervals.

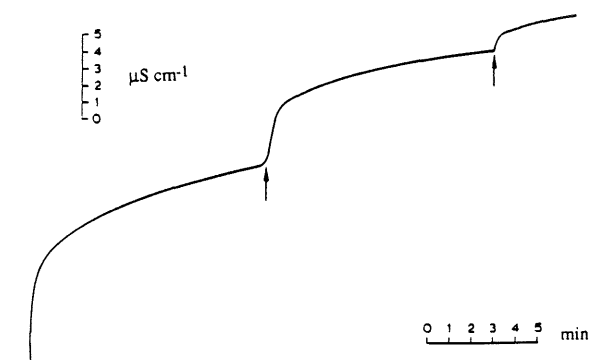


Fig. 3. Stimulation of root efflux rate in response to removing the plants from the incubation system and then immediately returning them to the original position. The moments of the start of the treatment are indicated by arrows.

Results

Electrolyte efflux, and nature and half-life of the Donnan phase

Since there were no significant differences between the two potato cultivars, the time course data of the anion plus cation efflux [$\text{neq (g DW)}^{-1} \text{ s}^{-1}$] of both cultivars were combined (Fig. 2). Damaging the roots induced high ion efflux into demineralized water (data not shown). Gently removing the plants from this medium and immediate return to the original position also increased efflux (Fig. 3). However, comparison with efflux kinetics of untreated roots (Fig. 2) revealed that this increase was transient (duration less than 10 min). The high initial efflux is partly due to previous treatments (rinsing and transfer) of the roots (cf. Fig. 3).

A comparison of the relative contribution of the cations released into the efflux medium with those in extracts of the roots showed that, although potassium was quantitatively the most important cation in the root (ca 77%), calcium was the most abundant one in the efflux medium (ca 67%). This calcium is unlikely to originate from the cytosol, because the cytosolic calcium concentration is tightly regulated at a very low level (ca $1 \times 10^{-6} \text{ M}$; Lühring and Tazawa 1985, Williamson and Ashley 1982). Cell walls however, contain a large amount of calcium (Demarty et al. 1984), suggesting that the calcium in the efflux medium originated predominantly from the free space. Efflux of ions from the free space is not compensated for by active transport and was, therefore, excluded in calculations of respiratory costs of re-uptake balancing efflux.

Reversible exchange for magnesium showed that the Donnan phase contained calcium (Figs 4 and 5), with a half-life of ca 9 min (cf. eq. 2) as it decreased by at least 90% within half an hour (Figs 4A and 5). The exchange could not be explained by adhering solution, because only little calcium was found in demineralized water after a CaCl_2 treatment (Fig. 4A). Thus the adhering solution was effectively removed during rinsing between

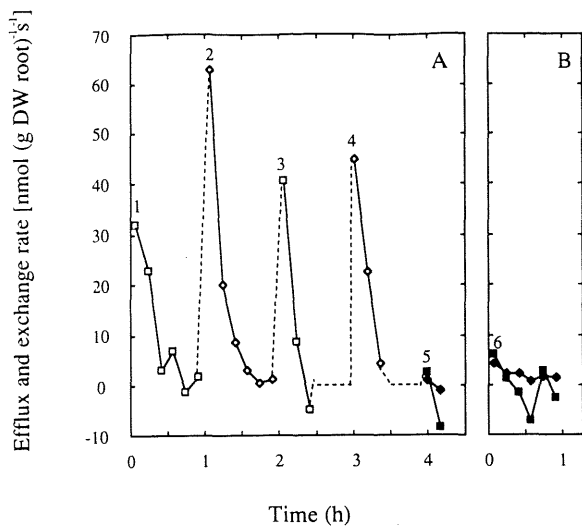


Fig. 4. The rate of exchange of calcium in $MgCl_2$ (\square ; 1 and 3) or magnesium in $CaCl_2$ (\diamond ; 2 and 4) and the efflux of calcium (\blacksquare) and magnesium (\blacklozenge) in demineralized water (5 and 6). The results of the different plants are shown in the figures A and B, respectively. Full details are given in Materials and methods.

treatments. Negative values for the efflux in demineralized water (Fig. 4A,B) are due to the variation in the samples combined with the correction for the ion content of control samples of demineralized water.

Calculation of respiratory costs of re-uptake

Respiratory costs for re-uptake of anions lost by efflux (m_c) were estimated according to equations 3 and 4 (Tab. 1). Efflux from the free space was excluded in calculations of respiratory costs by using the electrolyte efflux as measured after 30 min. At this moment the remaining efflux originating from the free space is found to compensate approximately for the decrease in efflux from the symplast (details in discussion). Efflux data after 10 min and 2 h are presented to show the effect of a higher and lower efflux rate on respiratory costs (Tab. 1). In the calculations we assumed that (1) all calcium originates from the WFS and Dp, (2) the symplasmic efflux consists of potassium accompanied by anions, resulting in overall electroneutrality and (3) the anions consist predominantly of nitrate. The calculated respiratory costs for re-uptake of anions lost by efflux (m_c) after 10 min, 0.5 h and 2 h were 88, 35 and 19 nmol ATP $(g\ DW)^{-1}\ s^{-1}$ or 18, 7 and 4 nmol $O_2\ (g\ DW)^{-1}\ s^{-1}$, respectively (Tab. 1).

Discussion

Evaluation of the electrical conductivity technique

Isotopic labelling is commonly used for examining in- and efflux kinetics. A limitation of this laborious technique is the small number of ion species that can be

handled in one experiment. Therefore, no overall measure of ion efflux is obtained. Electrolyte leakage, measured by electrical conductivity (EC), is a commonly used index of membrane integrity and thus potentially suitable for monitoring total ion efflux from intact plant roots. It has been used for the evaluation of the extent of (1) senescence of plant tissues (Borochoy and Faragher 1983), (2) chilling injury (Hetherington et al. 1988), (3) seed germination capacity (Senaratna et al. 1988) and (4) injury caused by herbicide surfactants (O'Donovan et al. 1983). Here we discuss the validity of measuring the efflux rate as change of EC in demineralized water, and consider the experimental problems in comparison with the labelling technique.

Calcium stabilizes membranes and shields negative charges (Ferguson 1984, Hanson 1984). No calcium was added to the efflux medium, because it lowers the sensitivity for measuring EC due to an increased background signal. In labelling experiments this technical limitation is absent. This lack of external calcium was not considered to be a problem as plants were grown with ample nutrients, the experiments were short, and requirements are at millimolar activities (Hanson 1984). Moreover, considering that the initial efflux consists mainly of calcium from the cell wall, the calcium concentration in the medium would reach the millimolar range within 10 min.

The present method provides information on the kinetics of *net* ion efflux rates. Therefore, ion efflux may have been underestimated for two reasons. Firstly, total

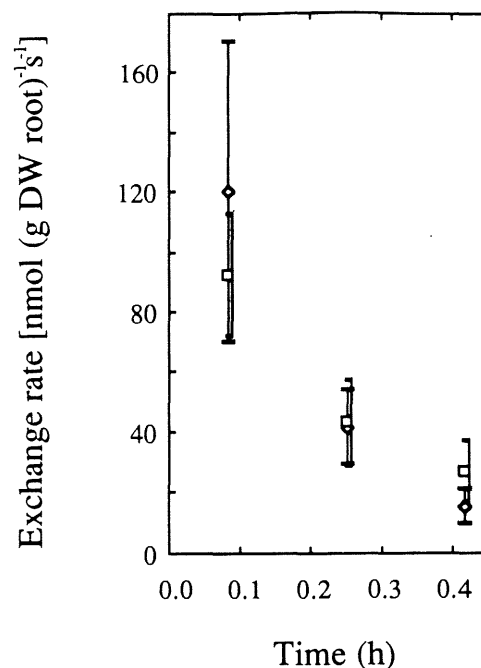


Fig. 5. The rate of efflux or exchange of magnesium in $0.023\ M\ CaCl_2$ (\diamond) and of calcium in $0.023\ M\ MgCl_2$ (\square) after washing for 60 min in (a) $0.023\ M\ MgCl_2$ and (b) $0.023\ M\ CaCl_2$. Bars indicate 95%-probability intervals.

Tab. 1. Calculation of (1) the ATP costs, (2) the respiratory costs and (3) the fraction of literature values for total or maintenance respiration (references in last column), which are explained by re-uptake balancing efflux in roots (maintaining gradients). In the calculations we used an anion-valency (z_i), proton/ion ratio (H/I_i) and the proton: ATP-ratio (H/P) of 1, 2 and 2, respectively.

Parameter	Time (min)			ADP/O -ratio	References
	10	30	120		
Leakage rates					
$\text{nS m}^2 (\text{g DW})^{-1} \text{s}^{-1}$	1.13	0.44	0.25		
$\text{neq} (\text{g DW})^{-1} \text{s}^{-1}$	176	69	38.8		
$\text{neq} (\text{g FW})^{-1} \text{s}^{-1}$	11.8	4.6	2.6		
Costs for re-uptake (absolute values)					
$\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	88	34.5	19.4		
$\text{nmol O}_2 (\text{g DW})^{-1} \text{s}^{-1}$	14.7	5.8	3.2	3	
$\text{nmol O}_2 (\text{g DW})^{-1} \text{s}^{-1}$	18.4	7.2	4.1	2.4	
Fraction of root respiration					
% of 355 $\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	24.7	9.7	5.5	2.4	Bouma (unpublished data)
% of 536 $\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	16.5	6.4	3.6	2.6	Lambers et al. (1983)
% of 240 $\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	36.7	14.4	8.1	2.4	Poorter et al. (1991)
Fraction of root maintenance costs					
% of 48 $\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	186	73	41	2.4	Bouma et al. (unpublished results)
% of 24 $\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	367	144	81	2.4	Poorter et al. (1991)
% of 18 $\text{nmol ATP} (\text{g DW})^{-1} \text{s}^{-1}$	490	190	107	2.3	Veen (1980)

efflux is underestimated if re-uptake from the medium occurs. In labelling studies this problem is regarded as being small, because re-uptake of the labelled ions is statistically less likely due to the excess of unlabeled ions (Jackson et al. 1976). In EC measurements this stochastic process is absent. Re-uptake will depend on (1) the external concentration (and thus measuring period) and (2) the K_m and V_{max} of the uptake system. These uptake parameters vary with nutrient supply over days (Lee and Drew 1986, Siddiqi et al. 1990). However, large changes are not likely to occur within 30 min. For example, Clarkson (1986) mentions that cells that have never been exposed to nitrate, upon exposure increase their nitrate uptake capacity from the constitutive level [$1 \mu\text{mol} (\text{g FW})^{-1} \text{h}^{-1}$] so that, after a lag period of 0.5 to 1.5 h, they reach a 2 to 5 times higher steady state level after 4 to 6 h (Fig. 1 in Clarkson 1986). De novo protein synthesis is involved in these adaptations (Fig. 2A in Clarkson 1986). Although the external anion concentration rises to about $100 \mu\text{M}$ after 2 h (cf. Fig. 2), this concentration is only 1% of that of the

growth medium (9.0 mM anions). Therefore, influx will be much lowered under these conditions. This notion is supported by our preliminary comparison, where the open and the closed system (Fig. 1) did not show different efflux rates. The second possible cause of underestimating the efflux rate, is a fast re-uptake within the tissue (from the cell wall) relative to the exchange between tissue and well-stirred medium. This possibility is more likely in a complex tissue than in a unicellular system. However, this problem relates to both labelling studies (described in a model by Morgan et al. 1972) and EC measurements alike, and cannot be resolved with present-day techniques.

In summary, EC measurements have the advantage that an overall measure for all ions is obtained. Incubation for more than about an hour should be avoided, as efflux will be affected both by the lack of calcium and by re-uptake from the medium. However, during short-term experiments these problems are expected to be small. Re-uptake from within the tissue cannot be assessed in any of the experimental ap-

Tab. 2. Half-life ($t_{1/2}$) of ions in different plant compartments as derived from the literature and measured for calcium in the present experiments. The ratio Cyt/Dp indicates the ratio between the half-life of the cytosolic ions and the Donnan phase (P_i and $^{15}\text{NH}_4^+$), or the free space when no data on the Donnan phase were available ($^{13}\text{NO}_3^-$).

Ions	$t_{1/2}$ (min)						Ratio Cyt/Dp	References
	WFS	Dp	FS	Cell	Cyt	Vacuole		
P_i	2.69			25.9				Lefebvre and Clarkson (1984)
	2.2	11.5			111	26.3×60	9.7	Ron et al. (1988)
$^{15}\text{NH}_4^+$	1.89	11.5			35.5	15.5×60	3.1	Macklon et al. (1990)
$^{15}\text{NO}_3^-$	5.8				126	140×60		Macklon et al. (1990)
$^{13}\text{NO}_3^-$			0.5		7		14.0	Siddiqi et al. (1991)
Ca^{2+}		9						Present paper

Tab. 3. Leakage rates for anions as derived from the literature for various species and experimental conditions. The literature data were obtained for roots by labelling techniques. Some authors indicate the origin of the efflux. This is shown by the superscripts, ^c, ^{c+v} and ^m for cytosol, cytosol with vacuole and inner space, respectively. For some data, indicated by *, it is not clear whether weights are for fresh or dry matter.

Anion and species	Anion leakage rate from roots		References
	[nmol (g DW) ⁻¹ s ⁻¹]	[nmol (g FW) ⁻¹ s ⁻¹]	
Nitrate			
<i>Pisum sativum</i>	0.47±0.03		Oscarson et al. (1987)
<i>Pennisetum americanum</i>	29.7 ±5.64		Pearson et al. (1981)
<i>Zea mays</i>	12.4 ±0.81		Pearson et al. (1991)
<i>Hordeum vulgare</i>		1.03 ± 0.14	Lee and Clarkson (1986)
<i>Hordeum vulgare</i>		3.44	Clarkson et al. (1989)
<i>Allium cepa</i>		0.22–1.11 ^{c+v}	Macklon et al. (1990)
<i>Hordeum vulgare</i>		0.0083–0.41 ^c	Siddiqi et al. (1991)
<i>Hordeum vulgare</i>		0.07–0.24	Wieneke and Nebeling (1990)
<i>Curcubita pepo</i>		0.41–0.53	Wieneke and Nebeling (1990)
Phosphate			
<i>Carex rostrata</i>	0.30–0.45		Atwell et al. (1980)
<i>Carex acutiformis</i>	0.20–0.32		Atwell et al. (1980)
<i>Triticum aestivum</i>	0.52–11.46 ^m		Cogliatti and Santa Maria (1990)
<i>Zea mays</i>		0.0033–0.0097 ^m	Elliott et al. (1984)
<i>Hordeum vulgare</i>		0.06 ^{c+v}	Lefebvre and Clarkson (1984)
<i>Allium cepa</i>		0.13 ^{c+v(*)}	Ron et al. (1988)
<i>Hordeum vulgare</i>		0.07–0.16	Schjørring and Jensén (1984)
<i>Brassica napus</i>		0.15–0.37	Schjørring and Jensén (1984)
Sulphate			
<i>Daucus carota</i> L.		0–3.10 ^{c(*)}	Cram (1983)
Parameter	Anion leakage rate from roots		References
	[neq (g DW) ⁻¹ s ⁻¹]	[neq (g FW) ⁻¹ s ⁻¹]	
Total			
Minimum	0.64	0.0116	References above
Maximum	47	7.7	References above
Measured by EC			
After 10 min	88	5.9	Present paper
After 30 min	34.5	2.32	Present paper
After 2 h	19.4	1.31	Present paper

proaches presently available. Therefore, we regard the present data as an adequate estimate for calculating the energy costs for maintenance of ion concentrations in roots.

Interpretation of efflux kinetics

Efflux from the free space needs to be excluded from total root efflux when calculating energy costs of re-uptake balancing efflux. From the calcium exchange rate, we calculated the half-life of the slowest component of the free space (Donnan phase) to be 9 min. This half-life agrees well with literature values (Tab. 2) obtained by compartmental analysis (Baker and Hall 1988) in labelling experiments. Because these data have been derived for multi-cellular systems (intact roots or root segments), results should be considered with care. The internal localization of the cellular compartments within the tissue might hamper a straightforward interpretation; numerous conditions must be met for a

proper analysis (Baker and Hall 1988, Zierler 1981). Also, the half-life values vary strongly, e.g. depending on whether ¹⁵N or ¹³N label is used (Macklon et al. 1990). However, we regard our estimated half-life reliable, as it is obtained by an alternative method, agreeing well with most of these literature values (Tab. 2).

The overall initial efflux [ca 250 neq (g DW)⁻¹ s⁻¹] originates from Dp, WFS, cytosol and vacuole. As the half-life of the Donnan phase is 9 min, after 30 min at most 25 neq (g DW)⁻¹ s⁻¹ originates from the free space and thus at least 44 neq (g DW)⁻¹ s⁻¹ (i.e. 69 less 25; Fig. 2) from the symplast. Using the ratio between the half-lives of cytosol and of Donnan phase as calculated in Tab. 2, the half-life of efflux from the cytosol is expected to be between 126 (i.e. 14 × 9) and 28 (i.e. 3.1 × 9) min. Regarding these extremes, after 30 min the initial cytosolic efflux rate would be underestimated by ca 8 to 49 neq (g DW)⁻¹ s⁻¹ (i.e. 18 to 110% of the cellular efflux after 30 min). Because this expected underestimation overlaps the expected overestimation due

to efflux from the free space [i.e. at most 25 neq (g DW)⁻¹ s⁻¹], we regard the efflux after ca 30 min as an adequate estimate of the efflux rate from the symplast, and thus suitable as input for calculating the energy costs of maintaining concentrations. Efflux rates after 10 min and 2 h were used to illustrate the relative effects of increase or decrease of the efflux on the costs for re-uptake of ions lost by efflux (Tab. 1).

The efflux calculated after 30 min is relatively high (Tab. 3), especially when considering that part of the literature data from the labelling studies were not corrected for initial efflux rates from the free space. Possible reasons for this high value are: (a) all anions are included in efflux, (b) nutrient supply was high during growth, (c) some organic ions may have been present, or (d) perturbation effects lasted for more than 30 min, but do not occur in labelling studies. Treatments can influence membrane potential and thus ion transport (Rincon and Hanson 1986), ion uptake (Bloom and Sukrapanna 1990) and root pressure (Miller 1987). The labelling studies in the literature show a large variation in ion efflux from roots (Tab. 3), probably partly due to different experimental conditions and interpretation of efflux kinetics.

However, the obtained efflux expressed as fraction of influx agrees well with values determined by means of isotopes. Efflux amounted to 50–85% (³⁶ClO₃⁻; cited in Clarkson 1985), 63% (¹⁵N and ¹³N; Tab. 5 in Clarkson et al. 1989) or up to 88% (¹⁵N; Tab. 1 in Pearson et al. 1981). In the present experiments, root net N uptake was estimated from the product of plant RGR (0.15 g g⁻¹ day⁻¹), N content [4.13 mmol N (g DW plant)⁻¹] and plant to root ratio [7 g DW plant (g DW root)⁻¹] at 4.3 mmol N g⁻¹ day⁻¹ or 50 nmol N g⁻¹ s⁻¹. When assuming anion efflux to consist predominantly of nitrate and thus assuming other anions to be negligible, efflux rate equals 100 × 34.5/(50 + 34.5) = 41% of nitrate influx (Influx = |Efflux| + Net uptake). Comprising other anions yields a slightly lower value (e.g. 34% assuming phosphate and sulfate to be 10% of nitrate in- and efflux).

In summary, the efflux rate observed after 30 min is a good estimate of the initial efflux from the symplast. Although the absolute value of this efflux rate is high (Tab. 3), the relative efflux rate as fraction of the influx rate is lower than the highest values found in the literature.

Comparison of respiratory costs of re-uptake to respiration rates

Table 3 compares the calculated respiration rates for re-uptake with the average overall and maintenance respiration of roots. For the data of Poorter et al. (1991) we estimated an average value for the species with an RGR of less than 0.2 g (g DW)⁻¹ day⁻¹, as the efflux was measured on potato plants with a RGR of only 0.15 g (g DW)⁻¹ day⁻¹. The respiration rates of potato roots

were transformed to an ATP basis by assuming a P/O₂-ratio of 4.8 [after Tab. 3 in Poorter et al. (1991) for plants with a RGR less than 0.2 g (g DW)⁻¹ day⁻¹]. The overall root respiration of potato was measured on young roots with a higher RGR [i.e. 0.21 g (g DW)⁻¹ day⁻¹] than the plants used in the present experiments, whereas maintenance respiration was estimated on roots with a similar RGR. The data of Veen (1980) were transformed using a dry matter content of 7% (B. W. Veen, personal communication) and a P/O₂-ratio of 4.6 mol ATP (mol O₂)⁻¹ (B. W. Veen, personal communication).

The respiration rate calculated for re-uptake after 30 min can easily explain the total maintenance respiration rate indicated by Poorter et al. (1991) and Veen (1980), and up to 73% of the maintenance costs of potato (Tab. 1). Before concluding that maintenance of ion gradients is the most important maintenance process, it is necessary to consider the estimates of maintenance respiration. All these values are obtained by the multiple regression method, as introduced by Veen (1980). In this statistical method, respiration rate is attributed to growth, ion uptake and maintenance as far as it correlates with the rate of dry weight increase, ion uptake rate, or root biomass, respectively. Depending on the presumed mechanism of efflux (e.g. slip of carrier or membrane permeability), re-uptake balancing efflux is expected to correlate with either ion uptake or root biomass. Thus, in the regression approach, costs for re-uptake of ions lost by efflux might be incorporated in the costs for uptake or maintenance. Our present calculations suggest that either a (major) part of these costs is included in the uptake costs instead of in the maintenance coefficient, or maintenance of ion gradients is indeed the quantitatively most important maintenance process. The latter inference is highly unlikely as Van der Werf et al. (1992) calculated that protein turnover accounts for 24–48% of the maintenance costs of roots, indicating that the maintenance of gradients is not the only maintenance process in roots. Moreover, the specific energy costs of protein turnover as used by Van der Werf et al. (1992) may have been underestimated by a factor 2 to 4 (cf. De Visser et al. 1992). Therefore, we hypothesize that energy costs for re-uptake balancing efflux are predominantly included in the regression coefficients of uptake. This contention is supported by the findings of Van der Werf et al. (1988) and Clarkson (1985; based on the data of Veen, 1980), who both calculated uptake costs to be twice as high as theoretically expected.

The energy costs of re-uptake balancing efflux may be assessed relative to the costs of net uptake and influx. Clarkson (1985) presented a mechanistic account of the energy costs of root ion uptake. We revised some of his assumptions according to more recent findings. The proton/ATP stoichiometry was considered to be 2 (as measured for the tonoplast; Bennett and Spanswick 1984, Guern et al. 1989). Two active membrane pas-

sages were assumed for ion uptake into the xylem, as Marschner (1989) indicates that xylem loading might be active and most ions will be transported into the xylem. However, in re-uptake balancing efflux from the symplast to external medium, only one membrane interface is involved. At a net anion uptake rate of $16.7 \text{ nmol (g DW root)}^{-1} \text{ s}^{-1}$ ($60 \text{ } \mu\text{mol g}^{-1} \text{ h}^{-1}$; Clarkson 1985) and two membrane passages per ion, costs of net anion uptake are $35 \text{ nmol ATP (g DW root)}^{-1} \text{ s}^{-1}$. The calculated influx costs equal the sum of the costs for efflux (Tab. 1; assuming efflux of nitrate only) and net uptake, i.e. $69.5 \text{ nmol ATP (g DW root)}^{-1} \text{ s}^{-1}$. In this case, efflux costs are ca 50% of influx costs. Considering the efflux of all anions, this value becomes negligibly lower [e.g. efflux costs become $32.8 \text{ nmol ATP (g DW root)}^{-1} \text{ s}^{-1}$ and thus 48% of the influx costs, when assuming phosphate and sulfate to be 10% each of the efflux]. In our plants, nitrate uptake rate was $50 \text{ nmol N (g DW root)}^{-1} \text{ s}^{-1}$, giving an ATP hydrolysis rate of $100 \text{ nmol ATP (g DW root)}^{-1} \text{ s}^{-1}$. Here, influx costs for nitrate equal $135 \text{ nmol ATP g}^{-1} \text{ s}^{-1}$. Efflux costs are 26% of influx costs, and ca 10% of total root respiration of young potato plants.

In conclusion, the energy costs of the re-uptake that balances efflux, are clearly a significant part of influx costs (ca 25–50%) and respiration in roots.

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References

- Amthor, J. S. 1984. The role of maintenance respiration in plant growth. – *Plant Cell. Environ.* 7: 561–569.
- Anonymous. 1978. CRC Handbook of Chemistry and Physics 59th Ed.; A Ready-reference Book of Chemical and Physical Data (R. C. Weast, M. J. Astle, eds), p. D-205. CRC Press Inc., Boca Raton, FL. ISBN 0-8493-0549-8.
- Atwell, B. J., Veerkamp, M. T., Stuiver, C. E. E. & Kuiper, P. J. C. 1980. The uptake of phosphate by *Carex* species from oligotrophic to eutrophic swamp habitats. – *Physiol. Plant.* 49: 487–494.
- Baker, D. A. & Hall, J. L. 1988. Introduction and general principles. – In *Solute Transport in Plant Cells and Tissues* (D. A. Baker and J. L. Hall, eds), pp. 1–27. Longman Scientific and Technical, Essex. ISBN 0-582-00580-9.
- Bennett, A. B. & Spanswick, R. 1984. H^+ -ATPase activity from storage tissue of *Beta vulgaris*. – *Plant Physiol.* 74: 545–548.
- Bloom, A. J. & Sukrapanna, S. S. 1990. Effects of exposure to ammonium and transplant shock upon the induction of nitrate absorption. – *Plant Physiol.* 94: 85–90.
- Borochoy, A. & Faragher, J. 1983. Comparison between ultraviolet irradiation and ethylene effects on senescence parameters in carnation flowers. – *Plant Physiol.* 71: 536–540.
- Bouma, T. J., Spitters, C. J. T. & De Visser, R. 1992. Variation in leaf respiration rate between potato cultivars: effect of developmental stage. – In *Molecular, Biochemical and Physiological Aspects of Plant Respiration* (H. Lambers and L. H. W. van der Plas, eds), pp. 512–522. SPB Academic Publishing, The Hague. ISBN 90-5103-079-7.
- Chang, R. 1981. Electrolyte solutions. – In *Physical Chemistry with applications to Biological Systems*, pp. 198–204. Macmillan Publishing Co., Inc., New York, NY. ISBN 0-02-321040-0.
- Clarkson, D. T. 1985. Factors affecting mineral nutrient acquisition by plants. – *Annu. Rev. Plant Physiol.* 36: 77–115.
- 1986. Regulation of the absorption and release of nitrate by plant cells: A review of current ideas and methodology. – In *Fundamental, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants* (H. Lambers, J. J. Neeteson and I. Stulen, eds), pp. 3–27. Martinus Nijhoff Publishers, Dordrecht. ISBN 90-247-3258-1.
- , Saker, L. R., Purves, J. V. 1989. Depression of nitrate and ammonium transport in barley plants with diminished sulphate status. Evidence of co-regulation of nitrogen and sulphate intake. – *J. Exp. Bot.* 40: 953–963.
- Cogliatti, D. H. & Santa Maria, G. E. 1990. Influx and efflux of phosphorus in roots of wheat plants in non-growth-limiting concentrations of phosphorus. – *J. Exp. Bot.* 41: 601–607.
- Cram, J. 1983. Characteristics of sulfate transport across plasmalemma and tonoplast of carrot root cells. – *Plant Physiol.* 72: 204–211.
- Demarty, M., Morvan, C. & Thellier, M. 1984. Calcium and the cell wall. – *Plant Cell Environ.* 7: 441–448.
- De Visser, R., Spitters, C. J. T. & Bouma, T. J. 1992. Energy cost of protein turnover: theoretical calculation and experimental estimation from regression of respiration on protein concentration of full-grown leaves. – In *Molecular, Biochemical and Physiological Aspects of Plant Respiration* (H. Lambers and L. H. W. van der Plas, eds), pp. 493–508. SPB Academic Publishing, The Hague. ISBN 90-5103-079-7.
- Elliot, G. C., Lynch, J. & Läuchli, A. 1984. Influx and efflux of P of roots in intact maize plants. – *Plant Physiol.* 72: 204–211.
- Ferguson, I. B. 1984. Calcium in plant senescence and fruit ripening. – *Plant Cell Environ.* 7: 477–489.
- Guern, J., Mathieu, Y., Kurdjian, A., Manigault, P., Manigault, J., Gillet, B., Beloeil, J. C. & Lallemand, J. Y. 1989. Regulation of vacuolar pH in plant cells. II. A ^{31}P NMR study of the modifications of vacuolar pH in isolated vacuoles induced by proton pumping and cation/ H^+ exchanges. – *Plant Physiol.* 89: 27–36.
- Hanson, J. B. 1984. The functions of calcium in plant nutrition. – In *Advances in Plant Nutrition*. Vol. 1 (P. B. Tinker and A. Läuchli, eds), pp. 149–208. Praeger Publishers, New York, NY. ISSN 0743-8931.
- Hedrich, R. & Schroeder, J. I. 1989. The physiology of ion channels and electrogenic pumps in higher plants. – *Annu. Rev. Plant Physiol.* 40: 539–569.
- Hetherington, P. R., Broughton, H. L. & McKersie, B. D. 1988. Ice-encasement injury to microsomal membranes isolated from winter wheat crowns. – *Plant Physiol.* 86: 740–743.
- Hoagland, D. R. & Snijder, W. C. 1933. Nutrition of strawberry plants under controlled conditions. – *Proc. Am. Soc. Hortic. Sci.* 30: 288–294.
- Jackson, W. A., Kwik, K. D., Volk, R. J. & Butz, R. G. 1976. Nitrate influx and efflux by intact wheat seedlings: effects of prior nitrate nutrition. – *Planta* 132: 149–156.
- Lambers, H. 1985. Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. – In *Encyclopedia of Plant Physiol.*, New Series, Vol. 18 (R. Douce and D. A. Day, eds), pp. 418–473. Springer-Verlag, Berlin. ISBN 3-540-13935-4.
- , Day, D. A. & Azcón-Bieto, J. 1983. Cyanide-resistant respiration in roots and leaves. Measurements with intact tissues and isolated mitochondria. – *Physiol. Plant.* 58: 148–154.
- Lauer, M. J., Blevins, D. G. & Sierzputowska-Graczyk, H. 1989.

- ³¹P-Nuclear Magnetic Resonance determination of phosphate compartmentation in leaves of reproductive soybeans (*Glycine max* L.) as affected by phosphate nutrition. – *Plant Physiol.* 89: 1331–1336.
- Lee, R. B. & Clarkson, D. T. 1986. Nitrogen-13 studies of nitrate fluxes in barley roots; I compartmental analysis from measurements of ¹³N efflux. – *J. Exp. Bot.* 37: 1753–1767.
- & Drew, M. C. 1986. Nitrogen-13 studies of nitrate fluxes in barley roots. – *J. Exp. Bot.* 37: 1768–1779.
- Lefebvre, D. D. & Clarkson, D. T. 1984. Compartmental analysis of phosphate in roots of intact barley seedlings. – *Can. J. Bot.* 62: 1076–1080.
- Lewis, J. C. & Powers, W. L. 1941. Antagonistic action of chlorides on the toxicity of iodides to corn. – *Plant Physiol.* 16: 393–398.
- Lühning, H. & Tazawa, M. 1985. Effect of cytoplasmic Ca²⁺ on the membrane potential and membrane resistance of *Chara plasmodium*. – *Plant Cell Physiol.* 26: 635–646.
- Macklon, A. E. S., Ron, M. M. & Sim, A. 1990. Cortical cell fluxes of ammonium and nitrate in excised root segments of *Allium cepa* L.; studies using ¹⁵N. – *J. Exp. Bot.* 41: 359–370.
- Marschner, H. 1989. Mechanism of ion release into the xylem. – *In* Mineral Nutrition of Higher Plants third printing, pp. 62–64. Academic Press Limited, London. ISBN 0-12-473541-X.
- Miller, D. M. 1987. Errors in the measurement of root pressure and exudation volume flow rate caused by damaging during the transfer of unsupported roots between solutions. – *Plant Physiol* 85: 164–166.
- Morgan, M. A., Volk, R. J. & Jackson, W. A. 1972. Simultaneous influx and efflux of nitrate during uptake by perennial ryegrass. – *Plant Physiol.* 51: 267–272.
- O'Donovan, J. T., O'Sullivan, P. A. & Caldwell, C. D. 1983. Basis for antagonism of paraquat phytotoxicity to barley by MCPA dimethylamine. – *Weed Res.* 23: 165–172.
- Oscarson, P., Ingemarsson, B., af Ugglas, M. & Larsson, C.-M. 1987. Short-term studies of NO₃⁻ uptake in *Pisum* using ¹³NO₃⁻. – *Planta* 152: 319–324.
- Pearson, C. J., Volk, R. J. & Jackson, W. A. 1981. Daily changes in nitrate influx, efflux and metabolism in maize and pearl millet. – *Planta* 152: 319–324.
- Penning de Vries, F. W. T. 1975. The costs of maintenance processes in plant cells. – *Ann. Bot.* 39: 77–92.
- Poorter, H., van der Werf, A., Atkin, O. K. & Lambers, H. 1991. Respiratory energy requirements of roots vary with the potential growth rate of a plant species. – *Physiol. Plant.* 83: 469–475.
- Rincon, M. & Hanson, J. B. 1986. Controls on calcium ion fluxes in injured or shocked corn root cells: importance of proton pumping and cell membrane potential. – *Physiol. Plant.* 67: 576–583.
- Ron, M. M., Macklon, A. E. S. & Sim, A. 1988. Cortical cell fluxes of phosphate in excised root segments of *Allium cepa* L. – *J. Exp. Bot.* 39: 1699–1707.
- Schjørring, J. K. & Jensen, P. 1984. Phosphorus nutrition of barley, buckwheat and rape seedlings. II. Influx and efflux of phosphorus by intact roots of different P status. – *Physiol. Plant.* 61: 584–590.
- Senaratna, T., Gusse, J. F. & McKersie, B. D. 1988. Age-induced changes in cellular membranes of imbibed soybean seed axes. – *Physiol. Plant.* 73: 85–91.
- Sentenac, H. & Grignon, C. 1981. A model for predicting ionic equilibrium concentrations in cell walls. – *Plant Physiol.* 68: 415–419.
- Siddiqi, M. Y., Glass, A. D. M., Ruth, T. J. & Rufty, T. W. 1990. Studies of the uptake of nitrate in barley. I. Kinetics of ¹³NO₃⁻ influx. – *Plant Physiol.* 93: 1426–1432.
- , Glass, A. D. M. & Ruth, T. J. 1991. Studies of the uptake of nitrate in barley. III. Compartmentation of NO₃⁻. – *J. Exp. Bot.* 42: 1455–1463.
- Tomos, A. D. & Wyn Jones, R. G. 1988. Some transport properties of cells within tissues. – *In* Solute Transport in Plant Cells and Tissues (D. A. Baker and J. L. Hall, eds), pp. 220–250. Longman Scientific and Technical, Essex. ISBN 0-582-00580-9.
- Van der Werf, A., Kooiman, A., Welschen, R. & Lambers, H. 1988. Respiratory energy costs for the maintenance of biomass, for growth and for ion uptake in roots of *Carex diandra* and *Carex acutiformis*. – *Physiol. Plant.* 72: 438–491.
- , Van den Berg, G., Ravenstein, H. J. L., Lambers, H. & Eising, R. 1992. Protein turnover: a significant component of maintenance respiration in roots. – *In* Molecular, Biochemical and Physiological Aspects of Plant Respiration (H. Lambers and L. H. W. van der Plas, eds), pp. 483–492. SPB Academic Publishing, The Hague. ISBN 90-5103-079-7.
- Veen, B. W. 1980. Energy costs of ion transport. – *In* Genetic Engineering of Osmoregulation. Impact on Plant Productivity for Food, Chemicals and Energy (D. W. Rains, R. C. Valentine and C. Halaender, eds), pp. 187–195. Plenum Press, New York, NY. ISBN 0-306-40454-0.
- Wieneke, J. & Nebeling, B. 1990. Improved method for ¹³N-application in short-term studies on NO₃⁻ fluxes in barley and squash plants. – *Z. Pflanzenernähr. Bodenk.* 153: 117–123.
- Williamson, R. E. & Ashley, C. C. 1982. Free Ca²⁺ and cytoplasmic streaming in the alga *Chara*. – *Nature* 296: 647–651.
- Zierler, K. 1981. A critique of compartmental analysis. – *Annu. Rev. Biophys. Bioeng.* 10: 531–562.