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In vivo Measurement of Cadmium (^{115m}Cd) Transport and Accumulation in the Stems of Intact Tomato Plants (*Lycopersicon esculentum*, Mill.)

I. Long Distance Transport and Local Accumulation

C.M. Petit*
Soil Science Department, Cath. Univ. Louvain, Place Croix du Sud 2, B-1348 Louvain-La-Neuve, Belgium and Association Euratom-ITAL, P.O. Box 48, Wageningen

S.C. van de Geijn
Association Euratom-ITAL, P.O. Box 48, Wageningen, The Netherlands

Abstract. Semiconductor radiation detectors have been used to study in vivo the long-distance transport and accumulation of cadmium (^{115m}Cd) in the stems of tomato plants. Long-distance transport proceeds at a speed of 0.35–0.60 m h^{-1}. The shape of the accumulation curve is characterized by a shoulder after about 6 h. This corresponds to the saturation of the xylem tissue. The effects of changes in the nutritional pattern have been considered as well. The cadmium content in the stem sharply decreases after a transfer of the plant to a nonlabeled solution of high ionic strength, whereas it tends to stabilize after a shift to a low ionic strength medium. These observations are explained by exchange processes between cadmium and other divalent cations.

Key words: Cadmium – Cation exchange – Repartition – Tomato – Xylem transport.

Introduction

Cadmium is known to be one of the most toxic heavy metals released into the environment. It presents a health hazard to man (Friberg et al., 1974). Recent studies have shown a high uptake of cadmium by several crops (Page et al., 1972; John, 1973; Bingham et al., 1975; Petterson, 1977). Moreover cadmium is readily transported to the shoots in several plant species whereas heavy metals such as lead (Malone et al., 1974; Koeppe, 1977) and chromium (Mytten-aere and Mousny, 1974; Skeffington et al., 1976) are not. Knowledge about the transport processes and distribution patterns can help to elucidate the differences between the amount of cadmium accumulated by various plant species or plant organs.

* *Mailing address*: Association Euratom-ITAL, P.O. Box 48, Wageningen, The Netherlands

Translocation of ^{45}Ca and its redistribution within oat leaves was measured in vivo using semiconductor radiation detectors (Ringoet et al., 1967, 1968; Melloni et al., 1969). Recently the applicability of this approach has been improved considerably (Van de Geijn, 1974a, b), and a new method has been developed allowing the in vivo determination of the internal repartition of a β-emitting tracer inside the tissue (Van de Geijn, 1976a).

At present a set of complementary methods can be applied to follow the transport processes at different levels in a single plant:

1. Count rate measurements
2. β spectrometry, involving

a) determination of the maximum energy of the transmitted β rays,

b) analysis of the complete β spectrum

In this study, these methods are applied to an investigation of the translocation and lateral repartition of cadmium in the stems of tomato plants. Changes in the distribution pattern, following a shift in the nutrient solution, have also been considered. The present paper and the succeeding one, respectively, will describe the results obtained by count rate measurements and β spectrometry.

Materials and Methods

Radioisotopes

Sources of ^{115m}Cd (0.1–1 mCi mg^{-1} Cd in 0.1 M HCl) were obtained from The Radiochemical Centre, Amersham (U.K.). The β spectrum of ^{115m}Cd ($T_{1/2}$=43 days) has a maximum energy E_{max}=1.63 MeV. The penetration power of these β rays is thus comparable to that of ^{32}P radiation (E_{max}=1.71 MeV) (Lederer et al., 1967).

Detectors and Electronic Equipment

The count rate of the β rays emerging from the material at the measuring position was recorded continuously using silicon surface

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barrier detectors (ORTEC, 25 $mm^2 \times 300$ μm thick). The associated electronic equipment, consisting of amplifiers, count rate meters, and a 4-channel stripchart recorder, has been described before (Melloni et al., 1969; Van de Geijn, 1974a, b).

Growth Conditions

Seeds of tomato plants (*Lycopersicon esculentum*, Mill., cv. Moneymaker) were germinated on wet filter paper for 5 days (28° C, darkness). The seedlings were grown for 26 days or 5 weeks on a nutrient solution (in mM: 6 KNO_3, 5 $Ca(NO_3)_2 \cdot 4H_2O$, 2 $MgSO_4 \cdot 7H_2O$, 1 KH_2PO_4, 9.1×10^{-3} $MnSO_4 \cdot H_2O$, 7.6×10^{-4} $ZnSO_4 \cdot 7H_2O$, 3.1×10^{-4} $CuSO_4 \cdot 5H_2O$, 10^{-4} $MoO_3 \cdot H_2O$, 4.6×10^{-2} H_3BO_3, 8.2×10^{-2} NaFe(EDTA)), renewed weekly, in a climate-controlled growth chamber (light intensity 13,000 lx; day (16 h) temp. 23° C; night temp. 17° C; constant rel. hum. 70%).

Experimental Procedure

One day before the experiment the selected plant was transferred to a growth vessel (0.7 l) provided with a system allowing a rapid replacement of the nutrient solution (within 2 min) without the plant being touched. All solutions, continuously aerated, were renewed at a constant rate of 120 ml h^{-1} using a peristaltic pump. This flow rate was sufficient to maintain the concentration of the nutrients at an acceptable level (depletion about 25%). When changing to an unlabeled solution, the roots were rinsed three times for 2–3 min with demineralized water.

During the experiment the light was kept on (13,000 lx) and the temperature constant (23° C) to avoid any alteration in uptake and translocation processes that might occur as a result of the darkness and a lowering of the temperature.

Two or three detectors were placed along the stem at selected positions facing one of the three vascular bundles. In addition, one detector was placed at the margin of a leaf.

The nutrient solution containing 5×10^{-6} M cadmium labeled with ^{115m}Cd (about 150 μCi l^{-1}) was applied to the plant for a short period (about 7 h) or a longer one (45–75 h). Subsequently this solution was replaced by an unlabeled medium of high or low ionic strength. The high ionic strength medium was either the complete nutrient solution (2.9×10^{-2} M) or a calcium nitrate solution of comparable ionic strength (3.9×10^{-2} M). Demineralized water with or without added stable cadmium (5×10^{-6} M $Cd(NO_3)_2 \cdot 4H_2O$) was used as a low ionic strength medium.

Autoradiography

In some experiments autoradiography was performed as a control of the results obtained with the semiconductor detectors. In this instance 26-day-old plants were transferred to a polypropylene tank (25 l). The nutrient solution contained 2×10^{-6} M Cd labeled with ^{115m}Cd (about 1 μCi l^{-1}) and was renewed continuously to limit the cadmium depletion to less than 5%. After 35 h two plants were harvested. The remaining plants were transferred to the unlabeled cadmium-free nutrient solution for 6 additional days and then harvested.

The roots were washed for 5 min, first with demineralized water, then with the unlabeled nutrient solution, and finally blotted with tissue paper. Each whole plant was placed between two sheets of blotting paper and quickly frozen between aluminum envelopes, filled with powdered dry ice (−70° C). All plants were freeze-dried (5 days at −5° C in vacuum, about 0.15 Pa) and simultaneously prepared for autoradiography according to the method of Levi (1966).

Results

Figure 1 shows a typical time course of local cadmium "accumulation" in the stem of a tomato plant receiving a long-term supply. Strictly speaking, the count rate cannot be interpreted directly in terms of absolute quantities, because the attenuation of the β rays inside the plant material cannot be completely neglected. The transmission of the high-energy ^{115m}Cd β rays through 1 mm material of density 1 g cm^{-3} is about 80%. Because so large a shift in the position of the bulk amount of tracer cannot be expected (Van de Geijn and Petit, 1978), corrections will generally be small.

Some common features of the accumulation curves in the various treatments deserve attention:

1. A time-lag of 30–50 min before any radiation could be detected at the base of the stem. The length of this delay increased with the distance from the root base as shown for a short-term cadmium supply

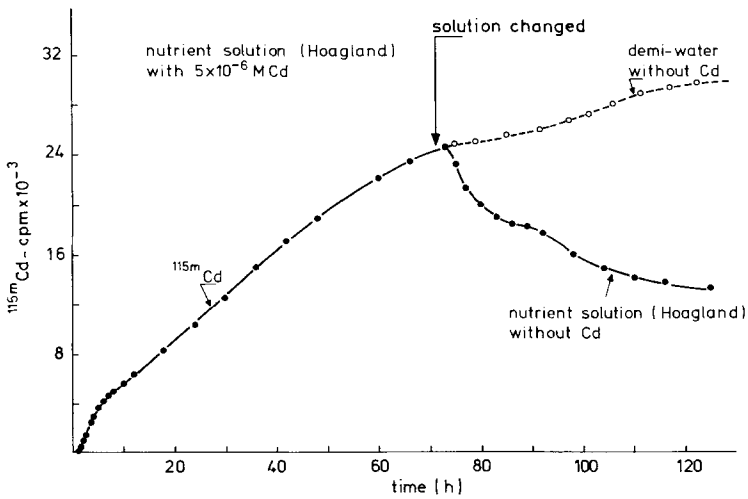

Fig. 1. Dependence of the count rate in a stem on time for a long-term supply of ^{115m}Cd. The nutrient solution labeled with ^{115m}Cd was replaced either by the unlabeled one or by demineralized water

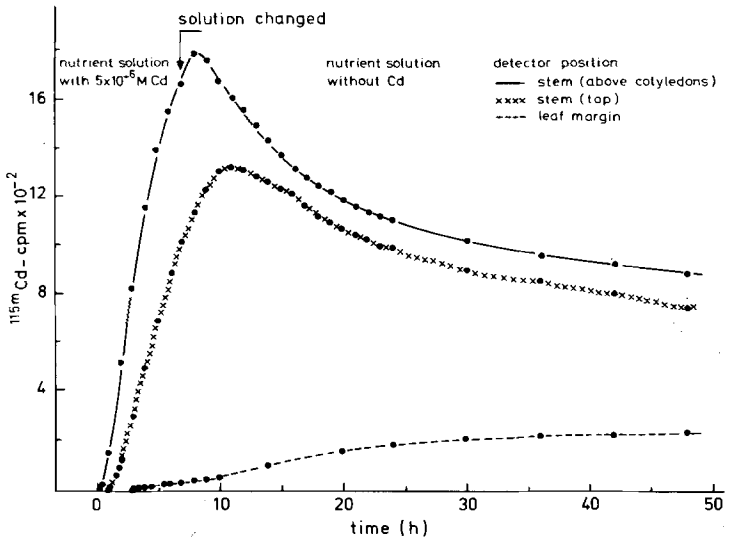

Fig. 2. Dependence of the count rate on time for a short-term supply of ^{115m}Cd, recorded simultaneously by three semiconductor detectors, fixed at the top and the base of the stem and at the margin of a developing leaf. The labeled nutrient solution was replaced by the unlabeled one after 7 h

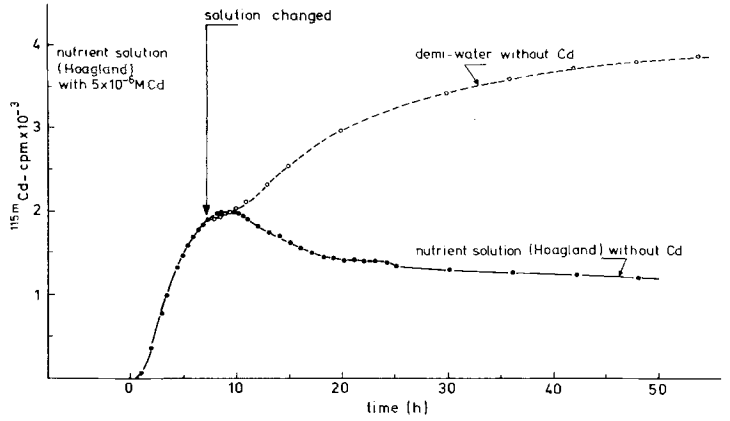

Fig. 3. Dependence of the count rate in a stem on time for a short-term supply of ^{115m}Cd. The labeled nutrient solution was replaced either by the unlabeled one, or by demineralized water

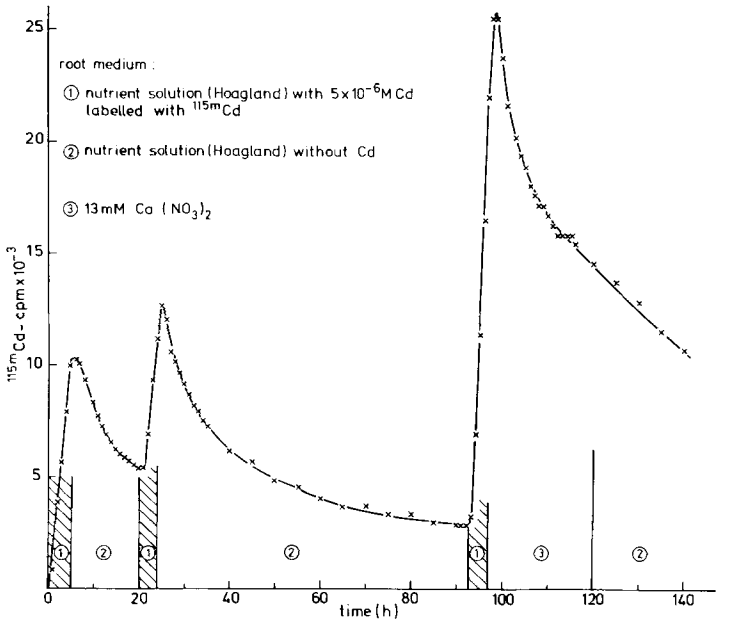

Fig. 4. Dependence of the count rate in a stem on time for a sequential supply of labeled (^{115m}Cd, dashed time periods) and unlabeled solutions

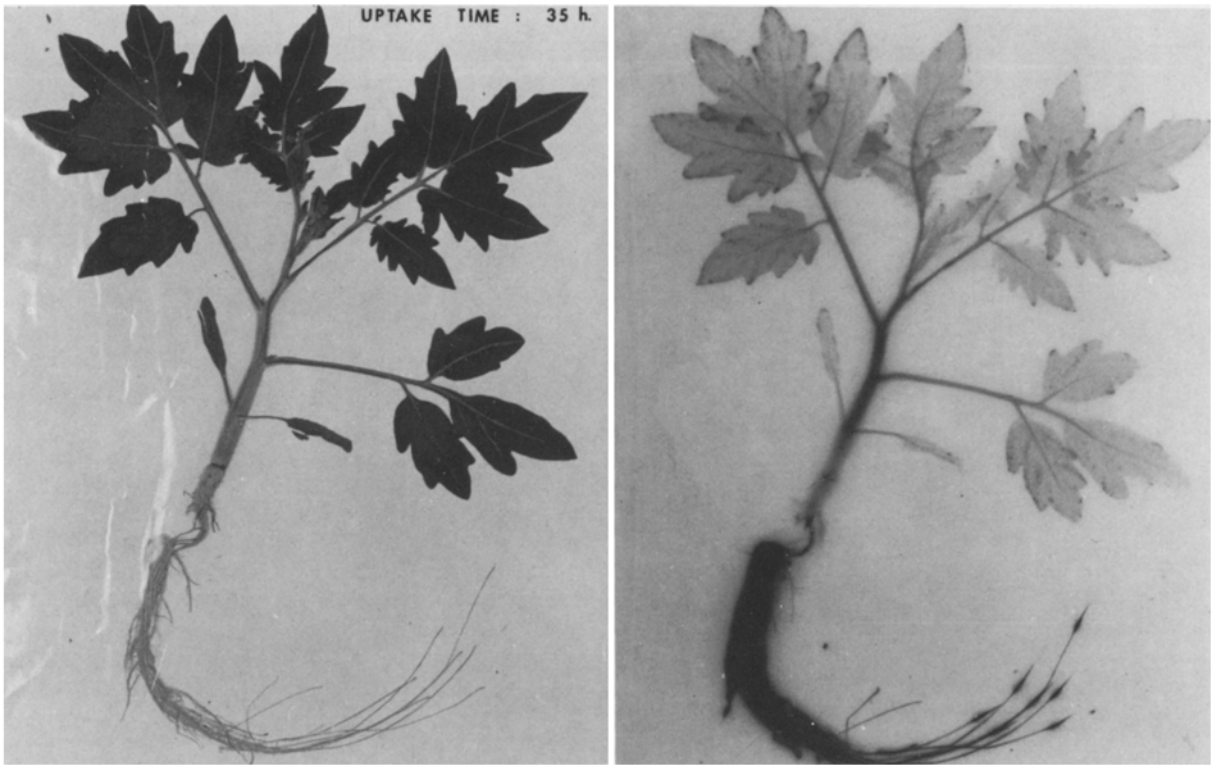

Fig. 5. Autoradiograph of a tomato plant (26 days old) after 35 h of cadmium uptake. *Left* plant; *right* autoradiograph

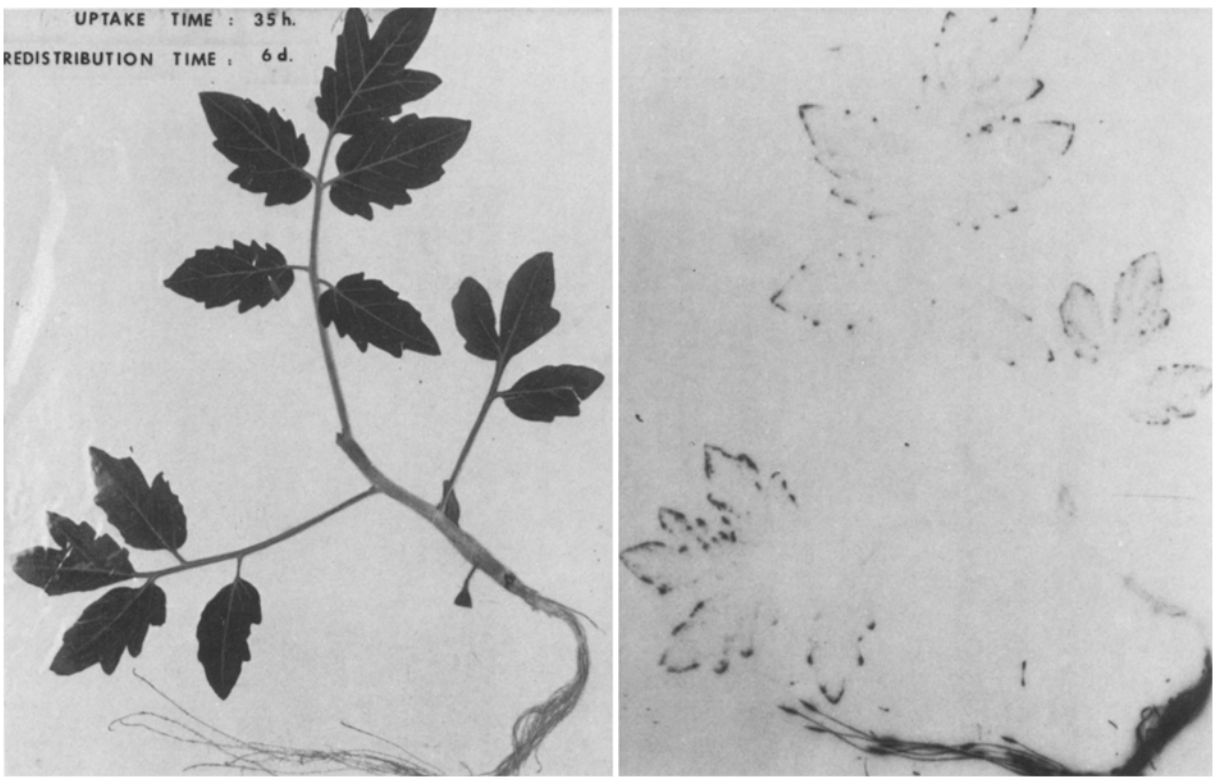

Fig. 6. Autoradiograph of a tomato plant (26 days old) after 35 h of cadmium uptake and 6 additional days of growth on a cadmium-free nutrient solution. *Left* plant with the first leaves; *right* autoradiograph

(Fig. 2). From this difference in time-lag the propagation speed of the "wave-front" of the tracer along the stem has been calculated. At the experimental concentration, values ranging from 0.35 to 0.60 m h^{-1} were obtained for different replicates.

2. A non-steady-state phase in which the time course of accumulation was not linear but contained a sigmoidal component (e.g. the first 6 h in Fig. 1).

3. A steady-state phase, characterized mainly by a constant rate of accumulation. After two days of cadmium supply (5×10^{-6} M) this rate gradually decreased.

4. A net local cadmium release occurred at the base of the stem less than 2 h after the labeled solution was replaced either by the nonlabeled nutrient solution (Figs. 1 and 3) or by the calcium nitrate solution. As illustrated in Figure 4 the cadmium accumulation and release could be repeated by supplying sequentially labeled and nonlabeled solutions. The time elapsed between the shift to a nonlabeled solution and the start of the release increased with the distance from the root base (Fig. 2). At the margin of the leaf no release was observed, but accumulation continued (Fig. 2). From the autoradiographs of whole plants treated or not with a nonlabeled nutrient solution (Figs. 5 and 6), it appeared that cadmium was released from the vascular tissues of the aerial part, stem, petiole, and veins. Because no efflux into the nutrient solution was detected, it was concluded that the cadmium migrated upward along the pathway of the transpiratory flux, eventually accumulating at the tip and margin of the leaves (Figs. 2 and 6).

5. Replacement of the labeled solution by demineralized water with or without added stable cadmium resulted in quite a different pattern. After replacement, the time course of cadmium accumulation tended to level, but no cadmium release could be observed.

However, this pattern was dependent on the duration of the preceding supply. After a long-term cadmium supply the rate of accumulation at first tended toward zero but subsequently increased, more than 10 h later. Finally it leveled again (Fig. 1). A similar pattern was observed in a short-term supply, except that the initial decrease of the rate of accumulation was smaller and lasted for less than 2 h (Fig. 3).

The water uptake of the plants, measured before and after the shift to demineralized water, remained constant for at least 6 h and then slowly decreased during the next 35 h to half the initial amount.

Discussion

Loading Phase

The speed of upward movement of the ^{115m}Cd, as calculated from the difference in time-lag of the first detection of the tracer at two points along the stem (0.35–0.60 m h^{-1}) is similar to that reported for the acropetal movement of calcium in the xylem of oat leaves (Ringoet et al., 1967). This value is in strong contrast to the speed of ^{32}P movement under similar conditions (6–10 m h^{-1}) (Van de Geijn, 1976b). Analogous observations were reported by Ferguson and Bollard (1976), who perfused isolated pieces of apple shoots with different solutions (^{45}Ca, ^{32}P). The contrasting results can be attributed to the difference in transport processes for both ions: chromatographic exchange transport at the negatively charged sites in the xylem (Charles, 1953) for cadmium, as opposed to bulk transport in the transpiratory flux for ^{32}P.

The sigmoidal shape of the count-rate curve in the initial six hours of application is related to the progressive saturation of the xylem tissue (Van de Geijn and Petit, 1978). The lateral accumulation starts simultaneously. The approximate rate of lateral accumulation can be calculated from the slope of the count-rate curve at the steady state (6–40 h, Fig. 1). It amounts to about 12% h^{-1} of the amount present in the xylem tissue at the steady state.

A quick initial depletion of the cadmium flux in the roots may also contribute to the initial non-steady-state phase. For heavy metals, two physicochemical mechanisms mainly come into play: adsorption at pectic sites of the cell walls (Cutler and Rains, 1974; Peterson, 1969) and complexation by hystidyl and thiol groups (Vallee and Ulmer, 1972) along the symplastic pathway in the roots. These processes may also be involved in the establishment of the initial time lag (30–50 min).

Unloading Phase

The importance of the exchange processes is stressed once more by the results of the experiments in which a plant was supplied sequentially with labeled and unlabeled solutions (Fig. 4). The unlabeled solution with a high cation concentration exchanges the ^{115m}Cd progressively upward along the pathway of the transpiratory flux. In contrast, if the unlabeled solution contains only a low concentration of cations, even with cadmium at the same concentration as before, the elution power will be slight or absent, and no cadmium efflux from the xylem will be observed (Van de Geijn and Petit, 1978) (Figs. 1 and 3). These results are in agreement with the observations of Bell and Biddulph (1963) for calcium, more recently confirmed by Jacoby (1967) and by Ferguson and Bollard (1976). This indicates that cadmium is translocated predominantly by exchange, either as a divalent cation or as a positively charged complex.

At the end of the labeling period the accumulation of cadmium continues for some time (Figs. 1, 2, and

3). Such an additional accumulation is related to the time needed for the upward shift of the labeled cadmium that is exchangeably bound in the vascular tissue below the stem segment under consideration and in the roots. During a switch to a normal nutrient solution at the steady-state phase, this additional accumulation is very small (Fig. 1). This confirms the high retention of the root tissue (Petit, 1976), also apparent in the autoradiograph obtained after a redistribution period of 6 days (Fig. 6). A discussion of the cadmium distribution along the roots, particularly the highly labeled spots at some distance from the apex (Figs. 5 and 6), is out of the scope of the present paper (Petit, 1976).

The restarting of the local accumulation of cadmium after a switch to demineralized water is a particular phenomenon that cannot be explained by ion exchange only. The cause of this phenomenon is not yet known. There are several possible explanations: 1. a change in the osmotic equilibrium in the tissue; 2. an increase in the permeability of membranes due to calcium deprivation (Epstein, 1972); 3. a washing effect on the tissue in contact with diluted sap (Van Steveninck, 1975).

The delay of the restarting of this accumulation is apparently longer after a long-term than after a short-term cadmium supply (Figs. 1 and 3). This suggests an increased capacity of the tissue below the measuring point to retain its cadmium when the plant is supplied with this element for a prolonged time. This is consistent with the increased number of binding sites at the root level induced by preceding cadmium uptake (Petit, 1976).

The decreasing rate of cadmium accumulation observed after more than 40 h supply (Fig. 1) can be explained also by the higher number of cadmium binding sites mentioned above, possibly in combination with a saturation of the stem tissue.

Conclusions

The count rate measurements allow the kinetics of penetration and accumulation of ^{115m}Cd in the stem of a single plant to be followed in vivo. Conclusions concerning the tissues involved in these processes should, however, be supported by data obtained by other methods (Van de Geijn and Petit, 1978).

The relatively low speed of cadmium penetration in the stem as well as the effect of posttreatment with a high or a low ionic strength medium emphasize the important role of the chromatographic exchange process for xylem transport. The total ionic concentration of the divalent cations in the medium rather than the presence or absence of stable cadmium plays a decisive role for the transport. The similarity between the kinetics of translocation observed in these experiments and that reported for calcium, indicates that anionic or neutral cadmium complexes, which would not participate in the exchange processes, play only a minor role in translocation.

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References

Bell, C.W., Biddulph, O.: Translocation of calcium. Exchange versus mass flow. Plant Physiol. **38**, 610–614 (1963)

Bingham, F.T., Page, A.L., Mahler, R.J., Ganje, T.J.: Growth and cadmium accumulation of plants grown on a soil treated with a cadmium-enriched sewage sludge. J. Environ. Qual. **4**, 207–211 (1975)

Charles, A.: Uptake of dyes into cut leaves. Nature **171**, 435–436 (1953)

Cutler, J.M., Rains, D.W.: Characterisation of cadmium uptake by plant tissue. Plant Physiol. **54**, 67–71 (1974)

Epstein, E.: Mineral nutrition of plants. Principles and Perspectives. New York: J. Wiley 1972

Ferguson, I.B., Bollard, E.G.: The movement of calcium in woody stems. Ann. Bot. **40**, 1057–1065 (1976)

Friberg, L., Piscator, M., Nordberg, G.F., Kjellström, T.: Cadmium in the environment. 2nd edn., Cleveland Ohio: Chemical Rubber Co. Press 1974

Jacoby, B.: The effect of the roots on calcium ascent in bean stems. Ann. Bot. **31**, 725–730 (1967)

John, M.K.: Cadmium uptake by eight food crops as influenced by various soil levels of cadmium. Environm. Pollut. **4**, 7–15 (1973)

Koeppe, D.E.: The uptake, distribution and effect of cadmium and lead in plants. Sci. Total Envir. **7**, 197–206 (1977)

Lederer, L.M., Hollander, J.M., Perlman, I.: Table of Isotopes. 6th edn. New York: J. Wiley 1967

Levi, E.: Handling plants for macro-autoradiography. In: Isotopes in weed research, pp. 189–194. Vienna: International Atomic Energy Agency 1966

Malone, C., Koeppe, D.E., Miller, R.J.: Localization of lead accumulated by corn plants. Plant Physiol. **53**, 388–394 (1974)

Melloni, M., Rechenmann, R.V., Ringoet, A., Van de Geijn, S.C., Wilthagen, R.J.C.: In-depth localization of beta-emitting isotopes with a semiconductor detector spectrometric assembly. Nucl. Instr. Meth. **74**, 101–108 (1969)

Myttenaere, C., Mousny, J.M.: The distribution of chromium-51 in lowland rice in relation to the chemical form and to the amount of stable chromium in the nutrient solution. Plant Soil **41**, 65–72 (1974)

Page, A.L., Bingham, F.T., Nelson, C.: Cadmium absorption and growth of various plant species as influenced by solution cadmium concentration. J. Environ. Qual. **1**, 288–291 (1972)

Petit, C.M.: Uptake, translocation and localization of cadmium in tomato plants (*Lycopersicon esculentum*, Mill.). Thesis. Fac. Agricult. Sci., Cath. Univ. Louvain, Belgium 1976

Peterson, P.J.: The distribution of Zinc-65 in *Agrostis tenuis* Sibth. and *A. stolonifera* L. tissues. J. exp. Bot. **20**, 863–875 (1969)

Petterson, O.: Difference in cadmium uptake between plant species and cultivars. Swed. J. Agr. Res. **7**, 21–25 (1977)

Ringoet, A., Rechenmann, R.V., Veen, H.: Calcium movement in oat leaves measured by semiconductor detectors. Rad. Bot. **7**, 81–90 (1967)

Ringoet, A., Rechenmann, R.V., Melloni, M.: Calcium localization within live oat leaves using a semiconductor detector assembly. Planta **81**, 280–286 (1968)

Skeffington, R.A., Shewry, P.R., Peterson, P.J.: Chromium uptake and transport in barley seedlings (*Hordeum vulgare* L.). Planta **132**, 209–214 (1976)

Vallee, B.L., Ulmer, D.D.: Biochemical effects of mercury, cadmium and lead. Ann. Rev. Biochem. **41**, 91–128 (1972)

Van de Geijn, S.C.: In-depth localization of beta-emitting isotopes. Dependence of the range of applicability upon maximum energy and complexity of the spectrum. Nucl. Instr. Meth. **117**, 109–113 (1974a)

Van de Geijn, S.C.: An improved method for the determination of the maximum energy of distorted beta-spectra and the in-depth localization of spatially distributed beta-emitting isotopes. Nucl. Instr. Meth. **120**, 107–112 (1974b)

Van de Geijn, S.C.: A non-destructive determination of the depth distribution profile of a radionuclide by analysis of its beta-spectrum. Nucl. Instr. Meth. **133**, 559–568 (1976a)

Van de Geijn, S.C.: A non-destructive spectrometric determination of the internal repartition of beta-emitting isotopes in live and inert material. Thesis, Fac. Sci., Univ. of Utrecht, The Netherlands, 1976b

Van de Geijn, S.C., Petit, C.M.: In-vivo measurement of cadmium (^{115m}Cd) transport and accumulation in the stem of tomato plants (*Lycopersicon esculentum*, Mill.). II. Lateral migration from the xylem and redistribution in the stem. Planta **138**, 145–151 (1978)

Van Steveninck, R.M.: The "washing" or "aging" phenomenon in plant tissues. Ann. Rev. Plant Physiol. **26**, 237–368 (1975)

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