Plant Physiol. (1993) 102: 467-472

Compartmentation Analysis of Paraquat Fluxes in Maize Roots as a Means of Estimating the Rate of Vacuolar Accumulation and Translocation to Shoots

Joseph M. DiTomaso*, Jonathan J. Hart, and Leon V. Kochian

Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, New York 14853 (J.M.D., J.J.H.); and United States Plant, Soil and Nutrition Laboratory, United States Department of Agriculture/ Agricultural Research Service, Cornell University, Ithaca, New York 14853 (L.V.K.)

Efflux analysis conducted after five loading periods of various lengths (2, 6, 12, 18, or 24 h) was used to investigate uptake, compartmentation, and translocation of [^{14}C]paraquat in maize (*Zea mays* L.) seedlings. The time course for net paraquat uptake (paraquat concentration in uptake solution = 25 μM) into maize roots was linear (56.7 nmol g^{-1} root fresh weight h^{-1}) for 24 h. Estimates of changes in paraquat content in the vacuole, cytoplasm, and cell wall after 2-, 6-, 12-, 18-, and 24-h loading periods indicated that the cell wall saturated rapidly, whereas accumulation of paraquat into the vacuole increased linearly (12.4 nmol g^{-1} root fresh weight h^{-1}) over 24 h. In contrast to vacuolar accumulation, cytoplasmic paraquat content appeared to approach saturation. The half-time for paraquat efflux from the cell wall (16.6 min ± 1.2 SD) and cytoplasm (58.8 min ± 8.9 SD) remained relatively constant regardless of the length of the loading period, whereas the half-time for efflux from the vacuole was considerably longer and increased linearly with increased loading time (6.1-18.7 h). The time course for paraquat translocation to the shoot was linear within a 24-h exposure to radiolabeled herbicide, but translocation did not begin until 5 h after initiation of treatment. The experimental approach used in these experiments provides a valuable method for examining the movement of paraquat in maize seedlings. Results indicate that the herbicide slowly accumulates in the vacuole of root cells but is also translocated to the shoot.

Paraquat is a foliar-applied herbicide that causes rapid membrane damage in shoot tissues by accepting electrons from PSI and subsequently donating these electrons to molecular oxygen to form toxic oxygen species (Summers, 1980). Although the vast majority of herbicides are passively transported across cell membranes as nonionic molecules (see Hess, 1985, for review), we have shown that uptake of paraquat appears to be through a membrane protein-mediated process (Hart et al., 1992a). Furthermore, we recently reported that this divalent cationic herbicide may enter plant cells via a carrier system that normally functions in the transport of diamines, such as putrescine and cadaverine (Hart et al., 1992b). Specificity for the transporter seems to be related to the spatial distribution of positively charged amine groups.

Kinetic studies characterizing the transport of ions across the plasmalemma are generally conducted using short-term uptake experiments. These types of studies have been widely used to provide an approximation of the unidirectional rate of movement of ions across the root-cell plasmalemma. By comparison, efflux analysis has been used frequently to approximate the accumulation of organic (Balke and Price, 1988) and inorganic ions (Cram, 1968; Jeschke and Jambor, 1981; Kasimir-Klemedtsson and Pettersson, 1990) into cellular compartments (i.e. cell wall, cytoplasm, and vacuole) and to quantify fluxes between each of these compartments. Using this same technique, we found that whereas paraquat accumulates in the vacuole of root cells, it can also be transported across the tonoplast and plasmalemma with half-times of 4.7 h and 29 min, respectively (Hart et al., 1992a). In that study, seedlings were loaded with radiolabeled paraquat for a fixed period (2 h) prior to initiation of the efflux experiment.

In the current investigation, we examined the kinetics of paraquat efflux from maize (*Zea mays* L.) seedlings following a series of loading periods (2, 6, 12, 18, or 24 h), which made it possible to estimate the net rate of paraquat accumulation in the vacuole. This approach also provides a measure of cytoplasmic and cell wall paraquat concentrations and fluxes, as well as a determination of the rate of translocation of the herbicide to shoot tissue. We also investigated the time course of vacuolar compartmentation of paraquat in roots of intact maize seedlings. In addition, we monitored the rate of paraquat translocation to shoots within a 24-h treatment period. Our results indicate that compartmental analysis can provide an effective means of estimating the net rate of paraquat accumulation in root cell vacuoles.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* L. Pioneer hybrid 3377) seeds were surface sterilized in 0.5% NaOCl and germinated in the dark on filter paper saturated with 0.2 mM $CaCl_2$. After 3 d, germinated seedlings were selected for uniform growth, transferred to polyethylene cups with perforated bottoms (two to three seedlings per cup), and covered with black polyethylene beads. Cups were then placed into precut holes in the covers of black, plastic boxes containing 5 L of aerated 0.2 mM $CaCl_2$

* Corresponding author; fax 1-607-255-2644.

solution. The seedlings were grown under lights at 25°C with a photon flux density of 150 μmol m^{-2} s^{-1}. After 2 additional days of growth, seedlings were removed from the cups and transferred to beakers for use in efflux and translocation experiments.

Paraquat Efflux and Translocation

Secondary seminal roots of maize seedlings were excised and primary roots of 60 intact seedlings were inserted through polyethylene mesh suspended above 900 mL of aerated solution consisting of 0.2 mM $CaCl_2$ and 1 mM Mes-Tris buffer (pH 6) in 1-L beakers. Aeration served to oxygenate the solution and to facilitate herbicide mixing. Paraquat loading was initiated by introducing 25 μM paraquat containing 10.8 μCi of [^{14}C]paraquat (Sigma Chemical Co.) to the uptake solution. Seedlings were allowed to accumulate paraquat in the dark for 2, 6, 12, 18, or 24 h. Paraquat at the same specific activity was added back to the uptake solution (12-, 18-, and 24-h treatments) every 6 h to maintain the external herbicide concentration at 25 μM. At no time did the concentration of paraquat fall below 20 μM. The loading of radiolabeled paraquat was terminated at each designated time by vacuum withdrawal of the uptake solution; the paraquat was immediately replaced with 900 mL of a solution containing 2.0 mM $CaCl_2$, 1 mM Mes-Tris (pH 6), and 25 μM unlabeled paraquat. Higher concentrations of Ca^{2+} were used in the efflux solution to facilitate rapid exchange of cell-wall-bound paraquat and to inhibit reabsorption of radiolabeled paraquat released from the root (see Hart et al., 1993). Immediately following the appropriate loading period, four seedlings were removed and roots and shoots were excised, blotted, and weighed. Roots were placed in scintillation vials and solubilized in 1 mL of Soluene 350 (Packard). After a 2-h solubilization period at 60°C, 5 mL of Hionic-Fluor (Packard) was added to each vial and the radioactivity was measured in a Beckman LS5000TD liquid scintillation counter. Shoots were oven-dried for 2 d at 60°C and were subsequently oxidized (Harvey Biological Oxidizer OX300, R.J. Harvey Instruments Corp., Hillsdale, NJ). Radiolabeled CO_2 was trapped in Carbon 14 Cocktail (R.J. Harvey Instruments Corp.) and subsequently measured by liquid scintillation counting. For efflux analysis studies, four seedlings were removed and roots were analyzed as described above at time intervals (5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, and 420 min) following the appropriate loading period (in low light; <0.1 μmol m^{-2} s^{-1}). Shoots were also analyzed at 1-h time intervals to determine the amount of paraquat translocated to the shoot. Efflux solution was vacuum withdrawn and fresh solution was added at each root sampling time.

RESULTS AND DISCUSSION

Root Uptake

The time course for paraquat uptake in maize seedlings was determined by combining the total root and shoot radioactivity from seedlings analyzed immediately following the removal of radiolabeled paraquat. Paraquat uptake, based on root fresh weight, appears to be linear ($r^2 = 0.976$) within the 24-h time period. The rate of paraquat uptake during the linear phase was determined to be 56.7 nmol g^{-1} root fresh weight h^{-1} (Fig. 1). It is unlikely that significant paraquat degradation occurred during the course of this experiment. Paraquat metabolism in maize and other plant species has not been shown to occur even after 3 weeks of foliar exposure (Slade, 1966; Funderburk and Bozarth, 1967).

Although paraquat is a foliar-applied herbicide, we investigated the kinetics of paraquat influx and efflux in maize roots for two reasons. Because roots lack chloroplasts, it was possible to minimize the short-term phytotoxic effect of paraquat. In addition, utilizing roots eliminated potential problems of herbicide uptake associated with cuticular penetration. Although we have made the assumption that transport processes in root cells are probably similar to those of leaf cells, it is possible that this is not true.

Secondary seminal roots were excised from maize seedlings to provide a more accurate estimate of paraquat uptake into primary roots from the treatment solution. From previous unpublished work (J.M. DiTomaso and J.J. Hart), we have shown that removing seminal roots has no effect on primary root growth. Based on this, we have made the assumption that cellular transport processes are similarly unaffected.

Paraquat Compartmentation

Efflux of paraquat from maize roots was determined by plotting the log dpm remaining in the root per g fresh weight versus time. The resulting data (not shown) yielded efflux curves similar to those we previously reported in maize roots exposed for 2 h to 100 μM [^{14}C]paraquat (Hart et al., 1992a), which could be dissected into three approximately linear phases. The slope of the straight line drawn through the data points representing the slowest exchanging phase (240–420 min) was used to calculate the rate constant and half-time for efflux from the vacuole. The intercept of this line yielded

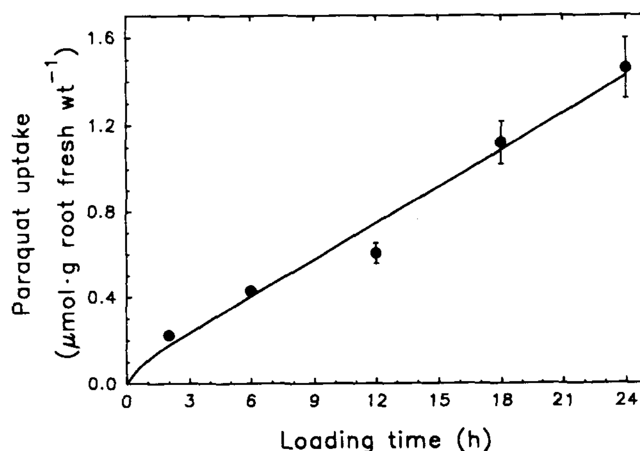

Figure 1. Paraquat uptake in maize seedlings at various time periods following root treatment with a solution containing 1 mM Mes-Tris (pH 6), 0.2 mM $CaCl_2$, and 25 μM [^{14}C]paraquat. Data points represent mean and SD values of four replicates. Error bars do not extend outside all data points.

an estimate of the amount of paraquat in the vacuole at the end of the loading period and was subsequently used to calculate the percentage distribution of paraquat within the cell at the termination of the loading period. Subtraction of this line from the total efflux data yielded a curve that was analyzed similarly but was interpreted as the time course of efflux from the cytoplasm (30–150 min) and cell wall (0–15 min). Subtraction of the straight line representing cytoplasmic paraquat from the remaining data resulted in a single straight line that we considered to represent cell-wall-localized paraquat.

The data presented in Figure 2 represents the amount of paraquat in each of the three cellular compartments at the end of each successive loading period, thus yielding a time course for paraquat compartmentation. Paraquat accumulation in the vacuole was linear over a 24-h loading period, with a first-order rate coefficient of 12.4 nmol g^{-1} root fresh weight h^{-1} (r^2 = 0.994). Vacuolar paraquat content, as a percentage of total accumulated paraquat in the roots, increased from 15% after 2 h of loading to 42% at the end of a 24-h loading period. In contrast to the vacuole, the total amount of paraquat in the cytoplasm increased curvilinearly. The level of paraquat associated with the cell wall fraction remained relatively constant at each loading period, suggesting that this phase saturated rapidly.

Compartmentation (efflux) analysis was initially developed to study the kinetics of ion movement in giant algal cells (see MacRobbie, 1971, for review). Using single-celled organisms, it was possible to correlate the kinetics of ion efflux with distinct compartments, the cell wall, cytoplasm, and vacuole. Subsequent to this early work, efflux analysis has been used to investigate transport processes in higher plants. It was assumed that the compartments of higher plant tissues, i.e. cell wall, cytoplasm, and vacuole, are functional in series. However, it is important to recognize the limitations of applying a technique based on flux analysis in single cells to a complex, multicellular tissue, such as a root. For this and other reasons, several researchers have questioned the use of compartmental efflux analysis in higher plants. For example, it has been argued that radiolabeled ions may be slowly released from cell wall binding sites (Spanswick and Williams, 1965; Jorgenson, 1966), chemically bound within the cytoplasm (Walker and Webb, 1981; Robinson and Jackson, 1986), or compartmentalized in other organelles, such as plastids (Cheeseman, 1986). Under these conditions, the use of the simplistic three-compartment model would lead to an overestimation of the accumulation of a compound in the cytoplasm or vacuole. Cheeseman (1986) also questioned the approach after analyzing simulated error-free, four-compartment data using computerized nonlinear regression techniques. He found that the analysis more often resolved the curve into three components rather than four, and he concluded that compartmental efflux analysis by itself may not provide reliable information on multicompartmental systems.

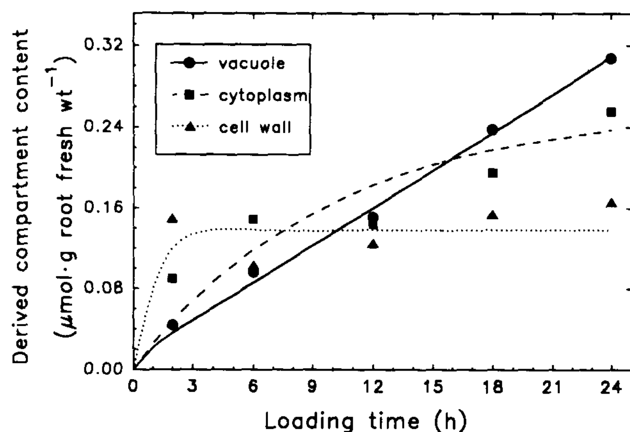

Figure 2. The estimated content of [^{14}C]paraquat in the vacuole, cytoplasm, and cell wall of maize roots after various loading periods. Data were derived from the t = 0 intercept of the straight lines representing efflux from the three compartments at each loading period.

Despite these criticisms, no other experimental methods are available to study ion or herbicide compartmentation in a semiquantitative manner. Although a number of researchers have recognized these limitations, they have utilized compartmental analysis to provide valuable information on the efflux and sequestration of several compounds, including the mono- and divalent cations K^+ (Kochian and Lucas, 1982), Na^+ (Pierce and Higinbotham, 1970), NH_4^+ (Macklon et al., 1990), Cd^{2+} (Rauser, 1987), Cu^{2+} (Thornton, 1991), Zn^{2+} (Santa Maria and Cogliatti, 1988), Co^{2+} (Macklon and Sim, 1987), and putrescine (DiTomaso et al., 1992), the anions Br^- (Pitmann, 1963), Cl^- (Cram, 1968), SO_4^{-2} (Peterson, 1987), PO_4^- (Ron et al., 1988), and NO_3^- (Macklon et al., 1990), and the nonionic herbicide atrazine and its metabolites (Balke and Price, 1988).

Many compartmentation studies have been conducted both with excised root segments (Cram, 1968; Balke and Price, 1988; Ron et al., 1988; Macklon et al., 1990) and with roots of intact seedlings (Jeschke and Jambor, 1981; Jeschke, 1982; Macklon and Sim, 1987, 1990; Rauser, 1987; Santa Maria and Cogliatti, 1988; Pettersson and Kasimir-Klemedtsson, 1990; Thornton, 1991). Although efflux analysis in intact seedlings may be complicated by translocation of the compound of interest to the shoots, it has been argued that energy reserves may become limiting in experiments conducted with excised root tissues (Jeschke and Jambor, 1981). This is particularly true when experiments are conducted at lengthy loading and efflux times. To account for radiolabeled paraquat that can move both into the external solution bathing the roots and also via translocation in the xylem to the shoot, we measured total radiolabeled paraquat remaining in the roots at various times during the course of the efflux experiment. This approach should provide a more accurate means of estimating the kinetics of paraquat movement from the cell wall, cytoplasm, and vacuole.

It should be noted here that for the compartmental analysis performed on seedlings loaded for short time periods, flux equilibrium was probably not achieved. Because the establishment of flux equilibrium is one of the requirements for conducting compartmental kinetic analysis, the approach used here can be subject to criticism. However, in earlier studies on compartmental analysis in oat coleoptiles (Pierce

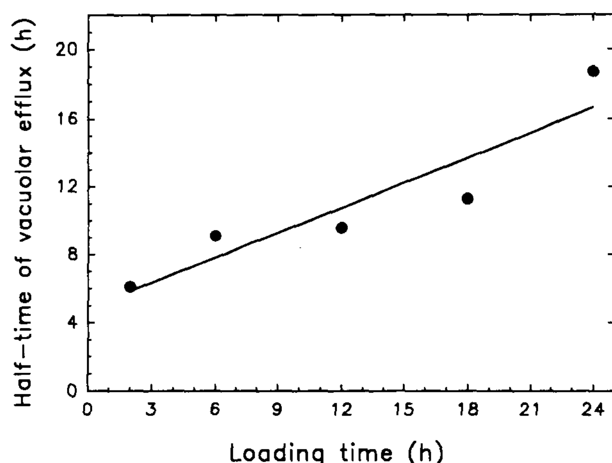

Figure 3. Estimates of the half-time of paraquat efflux from the vacuole in roots of intact maize seedlings. Data points were calculated from the slopes of the slowest exchanging component at each loading period.

and Higinbotham, 1970) and carrot root tissue (Cram, 1968), in which net ionic fluxes were occurring, the authors provided good arguments for the applicability of the technique if there were clear separation of the slopes and rate constants for the putative cytoplasmic and vacuolar components. As discussed below and presented previously (Hart et al., 1992a), the paraquat efflux kinetics for maize roots could be easily resolved into distinct cytoplasmic and vacuolar phases.

In the current study and in previous work (Hart et al., 1992a), we have used compartmental analysis to demonstrate that paraquat efflux from maize roots was consistent with efflux from three compartments in series: the vacuole, cytoplasm, and cell wall. This has not been the case with all divalent cations. Compartmental analysis for Cu^{2+} could not be performed on ryegrass (*Lolium perenne* L.) roots because a significant amount of the metal ion was bound to negatively charged cell wall sites and was only slowly released following removal of the radiotracer. This slowly desorbing Cu^{2+} interfered with measurements of Cu^{2+} efflux from the vacuole (Thornton, 1991). A similar situation, however, does not appear to exist with paraquat (Hart et al., 1992a). Although we found that the divalent cationic herbicide did bind to maize cell walls, radiolabeled paraquat loaded into living and dead (frozen-thawed) roots yielded distinctly different efflux kinetics. After 2 h of efflux, the total amount of radiolabeled paraquat in dead tissues was only 2% of that in living roots, indicating that the bulk of the herbicide in living tissues was localized in the cytoplasm or vacuole.

We are aware of only one other study that used the same approach as the current study to estimate the net rate of accumulation of an ion into the vacuole. Cram (1968) similarly reported that the net rate of $^{36}Cl^-$ accumulation in the vacuole of carrot roots was linear over a 90-min loading period. In contrast, he demonstrated that cytoplasmic $^{36}Cl^-$ content saturated after approximately 30 min of loading, and at a level of more than 1 order of magnitude below that of vacuolar $^{36}Cl^-$.

The results presented here support our previous findings for paraquat compartmentation (Hart et al., 1992a) and suggest that the herbicide is slowly sequestered away from the site of action in the cytoplasm. This is of primary importance in light of recent reports suggesting vacuolar sequestration as a possible mechanism of paraquat resistance in two weed species, *Conyza bonariensis* (Fuerst and Vaughn, 1990) and *Hordeum glaucum* (Bishop et al., 1987).

Paraquat Efflux

The half-time for vacuolar efflux increased linearly with extended exposure to radiolabeled herbicide (Fig. 3), which paralleled the increase in vacuolar paraquat concentration with time depicted in Figure 2. One possible explanation for the increasing half-times at extended periods would involve a putative paraquat tonoplast transport protein that can be saturated at intermediate vacuolar paraquat concentrations. At saturating paraquat concentrations, the rate of efflux from the vacuole would reach a maximum. Hence, at the longer loading periods where the vacuolar paraquat concentrations exceeded this saturating level, a constant (maximal) rate of paraquat efflux would result in longer efflux half-times. A similar scenario may explain the increased half-time for vacuolar putrescine efflux after a 24-h (half-time = 7.3 h) loading period compared with efflux across the tonoplast following a 2-h (half-time = 2.9 h) exposure (DiTomaso et al., 1992). In contrast to the data for vacuolar efflux, the half-time of paraquat efflux from the cytoplasm remained nearly constant (58.8 min ± 8.9 SD), regardless of the loading time, suggesting that the transport system involved in paraquat efflux across the plasmalemma was saturated relatively quickly. Similarly, the half-times for cell-wall efflux were relatively constant at each loading period (16.6 min ± 1.2 SD).

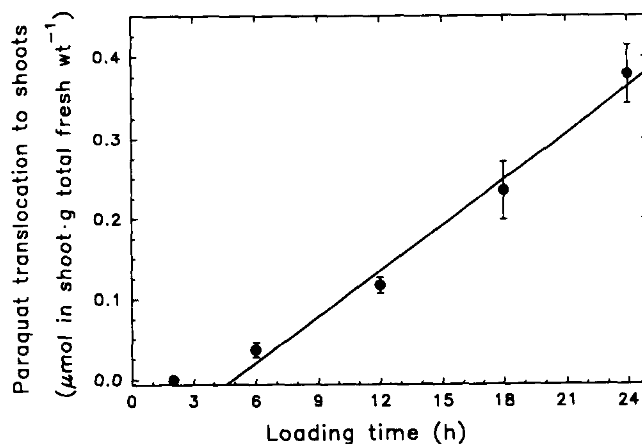

Figure 4. Translocation of [^{14}C]paraquat from roots to shoots of maize seedlings at several time intervals following a continuous 18-h exposure. Data points represent mean and SD values of four replicates.

Paraquat Translocation

When paraquat translocation was monitored at several time intervals during a 24-h exposure (Fig. 4), the amount of herbicide in the shoots increased linearly (19.0 nmol g^{-1} total fresh weight h^{-1}; $r^2 = 0.985$) during the course of the experiment. The rate of paraquat translocation to the shoots, based on root weight, was estimated to be 77% of the rate of uptake into roots. After 24 h, 60% of the total radiolabel in the plant tissue was localized in the shoots. Because paraquat that remained in the roots was used in the compartmentation analysis, efflux data represent both paraquat moving out of the roots into the external solution and paraquat translocating to the shoots. From these experiments, it was estimated that approximately 50% of the radiolabeled paraquat effluxing from roots cells was translocated to the shoot during the 7-h efflux period. Analysis of the data for paraquat translocation at loading times between 6 and 24 h indicated that the line intercepted the x axis at 4.6 h. This is in agreement with a number of other reports that found K^+ transport into the xylem translocation stream to be delayed by 1 to 4 h in low-salt-grown plants (see Kochian and Lucas, 1988, for review).

Although paraquat is a rapidly acting foliar-applied herbicide, it is considered to be relatively immobile in plants (Bishop et al., 1987). However, our results clearly indicate that paraquat is capable of significant translocation to the shoots following root exposure.

CONCLUSION

Efflux analysis conducted at various loading periods can provide a versatile method of approximating the movement within plants of a compound such as paraquat. This is particularly true when paraquat uptake and efflux in roots is measured concurrently with translocation to shoots. Using this approach, it is possible not only to measure paraquat translocation to shoots, but also to estimate simultaneously the net rate and time course of paraquat accumulation in the cell wall, cytoplasm, and vacuole, and the subsequent rate of efflux from these compartments. This is not possible when compartmental analysis is conducted at a single loading period, as is the customary approach.

ACKNOWLEDGMENT

We thank Robin Vanderpool for her technical assistance in these experiments.

Received January 11, 1993; accepted March 18, 1993.
Copyright Clearance Center: 0032-0889/93/102/0467/06.

LITERATURE CITED

Balke NE, Price TP (1988) Relationship of lipophilicity to influx and efflux of triazine herbicides in oat roots. Pestic Biochem Physiol **30:** 228–237

Bishop T, Powles SB, Cornic G (1987) Mechanism of paraquat resistance in *Hordeum glaucum*. II. Paraquat uptake and translocation. Aust J Plant Physiol **14:** 539–547

Cheeseman JM (1986) Compartmental efflux analysis: an evaluation of the technique and its limitations. Plant Physiol **80:** 1006–1011

Cram WJ (1968) Compartmentation and exchange of chloride in carrot root tissue. Biochim Biophys Acta **163:** 339–353

DiTomaso JM, Hart JJ, Kochian LV (1992) Transport kinetics and metabolism of exogenously applied putrescine in roots of intact maize seedlings. Plant Physiol **98:** 611–620

Fuerst EP, Vaughn KC (1990) Mechanisms of paraquat resistance. Weed Technol **4:** 150–156

Funderburk HH, Bozarth GA (1967) Review of the metabolism and decomposition of diquat and paraquat. J Agric Food Chem **15:** 563–567

Hart JJ, DiTomaso JM, Linscott DL, Kochian LV (1992a) Characterization of the transport and cellular compartmentation of paraquat in intact maize seedlings. Pestic Biochem Physiol **43:** 212–222

Hart JJ, DiTomaso JM, Linscott DL, Kochian LV (1992b) Transport interactions between paraquat and polyamines in roots of intact maize seedlings. Plant Physiol **99:** 1400–1405

Hart JJ, DiTomaso JM, Linscott DL, Kochian LV (1993) Investigations into the cation specificity and metabolic requirements for paraquat transport in roots of intact maize seedlings. Pestic Biochem Physiol **45:** 62–71

Hess FD (1985) Herbicide absorption and translocation and their relationship to plant tolerances and susceptibility. *In* SO Duke, ed, Weed Physiology, Vol II. CRC Press, Boca Raton, FL, pp 191–214

Jeschke WD (1982) Shoot-dependent regulation of sodium and potassium fluxes in roots of whole barley segments. J Exp Bot **33:** 601–618

Jeschke WD, Jambor W (1981) Determination of unidirectional sodium fluxes in roots of intact sunflower seedlings. J Exp Bot **32:** 1257–1272

Jorgenson CK (1966) Symmetry and chemical bonding in copper-containing chromophores. *In* J Peisach, P Aisen, WE Blumberg, eds, The Biochemistry of Copper. Academic Press, New York, pp 1–14

Kasimir-Klemedtsson A, Pettersson S (1990) Compartmentation analysis of efflux of K^+ ($^{86}Rb^+$) and Rb^+ ($^{86}Rb^+$) from roots of intact plants of barley (*Hordeum vulgare*) of high and low K^+ status, and after excision of the shoot. Physiol Plant **83:** 511–517

Kochian LV, Lucas WJ (1982) Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear component. Plant Physiol **70:** 1723–1731

Kochian LV, Lucas WJ (1988) Potassium transport in roots. Adv Bot Res **15:** 93–178

Macklon AES, Ron MM, Sim A (1990) Cortical cell fluxes of ammonium and nitrate in excised root segments of *Allium cepa* L: studies using ^{15}N. J Exp Bot **41:** 359–370

Macklon AES, Sim A (1987) Cellular cobalt fluxes in roots and transport to the shoots of wheat seedlings. J Exp Bot **38:** 1663–1677

Macklon AES, Sim A (1990) Cortical cell fluxes of cobalt in roots and transport to the shoots of ryegrass seedlings. Physiol Plant **80:** 409–416

MacRobbie EAC (1971) Fluxes and compartmentation in plant cells. Annu Rev Plant Physiol **22:** 75–96

Peterson CA (1987) The exodermal casparian band of onion roots blocks the apoplastic movement of sulphate ions. J Exp Bot **38:** 2068–2081

Pettersson S, Kasimir-Klemedtsson A (1990) Influx and efflux of K^+ in sunflower roots after transfer between solutions with different K^+ concentrations. Physiol Plant **79:** 686–692

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

Pitmann MG (1963) The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. Aust J Biol Sci **16:** 647–668

Rauser WE (1987) Compartmental eflux analysis and removal of extracellular cadmium from roots. Plant Physiol **85:** 62–65

Robinson NJ, Jackson PJ (1986) 'Metallothionein-like' metal complexes in angiosperms; their structure and function. Physiol Plant **67:** 499–506

Ron MM, Macklon AES, Sim A (1988) Cortical cell fluxes of phosphate in excised root segments of *Allium cepa* L. J Exp Bot **39:** 1699–1707

Santa Maria GE, Cogliatti DH (1988) Bidirectional Zn-fluxes and compartmentation in wheat seedling roots. J Plant Physiol **132:** 312-315

Slade P (1966) The fate of paraquat applied to plants. Weed Res **6:** 158–167

Spanswick RM, Williams EJ (1965) Ca fluxes and membrane potentials in *Nitella translucens*. J Exp Bot **16:** 463–473

Summers LA (1980) The Bipyridinium Herbicides. Academic Press, London

Thornton B (1991) Indirect compartmental analysis of copper in live ryegrass roots: comparison with model systems. J Exp Bot **42:** 183–188

Walker CD, Webb J (1981) Copper in plants: forms and behaviour. *In* JF Loneragan, AD Robinson, RD Graham, eds, Copper in Soils and Plants. Academic Press, Sydney, pp 189–212