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RESEARCH PAPER

A new, non-perturbing, sampling procedure in tracer exchange measurements

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Abstract

An isotope procedure for the tracing of ion fluxes and rate constants in intact plants is presented and applied to ^{42}K-labelled potassium fluxes in cells of intact barley (*Hordeum vulgare* L.) roots. This procedure differs from conventional tracer efflux protocols in that tracer accrual in the external solution bathing the labelled roots is continually monitored by solution subsampling, whereas conventional protocols involve monitoring the specific-activity decline in a sequence of eluates that wash out tracer released by roots. The new technique minimizes physical disturbance to the plant system, while permitting excellent time resolution of efflux kinetics. In the high-affinity transport (HATS) range, the flux and exchange parameters determined using this method showed close agreement with those found using a conventional protocol. However, in the low-affinity transport (LATS) range, substantially higher influx and efflux were seen than are normally observed with conventional tracer techniques. It is shown that this difference is attributable to the greater disturbance-sensitivity of LATS transport, and conclude that the measurement of fluxes is much more difficult in this transport range than in the disturbance-resistant HATS range.

Key words: Barley, compartmental analysis, efflux, influx, ion transport, potassium.

Introduction

Although all plants extract nutrient ions from their root environments, a significant fraction of transported ions returns from within root cells to the external medium, at rates sometimes approaching the unidirectional influx of these ions (Nielsen and Schjoerring, 1998; Britto *et al.*, 2001; Kronzucker *et al.*, 2001; Essah *et al.*, 2003). Knowledge about ion efflux at the plasma membrane is therefore central to the measurement of unidirectional influx of ions into plant tissues, which can be severely underestimated due to the simultaneously occurring efflux (Cram, 1969; Lee and Ayling, 1993; Britto and Kronzucker, 2001; Essah *et al.*, 2003; Britto *et al.*, 2004). However, only a few procedures exist to quantify efflux in complex systems such as plant organs. The leading procedure is that of Compartmental Analysis by Tracer Efflux (CATE), which involves the kinetic analysis of tracer release from a labelled root system to the external medium (Cram, 1968; MacRobbie, 1971; Walker and Pitman, 1976; Behl and Jeschke, 1982; Lee and Clarkson, 1986; Siddiqi *et al.*, 1991). In conjunction with initial tracer release rates, the rate of change of tracer release allows the quantification of the chemical efflux represented by the tracer; efflux can then be added to a net accumulation rate to arrive at a more accurate estimate of influx than can be determined by misleadingly 'direct' influx measurements that do not involve efflux analysis (Cram, 1968; Walker and Pitman, 1976; Behl and Jeschke, 1982; Lee and Clarkson, 1986; Siddiqi *et al.*, 1991; Kronzucker *et al.*, 2003).

CATE methodology, however, is subject to a number of criticisms. Several of these, such as issues of compartmental tracer origin, cellular heterogeneity in intact plant organs, and root-to-shoot translocation, have been discussed elsewhere (Jeschke, 1982; Kronzucker *et al.*, 1995, 2003; Britto and Kronzucker, 2003*a*,*b*). An outstanding concern relates to the potential problems associated with the physical handling of the plant during CATE experiments. It has been shown in several cases that such handling can have pronounced effects on ion fluxes, even in intact plants, and could result in a drastic deviation from the steady-state conditions that are assumed to exist in a CATE experiment (Hommels *et al.*, 1990; Aslam *et al.*, 1996; ter Steege *et al.*, 1998; Britto and Kronzucker,

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2003*b*). In particular, Bell *et al.* (1995) noted that increasing the frequency of elution in CATE experiments resulted in a variable stimulation of efflux. Field studies have also suggested that plant handling by investigators can have profound effects on plant growth and survival (Cahill *et al.*, 2001). Resolution of this is hampered by a lack of methods with which to corroborate flux parameters determined using CATE.

In the present work, a novel procedure for compartmental analysis is demonstrated that addresses this specific problem, and this procedure is used to examine $^{42}K^+$ fluxes in seedlings of the model system, barley (*Hordeum vulgare* L.). In conventional CATE methodology, the entire solution bathing the roots is exchanged at intervals, and the declining tracer activity in each eluate is monitored. In the present work, instead of complete, periodic solution changes, we use the frequent subsampling of an external, aqueous medium in which the labelled plant roots are immersed, and the accrual of tracer in this medium is monitored. Therefore, this procedure was termed 'SCATE' – or 'Subsampling-based Compartmental Analysis by Tracer Efflux'. As will be shown, this new procedure confirms the accuracy of the conventional CATE protocol in the high-affinity K^+ transport (HATS) range, while revealing some unexpected differences in the low-affinity (LATS) range. These differences require explanation, which the latter part of the paper provides.

Materials and methods

Plant culture

Seeds of barley (*Hordeum vulgare* L. cv. 'Klondike') were germinated on sand for 3 d prior to placement in aerated hydroponic growth medium containing modified quarter-strength Johnson's solution for an additional 4 d. The solution was modified to provide two concentrations of potassium (as K_2SO_4), 0.1125 mM and 40 mM, in order to investigate high- and low-affinity K^+ transport ranges (HATS and LATS), respectively. Plants were cultured in walk-in growth chambers equipped with fluorescent lights (Philips Econo-watt, F96T12), providing an irradiation of 200 μmol photons m^{-2} s^{-1} at plant height, for 16 h d^{-1}. Daytime temperature was 20 °C, night-time temperature was 15 °C, and relative humidity was approximately 70%.

Flux experiments

Each replicate consisted of five intact plants that were bundled together 1 d prior to the experiment (6 d after the start of germination), by means of a plastic collar made from a 0.5 cm length of tubing. This collar was attached to the plants just above the junction of seed and stem. A wire clasp was constructed to attach to the collar, so that the plant could be hooked to the rim of a beaker sufficiently filled with experimental solution to immerse the roots. This procedure was used to minimize physical disturbance to the plant root system during plant transfer from one vessel to another, and to minimize transfer times.

All experimental solutions were of the same chemical composition as the growth solution, with the labelling solutions containing the potassium radioisotope ^{42}K ($t_{½}$=12.36 h), provided by the McMaster Nuclear Reactor, in Hamilton, Ontario, Canada. Roots of bundled plants were exposed for 1 h to radioactive solution, then dipped in 30 ml of non-radioactive growth solution for 5 s, then to the first of two 50 ml beakers (desorption vessels) also containing 30 ml of non-radioactive growth solution. Every 30 s for 5 min, a 3 ml subsample of the solution was removed for gamma counting, using a Canberra-Packard, Quantum Cobra Series II, Model 5003 gamma-counter, and replaced with an equal volume of non-radioactive solution. Plants were then transferred to a second desorption vessel, from which 3 ml subsamples were again removed and replaced, at 30 s intervals for the first 5 min, and 1 min intervals for the remaining 20 min of desorption. This protocol allowed for minimal plant disturbance during the entire experimental procedure. Calculations based on previously published K^+ flux estimates (Kronzucker *et al.*, 2003), and on desorption-solution specific activity, indicated that re-absorption of tracer during desorption was negligible. This was confirmed by the observation that the varying of desorption volumes over two orders of magnitude had no effect on K^+ fluxes and exchange parameters (not shown).

Cumulative radioactivity released by plants over time in the two desorption vessels was quantified by gamma-counting, using the following formula:

$$cpm_{\text{released}} = cpm_{\text{n}}\left(\frac{v_{\text{vessel}}}{v_{\text{sample}}}\right) + \sum_{i=1}^{n-1} cpm_{\text{i}}$$

This relationship gives the cpm released at the time that the *n*th sample was taken from the desorption solution, and during which *n−1* previous samples had already been taken from the desorption vessel. It accounts for the ratio of subsample volume (v_{sample}) to total beaker volume (v_{vessel}), and for the sum of cpm previously removed from the beaker. After all desorption phases were complete, plants were harvested, with roots separated from shoots and spun in a low-speed centrifuge for 30 s, to remove surface water prior to weighing. Roots and shoots were gamma-counted for retained radioactivity.

Radioactivity released from plants was plotted against time, and exponential equations of the form A_t=A_0 $(1-e^{-kt})$ were fitted using the non-linear, least-squares regression method provided with Microcal Origin software (version 6.0). In this equation, A_t refers to accumulated tracer release at time t following desorption, A_0 is the maximal amount of released tracer, and k is the kinetic exchange constant for the cytosolic K^+ pool. The parameters A_0 and k were then used to differentiate the best-fit curve, yielding initial rates of tracer efflux for the second desorption phase (Lee and Clarkson, 1986; Siddiqi *et al.*, 1991). Because this phase began immediately after a preliminary 5 min desorption, the differentiated curve was extrapolated back in time by 5 min, to determine the initial tracer efflux from this phase at the end of the labelling period. Where a bolus subtraction (see Results and discussion) was used in the LATS range, an additional 30 s extrapolation was used to correct for steady-state cytosolic tracer release in the first 30 s of the second desorption phase.

As with conventional CATE procedures, the specific activities of the tracer-releasing compartments were determined from labelling times and the kinetic constants (k values) and these values were used to calculate chemical efflux from tracer efflux (Siddiqi *et al.*, 1991; Kronzucker *et al.*, 2001). Net flux was determined using k values and tracer retention in tissue at the end of the experiment (Kronzucker *et al.*, 2001). Influx was determined by summing efflux and net flux terms.

For comparative, system-testing purposes, three variations on this basic protocol were employed for the first of the two desorption periods, as discussed in the main text: (i) 4.0 l of solution were used in place of the standard 30 ml; (ii) a 10 min period was used instead of 5 min (with the differentiated curve from the second desorption extrapolated back by 10 min instead of 5); and (iii) instead of a 5 min desorption, a series of five, 1 min desorption periods were used, involving plant transfer from one beaker to the next.

CATE experiments, involving complete solution exchange at each time interval (and as seen in Fig. 1), were conducted using efflux funnels, as described elsewhere (Kronzucker *et al.*, 2003; also see Introduction). In the case of one experiment, the labelled plant was kept in a desorption solution for 10 min prior to transfer to an efflux funnel, at which point the CATE protocol commenced.

Results and discussion

Figure 1 shows the results of a CATE protocol with intact, $^{42}K^+$-labelled barley roots, grown and assayed at external $[K^+]$ of 0.1125 mM (trace A; HATS condition) and 40 mM (trace B; LATS condition). The rate of decline in the radioactivity content of eluates collected in the sequential washing of the root tissue reveals the decline in specific activity of the compartment(s) releasing tracer into the eluates (see Introduction). Under steady-state conditions, this decline will follow exponential kinetics determined by the size of the exchanging pool and by the activities of the transport and/or metabolic systems that move the traced substance (in this case, K^+) to, or from, the pool. Traces similar to those in Fig. 1 have been interpreted elsewhere (Behl and Jeschke, 1982; Memon *et al.*, 1985; Kronzucker *et al.*, 1995, 2003; Britto and Kronzucker, 2003*b*) as compound exponentials representing three compartments, identified as surface film, cell wall, and cytosol; the vacuole was essentially unlabelled under the 60 min labelling regime of the experiments in Fig. 1, as it is a much more slowly-exchanging compartment (Behl and Jeschke, 1982; Jeschke, 1982; Memon *et al.*, 1985; Britto and Kronzucker, 2001).

In the present work, a new way has been developed to observe efflux, by subsampling the solution in which the labelled plant roots are immersed. This is distinct from the complete exchange of bathing solution at each time point, with its potential disturbances to the plant system, that comprises the experimental protocol underlying not only Fig. 1, but nearly all tracer efflux studies in plants conducted until the present time. For the new system, it was predicted that, after sufficiently long initial desorption, during which label should be cleared from the more rapidly exchanging extracellular compartments (see below), the kinetics of tracer efflux from a single compartment, the cytosol, would become visible. Because tracer efflux is an exponential process, the graphical result of such a procedure was predicted to resemble the ideal asymptotic curve described by the equation $A_t=A_0\ (1-e^{-kt})$ (Walker and Pitman, 1976; Britto and Kronzucker, 2001).

The 5 min duration of the initial desorption series, required for the clearance of tracer from the surface film and cell wall phases of the root system, was chosen by reference to previous work that quantified tracer exchange between the cell wall and the external medium (Pierce and Higinbotham, 1970; Memon *et al.*, 1985; Szynkier and Kylin, 1976; Kronzucker *et al.*, 2003). Calculations using rates of efflux from extracellular spaces, exchange constants for the cell wall, and specific radioactivities of labelling solutions indicated that these spaces would be 95–99% cleared of tracer after 5 min. The efficacy of this procedure was confirmed by experiments showing that a 10 min desorption period yielded results not significantly different from those found with the 5 min duration (when the efflux trace was extrapolated backwards in time by an additional 5 min, see Materials and methods; data not shown). The 5 min duration was further justified by the observation that this period is typically chosen to clear the free space of tracer, in potassium influx studies (Leonard *et al.*, 1975; Vale *et al.*, 1987; Wang *et al.*, 1998).

Once tracer release from the extracellular phase was accounted for, the intracellular release became amenable to study. The results of a representative experiment for this slower phase in the HATS range are shown in Fig. 2. The

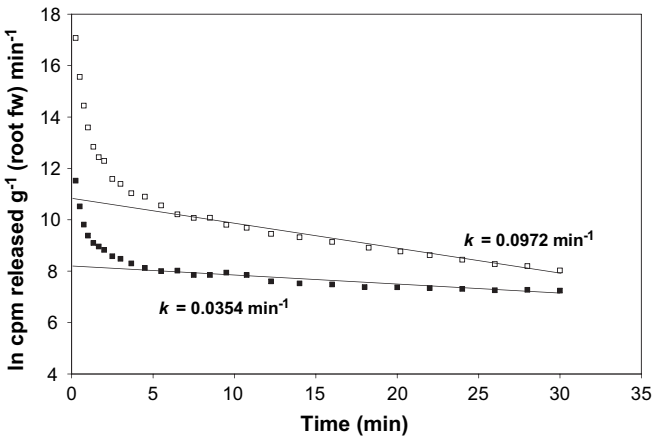

Fig. 1. Representative plots from conventional tracer elution (CATE) experiments with barley seedlings grown and assayed at 0.1125 mM (filled squares) and 40 mM (open squares) $[K^+]_{external}$. Kinetic constants (k), based on the slopes of the terminal, cytosolic, lines, are shown for each condition. Note that, unlike the subsampling (SCATE) plots that follow, the ordinate represents a rate of tracer release, rather than accumulated tracer release, from the plant system.

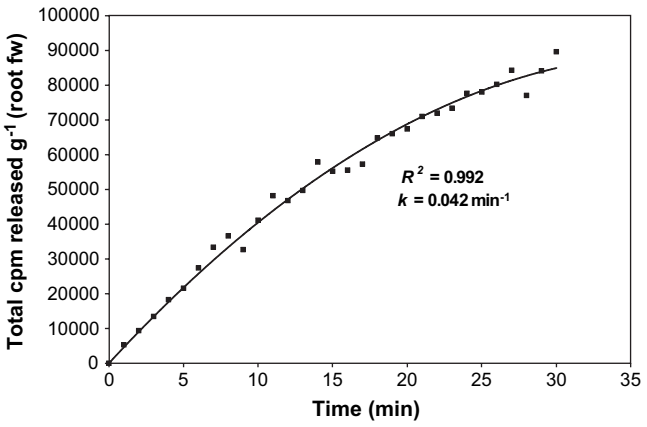

Fig. 2. Representative plot of $^{42}K^+$ released from the slow (cytosolic) phase of labelled barley roots, grown and assayed at 0.1125 mM $[K^+]_{external}$. Cpm release was determined using subsampling.

resemblance of this data set to an ideal, single-exponential graph of the form $A_t=A_0\ (1-e^{-kt})$ (see Materials and methods) is striking, and confirmed by a non-linear regression that tested the goodness-of-fit of this data to the predicted form (on average, R^2=0.98).

The chief advantage of the SCATE protocol is that it maintains the plant system in a condition of minimal physical disturbance, with only three changes of solution following labelling, as compared to 30 changes in the CATE protocol shown in Fig. 1. The low physical impact of subsampling, in turn, permits high sampling frequency and high time resolution of the undisturbed system. Low disturbance during measurement is of potentially great importance, as it has been shown that physical manipulation can substantially alter plasma membrane fluxes of nitrate (Bloom and Sukrapanna, 1990; Aslam *et al.*, 1996; ter Steege *et al.*, 1998), sulphate (Bell *et al.*, 1995), ammonium (Britto and Kronzucker, 2003*b*), and protons (Chastain and Hanson, 1982), in addition to potassium itself (Gronewald and Hanson, 1980; Bloom and Sukrapanna, 1990; Hommels *et al.*, 1990). Interestingly, despite such expectations, the key parameters determined from a SCATE analysis in the HATS condition were not significantly different from those found using CATE protocols, as shown in Table 1.

By contrast, with the graphical congruence in the HATS condition the raw data from plants grown and assayed under LATS conditions lacked congruence with ideal exponential plots (Fig. 3). An attempt to resolve kinetic parameters by exponential regression of the raw plot in Fig. 3 resulted in a discrepancy between the early points of the graph and those predicted by the best-fitting regression curve, with the initial data values exceeding predicted values in all cases (n=10). Compared with the HATS condition, the resulting regression yielded relatively low correlation values due to this discrepancy (on average, R^2=0.93). However, upon subtraction of the first of these outlying values from all data points, a nearly perfect non-linear fit was achieved (Fig. 4; on average, R^2=0.97; these plots also had a significantly higher goodness-of-fit based on chi-square analysis at P=0.95). This suggested that there was a non-steady-state discharge or 'bolus' of additional counts that were released from the root tissue, in immediate response to the physical disturbance associated with the transfer of plants. Importantly, in a CATE series, early deviations from first-order kinetics would be hidden in the early phases of elution, masked by the large amounts of tracer that are initially released from the cell wall (Fig. 1). A modified CATE protocol, in which the elution series began only after the labelled roots had rested in growth solution, undisturbed, for 10 min (to desorb the cell wall), revealed a similar burst of tracer released after transfer of the plant to the elution funnel (Fig. 5), verifying that the appearance of the bolus is a short-term effect of plant handling.

It was essential to validate the initial-point subtraction that transformed the raw data in Fig. 3, into the high-fidelity exponential plot shown in Fig. 4, by testing the assumption that the bolus is released from a root compartment in a manner independent of the steady-state fluxes. The hypothesis that these counts originated from extracellular spaces was initially tested by doubling the desorption time in the first of the two desorption steps from 5 min to 10 min. This would all but eliminate bolus counts, were they to have originated from this space or other rapidly-exchanging spaces. However, the deviation shown in Fig. 3 was not affected by this treatment (not shown), indicating that the origin of the bolus is indeed intracellular, as confirmed by the bolus appearing after a 10 min resting period in the modified CATE protocol described above (Fig. 5). The appearance of the bolus also clearly results from plant transfer to a fresh desorption medium. To test this further, the first 5 min desorption period was subdivided into five 1 min units, each entailing a transfer of plants to a fresh desorption vessel; this was followed by a 25 min SCATE series in a single desorption vessel, The experiment resulted not only in a complete disappearance of the bolus, but also in the generation of flux parameters not significantly different from those determined after subtraction of the initial point (data not shown).

These experiments provide clear evidence that bolus counts appear from within cells, stimulated by plant handling, and may result from the activity of mechanically-activated or stretch-activated efflux channels (Cosgrove and Hedrich, 1991; Hamill and Martinac, 2001). Interestingly, the bolus was only seen in the LATS range, which is consistent with the high ratios of efflux to influx demonstrated in this range (Table 1; Kronzucker *et al.*,

Table 1. *Flux and turnover (exchange half-time) parameters for high- and low-affinity K^+ transport systems, as determined using two procedures of compartmental analysis*

Fluxes are given in μmol g^{-1} FW h^{-1} and exchange half-times in min. Each datum is the mean ±SEM of 10–14 replicates. Different letters within a column refer to significantly different values (P=0.95).

Procedure	HATS (0.1125 mM $[K^+]_{external}$)			LATS (40 mM $[K^+]_{external}$)		
	Influx	Efflux	$t_{1/2}$	Influx	Efflux	$t_{1/2}$
Conventional (CATE)	9.66±0.37 a	2.41±0.24 a	16.45±0.67 a	37.54±3.33 a	31.18±3.18 a	8.61±0.40 a
Subsampling (SCATE)	9.29±0.22 a	2.40±0.13 a	15.96±1.22 a	58.39±5.73 b	52.77±5.62 b	6.89±0.64 b

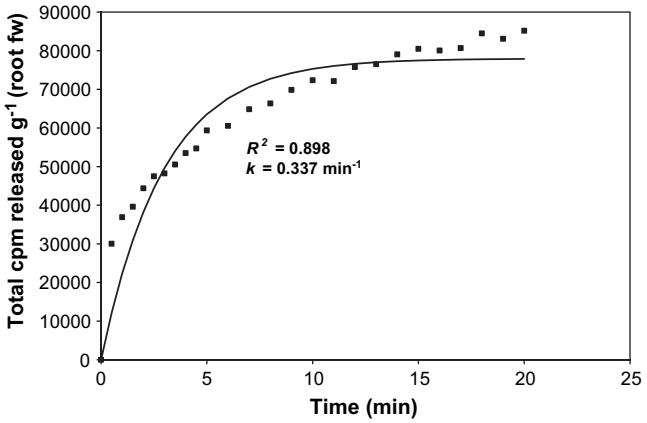

Fig. 3. Representative plot of $^{42}K^+$ released from the slow (cytosolic) phase of labelled barley roots, grown and assayed at 40 mM $[K^+]_{external}$, prior to subtraction of initial tracer burst. Cpm release was determined using subsampling.

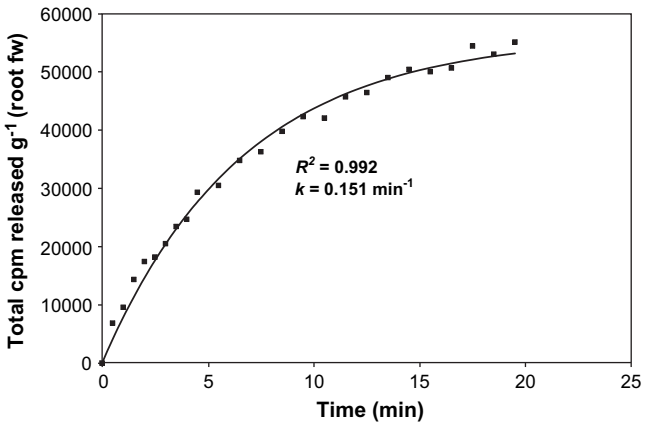

Fig. 4. Data as in Fig. 3, subsequent to subtraction of initial tracer burst.

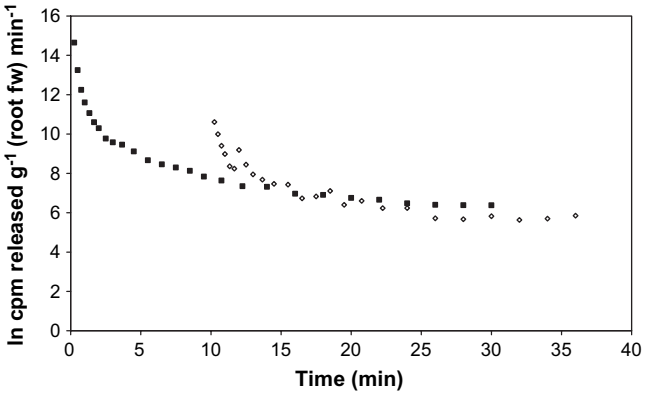

Fig. 5. Tracer burst visible in a modification of the conventional elution protocol, in which plants had been continuously desorbed for 10 min prior to elution (open squares). Closed squares indicate an unmodified, conventional elution series.

2003), suggesting that outwardly-directed K^+ channels operate at higher capacity under conditions of luxury consumption. This is in agreement with Hommels *et al.* (1990), who observed that physical disturbance of plants of several *Taraxacum* species resulted in significantly enhanced K^+ efflux, and that this effect was much more pronounced in high-salt plants. Furthermore, it was found that root-tip excision did not eliminate bolus counts, indicating that bolus counts originate from a more basal region of the root, possibly from tissues near the root-shoot boundary, which retained as much as 42% of the total tracer remaining in the root (data not shown). Regardless of its region of origin, it appears that the bolus neither affects, nor is affected by, the predominating tracer-release kinetics of the bulk root system. Rather, it is a short-lived, handling-induced phenomenon that appears to act in parallel with the main source of tracer released during desorption of intracellular spaces; this explains why its subtraction yields excellent exponential plots (Fig. 4).

Surprisingly, the subsampling procedure in the LATS range yielded values of influx and efflux that were significantly higher than those determined using conventional means (Table 1). This difference would have been further, if slightly, accentuated if the subtracted bolus counts had been included in the calculation of net flux, increasing influx by about 5% (not shown). These results contradicted expectations that decreased handling would lead to decreased efflux, as suggested by Hommels *et al.* (1990), but agreed with other reports indicating a suppressive effect of handling on potassium influx (Gronewald and Hanson, 1980; Bloom and Sukrapanna, 1990). These contradictory examples from the literature suggest that at least two distinct mechanisms may be responsible for efflux in the LATS range, one which is disturbance-stimulated, and one which is disturbance-inhibited.

To conclude, the procedure presented here provides a simple means to a highly time-resolved view of minimally disturbed cytosolic ion turnover and efflux in intact plant roots. Such a view is critical to the determination of unidirectional ion fluxes and, therefore, to the characterization of transport systems. As a specific case study, this work presents a more complex picture of the effects of disturbance on K^+ fluxes in plant roots than previous work has shown. In particular, the data for the LATS range suggest that there are two components of potassium efflux that can become manifest depending on experimental protocol: (i) a disturbance-stimulated efflux that is short-lived and independent of predominant, steady-state, bulk-tissue fluxes; and (ii) a disturbance-inhibited efflux component that is observed in the steady state. These complicating factors, while absent in the HATS range, may provide insights into the nature of mechanically-activated channels in plants, and into the difficulties associated with measuring unidirectional influxes in plant systems, particularly under high-salt conditions (Britto and Kronzucker, 2001).

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References

Aslam M, Travis RL, Rains DW, Huffaker RC. 1996. Effect of root perturbation and excision on nitrate influx and efflux in barley (*Hordeum vulgare*) seedlings. *Physiologia Plantarum* **97,** 425–32.

Behl R, Jeschke WD. 1982. Potassium fluxes in excised barley roots. *Journal of Experimental Botany* **33,** 584–600.

Bell C, Fordham M, Richardson P, Cram J, Tomos D. 1995. Cellular and subcellular compartmentation of sulphate in leaves in relations to low sulphur mobility. *Zeitschrift für Pflanzenernahrung und Bodenkunde* **158,** 63–65.

Bloom AJ, Sukrapanna SS. 1990. Effects of exposure to ammonium and transplant shock upon the induction of nitrate absorption. *Plant Physiology* **94,** 85–90.

Britto DT, Kronzucker HJ. 2001. Can unidirectional influx be measured in higher plants? A mathematical approach using parameters from efflux analysis. *New Phytologist* **150,** 37–47.

Britto DT, Kronzucker HJ. 2003*a*. Ion fluxes and cytosolic pool sizes: examining fundamental relationships in transmembrane flux regulation. *Planta* **217,** 490–497.

Britto DT, Kronzucker HJ. 2003*b*. Trans-stimulation of $^{13}NH_4^+$ efflux provides evidence for the cytosolic origin of tracer in the compartmental analysis of barley roots. *Functional Plant Biology* **30,** 1233–1238.

Britto DT, Ruth TJ, Lapi S, Kronzucker HJ. 2004. Cellular and whole-plant chloride dynamics in barley: insights into chloride–nitrogen interactions and salinity responses. *Planta* **218,** 615–622.

Britto DT, Siddiqi MY, Glass ADM, Kronzucker HJ. 2001. Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. *Proceedings of the National Academy of Sciences, USA* **98,** 4255–4258.

Cahill JF, Castelli JP, Casper BB. 2001. The herbivory uncertainty principle: visiting plants can alter herbivory. *Ecology* **82,** 307–312.

Chastain CJ, Hanson JB. 1982. Control of proton efflux from corn root-tissue by an injury-sensing mechanism. *Plant Science Letters* **24,** 97–104.

Cosgrove DJ, Hedrich R. 1991. Stretch-activated chloride, potassium, and calcium channels coexisting in plasma-membranes of guard-cells of *Vicia faba* L. *Planta* **186,** 143–153.

Cram WJ. 1968. Compartmentation and exchange of chloride in carrot root tissue. *Biochimica et Biophysica Acta* **163,** 339–353.

Cram WJ. 1969. Short-term influx as a measure of influx across the plasmalemma. *Plant Physiology* **44,** 1013–1015.

Essah PA, Davenport R, Tester M. 2003. Sodium influx and accumulation in *Arabidopsis*. *Plant Physiology* **133,** 307–318.

Gronewald JW, Hanson JB. 1980. Sensitivity of the proton and ion-transport mechanisms of corn roots to injury. *Plant Science Letters* **18,** 143–150.

Hamill OP, Martinac B. 2001. Molecular basis of mechanotransduction in living cells. *Physiological Reviews* **81,** 685–740.

Hommels CH, Prins HBA, Kuiper PJC, Tanczos OG. 1990. K^+ (Rb^+) uptake by intact *Taraxacum* plants: effects of nutrient status on K^+ uptake. *New Phytologist* **114,** 685–694.

Jeschke WD. 1982. Shoot-dependent regulation of sodium and potassium fluxes in roots of whole barley seedlings. *Journal of Experimental Botany* **33,** 601–618.

Kronzucker HJ, Britto DT, Davenport RJ, Tester M. 2001. Ammonium toxicity and the real cost of transport. *Trends in Plant Science* **6,** 335–337.

Kronzucker HJ, Siddiqi MY, Glass ADM. 1995. Analysis of $^{13}NH_4^+$ efflux in spruce roots: a test case for phase identification in compartmental analysis. *Plant Physiology* **109,** 481–490.

Kronzucker HJ, Szczerba MW, Britto DT. 2003. Cytosolic potassium homeostasis revisited: ^{42}K tracer analysis in *Hordeum vulgare* L. reveals set-point variations in $[K^+]$. *Planta* **217,** 540–546.

Lee RB, Ayling SM. 1993. The effect of methionine sulphoximine on the absorption of ammonium by maize and barley roots over short periods. *Journal of Experimental Botany* **44,** 53–63.

Lee RB, Clarkson DT. 1986. ^{13}N studies of nitrate fluxes in barley roots. 1. Compartmental analysis from measurements of ^{13}N efflux. *Journal of Experimental Botany* **37,** 1753–1767.

Leonard RT, Nagahashi G, Thomson WW. 1975. Effect of lanthanum on ion absorption in corn roots. *Plant Physiology* **55,** 542–546.

MacRobbie EAC. 1971. Fluxes and compartmentation in plant cells. *Annual Review of Plant Physiology* **22,** 75–96.

Memon AR, Saccomani M, Glass ADM. 1985. Efficiency of potassium utilization by barley varieties: the role of subcellular compartmentation. *Journal of Experimental Botany* **36,** 1860–1876.

Nielsen KH, Schjoerring JK. 1998. Regulation of apoplastic NH_4^+ concentration in leaves of oilseed rape. *Plant Physiology* **118,** 1361–1368.

Pierce WS, Higinbotham N. 1970. Compartments and fluxes of K^+, Na^+ and Cl^- in *Avena* coleoptile cells. *Plant Physiology* **46,** 666–673.

Siddiqi MY, Glass ADM, Ruth TJ. 1991. Studies of the uptake of nitrate in barley. 3. Compartmentation of NO_3^-. *Journal of Experimental Botany* **42,** 1455–1463.

Szynkier K, Kylin A. 1976. Fluxes of Na^+, Rb^+ and Cl^- ions in excised roots of sugar-beet seedlings. *Physiologia Plantarum* **38,** 89–94.

ter Steege MW, Stulen I, Wiersema PK, Paans AJM, Vaalburg W, Kuiper PJC, Clarkson DT. 1998. Growth requirement for N as a criterion to assess the effects of physical manipulation on nitrate uptake fluxes in spinach. *Physiologia Plantarum* **103,** 181–192.

Vale FR, Jackson WA, Volk RJ. 1987. Potassium influx into maize root systems: influence of root potassium concentration and ambient ammonium. *Plant Physiology* **84,** 1416–1420.

Walker NA, Pitman MG. 1976. Measurement of fluxes across membranes. In: Lüttge U, Pitman MG, eds. *Encyclopedia of plant physiology*, Vol. 2, Part A. Berlin, Germany: Springer-Verlag, 93–126.

Wang T-B, Gassmann W, Rubio F, Schroeder JI, Glass ADM. 1998. Rapid up-regulation of *HKT1*, a high-affinity potassium transporter gene, in roots of barley and wheat following withdrawal of potassium. *Plant Physiology* **118,** 651–659.