

# Effect of citric acid on the transport of cadmium through xylem vessels of excised tomato stem–leaf systems

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## SUMMARY

The positive effects of citric acid on the transport of cadmium (Cd) through the xylem vessels of tomato stems was quantitatively studied by measuring the rate constant ( $k$ ) of the lateral escape from the vessels to surrounding tissues and the amount of Cd adsorbed to the xylem cell walls. These two transport parameters were shown to decrease after addition of citric acid to the Cd solution, probably due to the formation of uncharged and negatively charged Cd–citrate complexes, which show little binding to the mainly negatively charged xylem cell walls. Reduced affinity of metal complexes to the cell walls can explain the long-term positive effects of citric acid on longitudinal transport of Cd and other metals in the xylem.

*Key-words:* cadmium, cation adsorption, citric acid, lateral escape, tomato, xylem transport.

## INTRODUCTION

Long-distance transport of elements taken up from the soil mainly occurs through the xylem vessels. It can be described as a bulk flow mainly driven by transpiration. The substances present in this flow, which is essentially a movement through the apoplast of the xylem, become involved in processes by: adsorption to mainly negatively charged sites present in the walls (Bell & Biddulph 1963; Ferguson & Bollard 1976; Wolterbeek *et al.* 1984); and lateral escape from the moving solution in the vessels into the adjacent inter-cellular spaces and walls (free space) and eventually to cells of other tissues (Van Bel 1978).

Although cadmium (Cd) is considered to be a non-essential element for plants, it is effectively absorbed by both root and leaf systems. Its long-distance translocation as a metal ion is limited, probably due to the binding of cations to exchange sites located in the xylem cell walls (Bell & Biddulph 1963; Ferguson & Bollard 1976; Wolterbeek *et al.* 1984; Kabata-Pendias & Pendias 1985). The possible formation of complexes or metal chelates in soils and in plants, however, may result in easy availability of soil-Cd and effective transport of Cd–organic complexes in plants (Tiffin 1970, 1972; White *et al.* 1981a,b,c; Kabata-Pendias & Pendias 1985). Increased efficiency of longitudinal Cd transport in xylem vessels will lead to elevated levels of Cd in the leaves, with possible phytotoxic effects of Cd on photosynthesis, transpiration, etc. (Bazzaz *et al.* 1974; Lamoreaux & Chaney 1978; Marschner 1983; Kabata-Pendias & Pendias 1985).

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White *et al.* (1981a,b,c) reported the presence of metal complexes with carboxylic and amino acids in xylem fluid of soybean and tomato plants. Increased longitudinal transport is often qualitatively expressed by reduction of the retention time of the substance in the stem, and is generally attributed to reduced binding of the metal concerned to the fixed negative charges of the xylem walls (Tiffin 1970; Bradfield 1976; Ferguson & Bollard 1976, Van de Geijn & Pikaar 1982; McGrath & Robson 1984).

Among the compounds mentioned above citric acid has attracted much attention because it occurs in relatively high concentrations in xylem fluid and forms stable complexes with many divalent cations (Tiffin 1970; White *et al.* 1981a,b,c; McGrath & Robson 1984).

Formation of anionic or uncharged Zn-citrate complexes resulted in more Zn passing through the excised stem of *Pinus radiata* (McGrath & Robson 1984). Citric acid is reported to have the same effect on the transport of Ca in apple shoots (Bradfield 1976; Ferguson & Bollard 1976), Fe in soybeans (Tiffin 1970) and Cu in papyrus stems (Van de Geijn & Pikaar 1982).

Another, probably related, phenomenon is the lateral escape of the metal considered (Van Bel 1978), the rate of which may also change, due to the formation of organic complexes. This rate also quantitatively affects the quantitative upward movement of elements (Van Bel 1978; Wolterbeek 1986).

In the present paper experiments are described in which quantitative effects of complexation on the upward movement of Cd through the xylem of tomato stems were investigated. The influence of citric acid on the transport of Cd through the xylem vessels was described by the changes of the values of both the amount of Cd adsorbed to the xylem vessel walls and of the escape rate constant.

## MATERIALS AND METHODS

### *Plants and plant culture*

Tomato plants (an inbred line of the red cherry tomato *Lycopersicon esculentum* Mill, cv. Tiny Tim) were used throughout. They were cultivated in a glasshouse at *c.* 25°C and 75% relative humidity.

Uptake experiments were carried out with plant sub-systems, consisting of a stem part with one fully expanded leaf. Twenty-four hours prior to the experiment the stem segment to be used (only one internodium) with one petiole (length stem-internodium with petiole *c.* 200 mm) was cut under water and conditioned in tap water to laboratory conditions (30°C, 70% relative humidity). From the composite tomato leaf all leaflets except the top three ones were cut in order to ensure a homogeneous element distribution.

### *Element solutions*

Two different element solutions were used. In Cd<sup>2+</sup> transport experiments, the solutions applied contained the radiotracers <sup>115</sup>Cd (0.4 mM, in CdSO<sub>4</sub>) and <sup>122</sup>Sb (0.3 μM, in Sb(SO<sub>4</sub>)<sub>2</sub><sup>-</sup>, formed by adding SbCl<sub>3</sub> to a medium containing SO<sub>4</sub><sup>2-</sup>). The acidity of the solution was set to pH 6 with KOH.

In experiments where the effect of complex formation on metal transport was determined, a citric acid solution (2.5 mM, pH 6) was applied which also contained <sup>115</sup>Cd and <sup>122</sup>Sb (concentrations mentioned above).

Citric acid forms stable complexes with Cd (White *et al.* 1981a,b,c). The dissociation constants, pK<sub>1</sub>, pK<sub>2</sub> and pK<sub>3</sub> of citric acid are reported as 3.08, 4.39 and 5.49, respectively

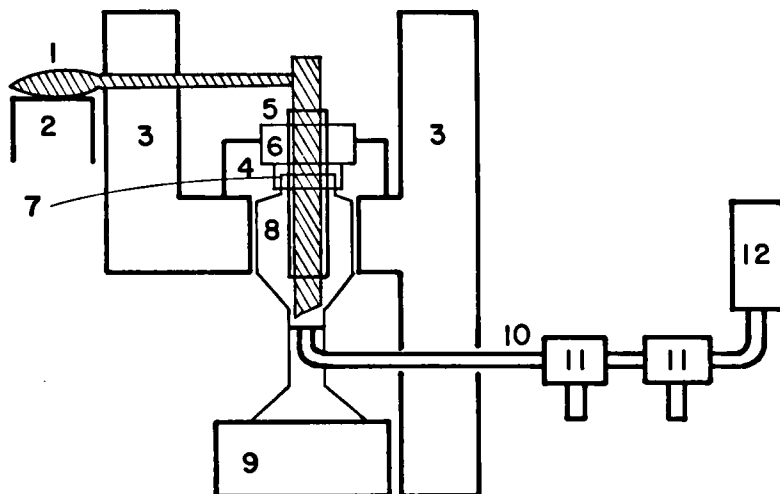


Fig. 1. Schematic representation of experimental set-up and position of the plant sub-system. 1: Fixed tomato leaf with petiole and stem part, 2: Ge(Li)-detector, 3: lead shield, thickness 5–7 cm, 4: fixation of the stem part, 5: perspex guidance tube, 6: guidance tube holder and free keg covering, 7: top of keg, 8: keg, 9: electronic balance, 10: tube 1.5 mm for application and removal of solutions, 11: taps, 12: syringe.

(Martell & Calvin 1959). For calculations of Cd–citrate complexes the values for the association constant ( $\log K$ ) for the formation of Cd–citrate<sup>−</sup> and Cd–H–citrate were taken as 3.98 and 2.28, respectively (Sillen & Martell 1964). Under the conditions applied, only 6–8% of the total Cd was present as Cd<sup>2+</sup>.

The radiotracer <sup>122</sup>Sb was added to both the solutions to permit the calculation of the total cross-sectional area of the xylem vessels involved in fluid and element transport (see section: Measurements and calculations).

#### *Production of radioisotopes and measurement equipment*

The radiotracers <sup>115</sup>Cd ( $t_{1/2} = 53.5$  h,  $\gamma$ -ray energy, 527 keV) and <sup>122</sup>Sb ( $t_{1/2} = 2.8$  days,  $\gamma$ -ray energy, 564 keV) were produced by neutron activation of SbCl<sub>3</sub> and CdSO<sub>4</sub> (see section: Element solutions) in a thermal neutron flux density of  $10^{17}$  m<sup>−2</sup>s<sup>−1</sup>, for 4 h. The activities used were 11.1 MBq and 0.4 MBq for <sup>115</sup>Cd and <sup>122</sup>Sb respectively.

Measurements were carried out using a  $\gamma$ -ray spectrometer with a semiconductor Ge(Li)-detector, coupled to a DEC PDP-11/44 computer through a CAMAC interface. Calibration, spectrum accumulation, control of measurement sequence and spectrum analysis were carried out according to the methods described by De Bruin & Korthoven (1972, 1974) and Korthoven & De Bruin (1977).

#### *Design*

The plant sub-system (stem–leaf) was placed in a lead shield (Fig. 1) which permitted the measurements of the radioisotopes in the leaf parts above the detector, with only minor interference from the activity in the solution and the shielded plant parts. The container with the radioactive solution was placed on an electronic balance to monitor the amount of water absorbed by the plant. One hour counting intervals permitted semi-continuous measurements of transport of elements into the leaf.

### *Measurements and calculations*

The amount of water transported to the leaf in time  $t$  could be described by the function  $F(t) = \alpha(1 - e^{-\beta t})$  with  $\alpha$  and  $\beta$  as constants (Wolterbeek *et al.* 1984).

The total cross-sectional area of xylem vessels ( $A_p$ ) was calculated from the length of the stem segment and the rate of transport of the negatively charged  $^{122}\text{Sb}(\text{SO}_4)_2^-$  (indicating xylem volume), which does not interact with the negatively charged xylem cell walls (Wolterbeek *et al.* 1984).

During the experiments, repeated 1-h countings were made of the absolute quantity of element accumulated in the leaf at time  $t$  at a distance  $l$  from the point of element introduction into the stem.

The lateral escape from xylem vessels was described as a first order process and expressed by the rate constant  $k$  ( $\text{h}^{-1}$ ). The rate constant  $k$  was calculated by fitting a mathematical accumulation function to the data obtained, using an iterative non-linear regression program (for function and full procedures, see Wolterbeek *et al.* 1984).

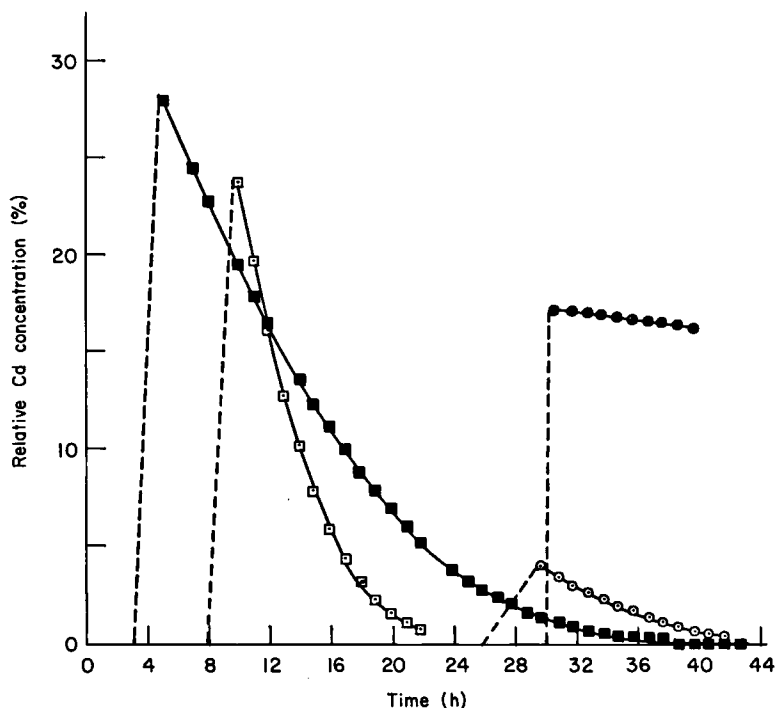
The amount of Cd adsorbed to the xylem cell walls was estimated from the value of  $t_1$  (time needed for the fluid to reach the leaf (determined with the help of  $^{122}\text{Sb}$ ) and for full equilibration of exchange sites) and the amount of Cd introduced into the plant sub-system during this period of time (Wolterbeek *et al.* 1985b).

The amounts of Cd adsorbed to the xylem cell walls were interpreted in terms of concentrations of fixed Cd, which could be derived by assuming a cell wall thickness of  $2 \mu\text{m}$  (Wolterbeek *et al.* 1985a,b).

## RESULTS

Figure 2 shows the Cd concentration in the xylem fluid transported into the leaf, relative to its concentration in the applied solution. Several experiments were conducted under identical conditions and the differences between measurements show the biological variability between plant sub-systems. The dotted line parts indicate the time required for full saturation or equilibrium of the exchange sites with Cd, starting from the time of first appearance of Cd in the leaf. The dotted line starts after an initial 'zero Cd' period (needed for the xylem fluid to reach the leaf and filling of exchange sites). Although obtained in the measurements, no data points are presented for this experimental period, since up to the time needed for reaching equilibrium, data were not processed in terms of relative concentrations. This period of time, and especially that prior to first detection of Cd in the leaf, not only depends on Cd concentrations applied, but also on the xylem volume flow. The full lines drawn show the experimental periods in which the transport could be described by a function reported earlier (Wolterbeek *et al.* 1984), yielding the value of  $k$ . The decrease with time of the relative Cd concentrations (Fig. 2) should be attributed to the characteristics of the xylem volume flow which also decreases with time. Consequently the maximal values of the relative Cd concentrations logically appear as soon as a steady state situation is reached with respect to adsorption processes.

Figure 2 indicates that the differences with respect to the time prior to the first Cd detection in the leaf, and with respect to the values of relative Cd concentrations in the xylem are the results of the mere absence or presence of citric acid in the solution applied. However, the times necessary for the exchange processes to reach an equilibrium situation are the result of both Cd concentrations and xylem volume flow. Thus, differences with respect to Cd adsorbed cannot be expressed on a time basis, but only on an 'amount of Cd absorbed' basis. Furthermore, differences in the relative Cd concentrations in the xylem



**Fig. 2.** Cd concentration in xylem fluid entering the leaf. Amounts are processed with respect to volume flow and are expressed as percentage of applied concentration. (□) and (■) are duplicate experiments with two plant subsystems in which both Cd and citric acid were applied. (○) and (●) are examples of experiments in which only Cd was applied. (---) Indicates the increase of the Cd-concentration with time up to the time  $t_1$ . The time  $t_1$  comprises a 'zero Cd' period, expressing the time needed for the xylem fluid to reach the leaf and initial filling of exchange sites and a 'dotted line' period, which represents the time needed for full equilibration of exchange sites. Note that these times are variables, depending on the rate of xylem flow and chemical form of Cd.

**Table 1.** Average values ( $\pm$ SE) of  $k$  and Cd adsorbed (Cd ads.) for the rate constant of lateral escape and the amount of Cd adsorbed during transport of Cd through xylem vessels in tomato

Applied Cd <sup>2+</sup> (mM)	Applied citrate (mM)	$k$ (h <sup>-1</sup> )	Cd ads. (meq l <sup>-1</sup> )	$n$
0.35 $\pm$ 0.05	—	1.89 $\pm$ 0.42	153 $\pm$ 82	3
0.32 $\pm$ 0.14	2.5	0.73 $\pm$ 0.38	25 $\pm$ 15	4

The values of  $k$  and Cd ads. under the two different conditions were significantly different at  $P < 0.05$  (Student's  $t$ -test).

fluid actually depend on differences in the xylem volume flow (Horwitz 1958), so that the only reliable means of expressing rates of lateral escape is by calculation of the escape rate constant  $k$ .

Table 1 presents the values for the escape rate constant  $k$  and adsorbed Cd, averaged for a number of experiments. The results clearly show reduced values for these two transport

parameters in the presence of citric acid, indicating that citric acid not only affects the adsorption of Cd to the xylem cell walls but also its rate of removal from the vessels in a lateral direction.

## DISCUSSION

Results shown in Fig. 2 indicate that, apart from the metal concentrations applied, the retention time in the stem should be regarded as a plant-dependent parameter, probably largely determined by xylem volume flow and anatomical xylem vessel characteristics such as available absolute quantity of exchange sites.

The required application of at least two plants for the determination of differences in transport may therefore lead to faulty interpretations of results, if these are expressed on a retention-time basis only. In the present paper, the values for the retention-times ( $t_1$  see Fig. 2) are used in combination with xylem volume flow, applied Cd concentration and xylem vessel characteristics (Wolterbeek *et al.* 1984), yielding concentrations of Cd actually adsorbed at the vessel walls (Table 1).

The presence of citric acid not only resulted in reduced amounts of Cd adsorbed to the xylem cell walls but also in smaller values for the lateral escape constant  $k$  (Table 1). The  $k$  values were invariably calculated on the basis of the total Cd concentration present in the xylem fluid, thus  $k$  values observed in the presence of citric acid will be a combination of the escape rates of free and complexed Cd. The free  $\text{Cd}^{2+}$  concentration in the applied solution in the presence of citric acid could be calculated as approximately 6–8% of the  $\text{Cd}^{2+}$  concentration in situations without citric acid. However, the complex relations between the various Cd chemical forms (ionic, complexed to cell walls and citrate) existing in the xylem channels do not permit a simple control experiment with strongly reduced free  $\text{Cd}^{2+}$  solution concentration. Furthermore, the time required for saturation of the exchange sites using a 0.02 mM  $\text{Cd}^{2+}$  solution amply exceeds the practical experiment time available in short-term uptake studies using plant sub-systems under the conditions described.

Essentially, the rate of lateral escape of  $\text{Cd}^{2+}$  from the xylem vessels may depend on concentration; the results of Wolterbeek *et al.* (1985b) indicate that the value of escape rate constant may be inversely related with the  $\text{Cd}^{2+}$  concentration. Therefore, the reduced value of  $k$  observed in the presence of citric acid shows the reduction of the rate of lateral escape of Cd due to complex formation.

The quantitative results of the present study clearly show that complex formation affects cation transport in the xylem, not only by reduced adsorption to the vessel walls but also by a decreased rate of lateral escape.

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