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Effects of cadmium on the longitudinal and lateral xylem movement of citric acid through tomato stem internodes

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SUMMARY

Short-term experiments (2·5 h) were performed to gain insight in the effects of cadmium (Cd) on the longitudinal and lateral xylem movement of ^{14}C-citric acid through tomato stem internodes (*Lycopersicon esculentum*, Mill, cv. Tiny Tim). The behaviour of citric acid was expressed by the concentrations of citric acid sorbed to the xylem walls (apparent anion exchange capacity AEC), and by the rate constant k for citric acid lateral escape.

For 0·05 mM citric acid applications, the presence of 1·0 mM cadmium resulted in approximately 2-fold increases in values for apparent AEC and k; for the 9·5 mM citric acid concentrations, cadmium resulted in less than 2-fold increase in AEC, but here k did not change.

In the presence of Cd, the stem-length profile of absorbed citric acid was shown to be in accordance with stem-length profiles of Cd xylem concentrations, rather than with the xylem concentrations of citric acid itself. These results were discussed in the light of the expected presence of Cd–citric acid complexes in the xylem sap; effects on k values were discussed with consideration for the possible processes of escape rate limitation in the stem.

Key-words: anion exchange capacity, cadmium, citric acid, lateral escape, tomato stem internodes.

INTRODUCTION

Organic and amino acids in the xylem are generally indicated to affect the behaviour of many metal ions during transport through the xylem channels (White *et al.* 1981a,b,c; Senden & Wolterbeek 1990). Among the organic acids, citric acid is considered as being of particular relevance, primarily due to the formation of relatively stable metal complexes (Tiffin 1970; Bradfield 1976; Ferguson & Bollard 1976; Van de Geijn & Pikaar 1982; White *et al.* 1981a; McGrath & Robson 1984; Senden *et al.* 1992a).

The effects of the formation of complexes on metal behaviour are mostly indicated by changes in the metal's longitudinal bulk movements, the latter expressed by data on

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stem retention, general movement efficiency (Tiffin 1970, 1972; White *et al.* 1981a,b,c; Kabata-Pendias & Pendias 1985), and apparent exchange capacity (Wolterbeek 1986; Senden & Wolterbeek 1990; Senden *et al.* 1992b). A related measure of the effects of complexation on the behaviour of the metal is the lateral escape (Senden & Wolterbeek 1990), expressed by the escape rate constant, which, for cadmium, was reported to be reduced due to the formation of cadmium–citric acid complexes (Senden & Wolterbeek 1990).

Remarkably, hardly any information exists on the effects of metals on the behaviour of xylem organic solutes. Effects of metals on the behaviour of organic solutes are described and used in various ways in chemistry, most especially in ligand-exchange chromatography (Helfferich 1961, 1962; Navratil *et al.* 1975; Davankov & Senechkin 1977; Matejka & Weber 1990; Sud *et al.* 1992), but in plant transport physiology the only information available is reported by Senden *et al.* (1992b, 1994). In the latter papers, cadmium, calcium and magnesium were shown to affect the sorption of citric acid into columns of isolated tomato xylem cell walls: the total citric acid sorption increased up to 7-fold the control values by metal treatments.

The present paper describes perfusion experiments with isolated stem internodes of tomato plants (*Lycopersicon esculentum* Mill, cv. Tiny Tim). The experiments were set up to investigate the persistence of effects of Cd on citric acid sorption in isolated xylem cell walls (see Senden *et al.* 1994), in a plant system which also allows lateral escape. The data indicate the effects of cadmium on both longitudinal movement (expressed by values on apparent anion exchange capacity, AEC) and lateral escape (expressed by the lateral escape rate constant) of citric acid during its transport through the xylem.

MATERIALS AND METHODS

Plant material

Tomato plants (an inbred line of the red cherry tomato *Lycopersicon esculentum* Mill cv. Tiny Tim) were grown in hydroculture (solution composition according to Senden *et al.* 1992a) in a heated glasshouse at 25°C and 75% relative humidity. Internodes (lengths and cross-sections approximately 120 mm and 3·5 mm, respectively) of 12-week-old plants were cut off under water and used throughout.

Preparation for perfusion

Silicone tubes were fitted around the morphological basal and apical ends of the internode, which was wrapped in aluminium foil to prevent stem-surface transpiration (Van Bel 1974). The internode was fixed and connected by the silicone tubing to a supply reservoir (morphological basis) and a pump (ip-12 Ismatec) (morphological apical end). The volume-flows during perfusions were about 0·85 ml h^{-1}. The perfusate (controlled as 50–2500 µl) was collected in a fraction collector (Retriever II ISCO), under permanent time registration.

Internodes pre-perfusions

Before experiments, all internodes were standardized (Na-form) by pre-perfusion with 0·5 ml 10 mM Na_2EDTA solutions (pH=6·0); excess Na and metal-EDTA were removed by subsequent rinsing with 0·5 ml distilled water. The applied 5 µm of EDTA was considered to remove metal ions from xylem vessel walls without significant effects

on cell membranes in the internode tissues, assuming (i) the high xylem volume flow and corresponding small quantities of EDTA escaping in lateral direction, and (ii) the small amount of introduced EDTA relative to a total internode Ca of about 50 μM (see also Van Steveninck 1965).

Determination of the average cross-section A_p (Wolterbeek 1986) of the xylem vessels was carried out by pre-perfusion of 0·5 ml [^{3}H]inuline (Van Bel 1978). Based on calculated A_p values, average linear flow rates of about 0·75 mm s^{-1} were obtained.

Experiments

Perfusion experiments were carried out with 2·5 ml 0·05 mM and 9·5 mM citric acid solutions (spiked with ^{14}C-citric acid), both in the absence and presence of 1·0 mM $Cd(NO_3)_2$. Before use, all solutions were set at pH 6·0 with KOH; further buffering was not considered necessary (Van Bel 1978; Senden *et al.* 1992b). After perfusion, xylem vessels were flushed by 1·0 ml distilled water. For the 9·5 mM citric acid applications, citric acid associated with xylem vessel walls was exchanged by direct perfusion of 2·5 ml 20 mM $Ca(NO_3)_2$, after which the internode was cut into pieces of 10 mm each.

For the 0·05 mM citric acid applications, internodes were cut into pieces prior to the perfusion with 20 mM $Ca(NO_3)_2$; in this case 0·1 ml volumes were applied to internodal subsegments of 10 mm length. For this purpose, each of the pieces was connected by silicone tubing to a pump, similar to the routines applied in experiments with 100 mm internodes.

Cd perfusion was carried out using 2·5 ml of a 0·1 mM ^{115}Cd spiked $Cd(NO_3)_2$ solution. Here, after perfusion and rinsing with 1·0 ml distilled water, the internode was cut into 10 mm pieces, and directly analysed for ^{115}Cd. After analysis, each of the pieces was perfused with 100 μl 20 mM $Ca(NO_3)_2$; here both collected perfusates and the stem pieces were counted for ^{115}Cd.

Radioisotope production and measurement

The radiotracer ^{115}Cd ($t_{1/2}$=53·4 h, initial specific activity 0·9 GBq g^{-1}) was produced by neutron activation of $Cd(NO_3)_2$ in the Hoger Onderwijs Reactor at Interfaculty Reactor Institute (IRI), Delft, The Netherlands, at a neutron flux density of $5 \cdot 10^{16}$ m^{-2} s^{-1} for 4 h. ^{115}Cd measurements were carried out using a γ-ray spectrometer with a NaI-detector (Auto-Gamma Minaxi 5000 Packard, used settings 425–625 keV) coupled to a multichannel analyser (Canberra).

^{14}C-citric acid (1·85 MBq ml^{-1}, 20·8 GBq g^{-1}) and [^{3}H]inulin (54·1 GBq $mmol^{-1}$, 10.4 GBq g^{-1}) were purchased from Amersham International UK. For ^{14}C-analysis, internode segments (50–100 mg fresh weight) were oxidized in a Tri-Carb Sample Oxidizer Model 306 (Packard); $^{14}CO_2$ was trapped in an organic base Carbo-sorb (Packard), and mixed with ^{14}C-Scintillator Permafluor V (Packard). ^{14}C and ^{3}H in aqueous samples were radioassayed in 10 ml Lumagel (Lumac Landgraaf, The Netherlands) by liquid scintillation counting (Tri–Carb 1600 TR Packard). ^{14}C in organic base samples (Carbo-sorb) were radioassayed in ^{14}C-Permafluor V (Packard) by liquid scintillation counting (Tri–Carb 1600 TR Packard). ^{14}C-recoveries (in sample treatment and oxydation procedures) were determined as 96 ± 9% (n=24) by regular analysis of ^{14}C standards (Amersham International UK).

Calculations

Apparent anion exchange capacity. Absolute quantities of citric acid (Q) associated with the xylem vessel walls were determined from the $Ca(NO_3)_2$ flushes. The apparent anion exchange capacity AEC (concentration basis) was derived from the known average cross-sectional area (A_p) and the length of the translocation pathway (L), following Wolterbeek *et al.* (1985), assuming an average cell wall thickness d, as:

$$AEC=\frac{M\,Q}{A_p\,L\,d} \tag{1}$$

with M=0·013 mm, as derived from the frequency distribution of vessel diameters (Wolterbeek *et al.* 1985). In all calculations, d was taken as 2 μm (Van de Geijn & Petit 1979; Wolterbeek *et al.* 1985).

Lateral escape rate constant. Following the formulations by Horwitz (1958), for flow of solutes through plant transport channels, the solute concentration C_p after perfusion will be related to the introduced solute concentration C_0 by:

$$\frac{C_p}{C_0}=e^{\frac{-k.L}{v}} \tag{2}$$

with L as the length of the translocation pathway, v as the linear velocity of solution flow, and with k as a first order reaction constant, expressing the rate at which solutes laterally escape from the moving xylem sap.

The amount of solute in the surrounding tissues after perfusion time t and at length L, $X_{t,L}$ can be expressed as

$$X_{t,L}=A_p\,k\,C_0\,e^{\frac{-k.L}{v}}\,(t-\frac{L}{v}) \tag{3}$$

with A_p as the xylem vessel cross-sectional area, and where the term $(t-L/v)$ expresses the time needed for the fluid front to reach distance L.

In the present 3-h perfusion experiments, $t>>L/v$; therefore, $X_{t,L}$ was expressed in integrated form as:

$$X_t=\int_0^L X_{t,L}dL=A_p\,C_0\,v\,t\left(1-e^{\frac{-k.L}{v}}\right) \tag{4}$$

with X_t as the absolute amount of solute in the total internode at time t, from which k was calculated as:

$$k=\frac{-\ln\left[1-\frac{X_t}{(A_p\,C_0\,v\,t)}\right]}{\frac{L}{v}} \tag{5}$$

Speciation. The chemical speciation of Cd and citric acid was calculated with the help of the computer program SOILCHEM, developed at the University of California, Berkeley, USA (Sposito & Coves 1988), with consideration for mol balances, relevant thermodynamic equilibrium constants and ionic strengths.

Table 1. Amounts of citric acid adsorbed to xylem cell walls (AEC) and values for lateral escape rate constant (k) of citric acid solutions with and without cadmium during transport through tomato stem internodes

Applied citric acid (mM)	Applied cadmium (mM)	Total citric acid recovery (%)	A_p (mm^2)	k (h^{-1})	AEC (mM)
0·050	—	101	0·08	0·75	0·015
0·050	—	101	0·27	1·17	0·021
0·050	1·0	94	0·37	1·88	0·043
0·050	1·0	93	0·32	3·09	0·044
9·5	—	100	0·31	0·76	2·94
9·5	1·0	105	0·28	0·85	4·84

For speciation calculations in xylem fluid, the effects of the fixed wall charges were taken into account only indirectly: determinations were based on the calculated total concentrations in bulk xylem fluid, C_p which, of course, were affected by the fixed wall charges.

RESULTS

AEC

Application of 9·5 mM citric acid resulted in an AEC value for citric acid of 2·94 mM (Table 1). This value was calculated from the citric acid recoveries during $Ca(NO_3)_2$ flushing, assuming that d=2 μm. Similarly, under 1·0 mM $Cd(NO_3)_2$ perfusion conditions, the citric acid apparent AEC was determined as 4·84 mM (Table 1).

For the 0·05 mM citric acid perfusions, duplicate AEC-values were obtained in the absence and presence of 1·0 mM $Cd(NO_3)_2$ as (0·015 and 0·021 mM) and (0·043 and 0·044 mM), respectively. Obviously, Cd additions resulted in an about 2-fold increase in AEC values for citric acid, for both citric acid concentrations. The order of magnitude of AEC values agrees with values found in experiments with columns of isolated xylem cell walls as reported by Senden *et al.* (1994). The 2-fold increase in response to Cd perfusion corresponds to predictions distilled from earlier column experiments (Senden *et al.* 1994) for 0·05 mM citric acid perfusions.

Cell wall sorption versus internode length

Figure 1 shows the cell wall sorption of citric acid determined directly from ^{14}C-counting, in 0·05 mM citric acid experiments. The lines drawn were obtained by fitting of $F(L)=ae^{-b.L}+c$ functions, with constants a, b and c. The marked decline in citric acid sorption with stem length under Cd conditions corresponds with the escaped quantities of Cd rather than with the cell wall sorbed Cd concentrations (Fig. 2). From the Cd-distribution (Fig. 2), using equations 2–5, the C_p/C_0 values for xylem Cd could be calculated (Fig. 3), which, together with the citric acid C_p/C_0 values (Fig. 3), underline the importance of the presence of Cd in the xylem sap for the chemical speciation and subsequent cell wall sorption of citric acid (Senden *et al.* 1994). Based on Fig. 3, the fractional citric acid presence as xylem Cd-complexes calculated as 97%,

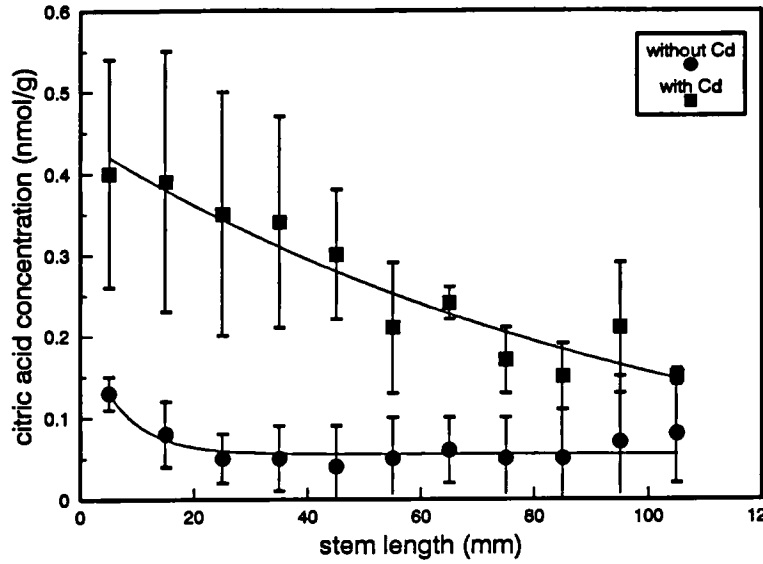

Fig. 1. Distribution of xylem cell wall sorbed citric acid, per stem length, for control 0·05 mM ^{14}C-citric acid concentrations. The stem length is given from basal to apical direction. The data are averaged values (± SD) from perfusions with two different stem internodes. The lines were drawn after curve-fitting by $F(L)=ae^{-bL}+c$ (control: $r^2=0{\cdot}79$, a=0·16 ± 0·07, b=0·15 ± 0·08, c=0·056 ± 0·05; with 1·0 mM Cd: $r^2=0{\cdot}91$, a=0·47 ± 0·22, b=0·010 ± 0·008, c= − 0·03 ± 0·25).

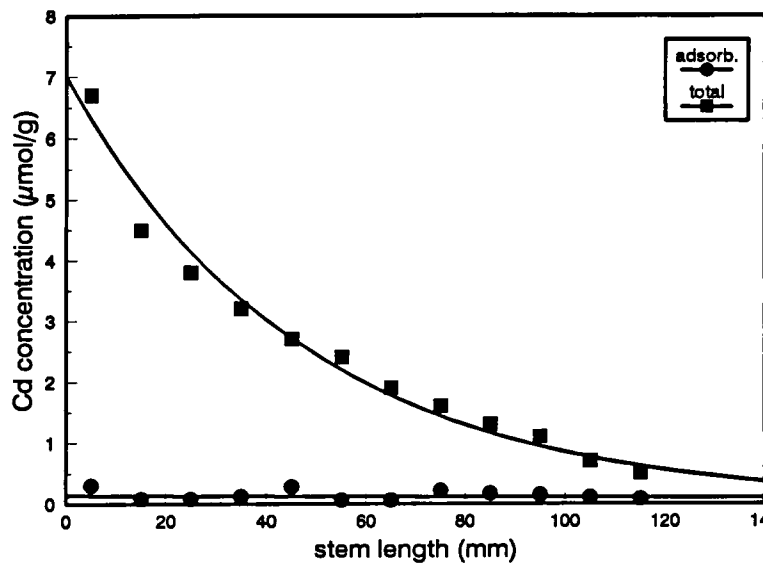

Fig. 2. Distribution of ^{115}Cd in the stem, after 1·0 mM $^{115}Cd(NO_3)_2$ applications. The stem length is given from basal to apical direction. Solid squares: total internode ^{115}Cd, the line drawn was fitted by $F(L)=ae^{-bL}+c$($r^2=0{\cdot}98$). Constants derived (± SD) were 8·98 ± 0·48 (a), 0·031 ± 0·004 (b) and 0·66 ± 0·24 (c). Solid circles: xylem cell wall adsorbed ^{115}Cd, line drawn was fitted by $F(L)=aL+b$ ($r^2=0{\cdot}08$), with constants—0·00064 ± 0·00069 (a) and 0·19 ± 0·05 (b).

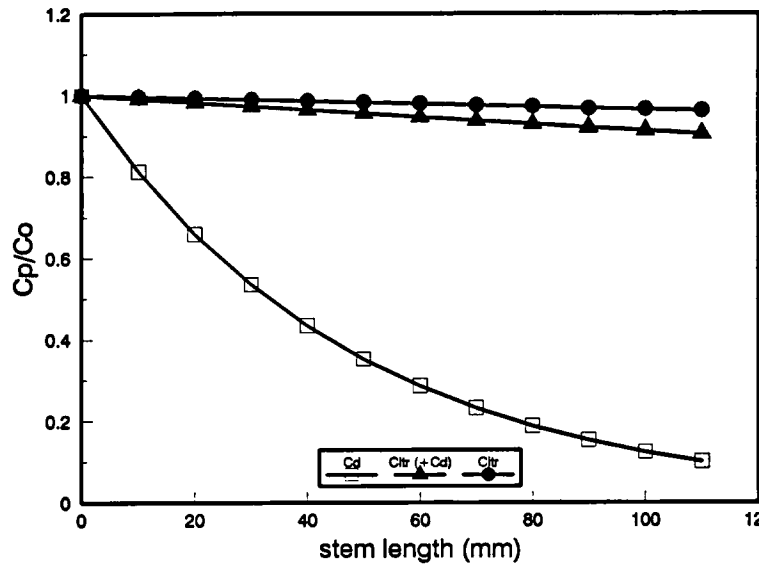

Fig. 3. Xylem concentrations C_p of cadmium (squares) and of citric acid (control: solid circles, with 1·0 mM Cd: solid triangles), relative to the introduced concentrations C_0, in relation to stem length. Introduced concentrations C_0 were 1 mM (Cd) and 0·05 mM (citric acid).

92% and 76%, for $L=0$, 50 and 110 mm, respectively. Here, it should be noted that effects from both fixed charges and lateral escape are expressed in the C_p values determined.

Lateral escape

For 9·5 mM citric acid, no appreciable Cd-related changes in k could be observed (Table 1). The absence of any response to Cd may be due to regulation of the escape by surrounding tissues rather than by the transport of citric acid through the xylem cell walls (Wolterbeek *et al.* 1985). As a result, effects of the presence of Cd on the lateral escape of citric acid may not be unequivocally determined by calculating rate constants k.

For the 0·05 mM citric acid concentrations, simultaneous application of $Cd(NO_3)_2$ roughly resulted in a 2·5-fold increase in the lateral escape of citric acid (Table 1). At this concentration, an increase in cell wall sorption of citric acid (AEC values, Table 1) concurs with an increase in the rate of lateral escape.

DISCUSSION

The present experiments were performed to investigate the persistence of effects of Cd on citric acid sorption in isolated xylem cell walls (Senden *et al.* 1994) in a plant system which allows lateral escape. Transport characteristics were expressed by apparent AEC and k transport parameters independent of variations in A_p, L, or v between individual plants (Wolterbeek 1986; Senden & Wolterbeek 1990).

The applied $Cd(NO_3)_2$ concentrations are in line with Cd concentrations used elsewhere in short-term experiments (Van de Geijn & Petit 1979; Van de Geijn *et al.* 1979; Senden & Wolterbeek 1990). Accumulated amounts of Cd in stem segments are in

Table 2. Citric acid and cadmium speciation in applied solutions (pH 5·7) in experiments with simultaneous addition of citric acid and Cd. Fractional presence rounded off to the nearest full %

Applied solution concentration		Fractional presence (%)												
Cd mM	cit mM	Cd^{2+}	$Cdcit^-$		$Cd(cit)_2^{4-}$		$CdHcit$		CdH_2cit^+		cit^{3-}	$Hcit^{2-}$	H_2cit^-	H_3cit
			Cd	cit	Cd	cit	Cd	cit	Cd	cit				
	$5{\cdot}0\ 10^2$										38	59	3	*
	9·5										59	40	1	*
1·0	$5{\cdot}0\ 10^2$	95	5	91	*	*	*	4	*	*	2	2	*	*
1·0	9·5	1	68	7	28	3	3	*	*	*	52	36	1	*

*Trace amount <0·5%.

the same order of magnitude as reported by Florijn (1993) and De Knecht (1994). Although these levels may be suspected to affect cellular metabolism (Kabata-Pendias & Pendias 1985; Adriano 1986), time aspects of Cd toxicity (Bazzaz *et al.* 1974; Lamoreaux & Chaney 1978; Wolterbeek *et al.* 1988; De Knecht 1994) mean that Cd effects on cellular metabolism were not taken into consideration in the present short-term experiments.

The AEC data in internode tissues (Table 1) clearly demonstrate the effects of Cd on cell wall sorption of citric acid, as shown earlier for columns of isolated xylem cell wall material (Senden *et al.* 1994). For 0·05 mM citric acid, the 2-fold increase corresponds to what was predicted by the column experiments. The less than 2-fold increase for 9·5 mM citric acid, however, is markedly less than the 7-fold increase in column experiments (Senden *et al.* 1994). This discrepancy cannot be explained at present, but effects of the relatively high acid concentrations are not expected (see Van Bel 1978; Van der Schoot 1989, and Senden *et al.* 1992b, for pH buffering in internodes and isolated xylem cell walls).

Column and internode experiments differed with respect to their pre-perfusion protocols. Columns were pre-perfused by subsequent rinses of HCl, NaCl and H_2O (Senden *et al.* 1994); the pre-perfusions in internodes were subsequent rinses of Na_2EDTA and H_2O. The latter rinsing solutions were adopted from Van de Geijn & Petit (1979) to prevent any possible cellular damage by HCl. Furthermore, and for similar reasons, although in column experiments the exchange capacity was determined by HCl flushes, in the present experiments use was made of 20 mM $Ca(NO_3)_2$. At first sight, the $Ca(NO_3)_2$ flushing may not have been as effective as the HCl in extracting all citric acid, especially at high citric acid applications, but the flushes were collected in seven fractions, of which the first showed about 55% of the total citric acid recovery during $Ca(NO_3)_2$ flushing. Furthermore, the total recovery (105%, see Table 1) does not indicate any loss of citric acid.

All k-data determined were based on application of equation 5. In principle, use of equation 2 is more convenient to yield k values, but here the accuracy of k depends on the ratio C_p/C_0. As relatively high linear velocities of flow were used, C_p/C_0 ratios were near unit ratio (see Fig. 3), leading to high variances in k. Therefore, k was invariably determined from stem data rather than from solution data.

The k-data (Table 1) indicate that $Cd(NO_3)_2$ increased the rate of citric acid lateral escape by a factor of about 2·5 for 0·05 mM citric acid, but hardly for 9·5 mM citric acid.

It should be noted that, in 0·05 mM additions, citric acid is present as $Cdcit^-$ for 91% (Table 2), whereas in 9·5 mM additions, only about 10% of the citric acid is complexed to Cd (Table 2: $Cdcit^-$, $Cd(cit)_2^{4-}$).

For 9·5 mM citric acid, the 2-fold increment in AEC (Table 1, 9·5 mM citric acid) does not appear to correspond with any increase in k value. As all escape rates (k-values) were based on total citric acid concentrations, effects expressed by k may be obscured by the small fractional presence of complexes between citric acid and Cd in 9·5 mM citric acid solutions. Moreover, the absence of effects may also be due to a shift in the regulation of the lateral escape from the rate of transport through the xylem cell walls to the processes of uptake in the surrounding tissues (Wolterbeek *et al.* 1985). The basis for this reasoning is a limited rate of uptake into cells (Michaelis Menten kinetics) whereas, at high applied concentrations, relatively high intrinsic citric acid fluxes through the xylem cell walls may be expected.

The present results indicate that Cd may affect the behaviour of citric acid during its transport through the xylem vessels, both by influencing its cell wall sorption and its rate of lateral escape. The physico-chemical nature of the processes involved, and the analogous results obtained with Ca and Mg in isolated tomato xylem cell walls (Senden *et al.* 1994), may suggest that the present data in internodes may have more general character than applicable for Cd only.

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