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Transport of Cadmium via Xylem and Phloem in Maturing Wheat Shoots: Comparison with the Translocation of Zinc, Strontium and Rubidium

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The toxic heavy metal cadmium is taken up by plants and may contaminate harvested parts of agricultural crops. In the experiments reported here, cadmium was introduced together with markers for phloem (rubidium) and xylem (strontium) transport, either into intact shoots via a flap below the flag leaf node, or into detached shoots via the cut stem. Cadmium introduced into intact plants was redistributed during maturation from the peduncle and the flag leaf lamina to the grain. In detached shoots, some cadmium was removed from the transpiration stream, as judged from the comparison of shoots steam-girdled below the ear and of controls with an intact phloem in the peduncle. A minor quantity of cadmium was transported to the grain via the phloem in control shoots while a high percentage of this element was retained in the peduncle. The cadmium content of the grain increased in response to the increased cadmium concentrations in the feeding solutions (0·1 to 10 μM). The cadmium content of the grain was slightly lower when zinc (> 10 μM) was introduced at the same time as cadmium (1 μM). © 1995 Annals of Botany Company

Key words: *Triticum aestivum* L., cadmium, phloem transport, wheat, zinc.

INTRODUCTION

Cadmium is a naturally occuring toxic element which is found in all soils in at least trace quantities (Page, Bingham and Chang, 1981). Phosphate fertilizers, sewage sludge and atmospheric deposition may cause elevated levels of cadmium in agricultural soils (Wagner, 1993). Concerns about cadmium entering the food chain are justified, since concentrations of cadmium in crops which are potentially harmful to man precede the concentrations that damage the crop itself (Davis, 1984). Therefore, healthy plants may contain levels of cadmium that are toxic to mammals.

The uptake of cadmium by plants strongly depends on soil properties (Cieslinski *et al*., 1996; Wenzel *et al*., 1996). The distribution in the plant organs may vary strongly between species and even between varieties of the same species (Cieslinski *et al*., 1996; Wenzel *et al*., 1996). Most plants retain more than 50% of the absorbed cadmium in the roots (Jarvis, Jones and Hopper, 1976; Obata and Umebayashi, 1993). Different mechanisms may play a role in this retention. For example, it might be due to a lateral diffusion and adsorption of the cadmium ion along the xylem (Petit and van de Geijn, 1978; van de Geijn and Petit, 1978).

Cadmium is chemically very similar to the micronutrient zinc (Chesworth, 1991). A reduction in cadmium uptake by ryegrass has been reported when zinc was added to the nutrient solution (Jarvis *et al*., 1976). The cadmium uptake by wheat plants was lower after the addition of zinc to the soil (Oberländer, Piatti-Fünfkirchen and Roth, 1989). Moreover, the addition of zinc to the soil was shown to reduce cadmium contents in wheat grain (Oliver *et al*., 1994).

While the zinc content of wheat grain was not increased by feeding high zinc concentrations to wheat shoots (Herren and Feller, 1996), a linear relationship was found between the cadmium content of the soil and of wheat grain (Mortvedt, Mays and Osborn, 1981; Davis, 1984; Grupe and Kuntze, 1988). Zinc introduced into the cut stem of wheat plants was shown to be removed from the transpiration stream in the peduncle, loaded into the phloem and transported to the maturing grains (Herren and Feller, 1994).

The aim of the work presented here was to investigate the retranslocation of cadmium via the long-distance transport systems in wheat shoots. The chemical similarities between zinc and cadmium suggest that a transfer of cadmium from the xylem to the phloem may occur in the peduncle. The question arises whether zinc may also interfere with the transport of cadmium to the grains.

MATERIAL AND METHODS

Redistribution of cadmium in intact plants

Winter wheat (*Triticum aestivum* L., cv. 'Arina') was grown in a field in Zollikofen near Bern (loamy soil). To investigate the redistribution of cadmium in intact plants, a flap was cut into the stem below the flag leaf node (according to Schenk and Feller, 1990) about 1 week after anthesis. The stem flap (2 mm wide, 40 mm long) was cut with a razor blade directly below the node. Tubes containing 1 ml of the solution were fixed to the stem of random plants to allow introduction of the solution into the cut xylem. Each tube contained 0·1 μmol $CdCl_2$ together with 5 μmol $SrCl_2$ and 5 μmol RbCl as markers for xylem and phloem transport, re-

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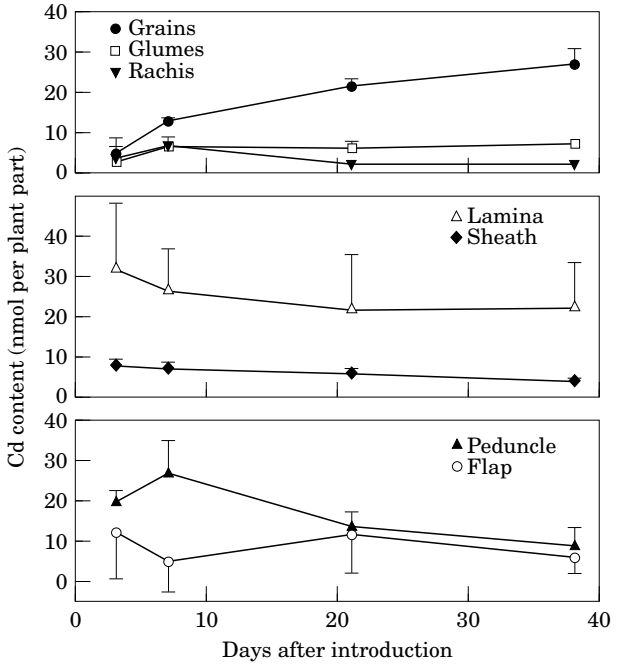

FIG. 1. Cadmium contents in different parts of wheat shoots 3, 7, 21 and 38 d after the introduction of cadmium, strontium and rubidium. The solution (1 ml) containing 0·1 μmol $CdCl_2$, 5 μmol $SrCl_2$ and 5 μmol RbCl was introduced into intact plants via a flap cut in the stem below the flag leaf node. The cadmium content before cutting the flap (day 0) was below the detection limit (< 0·5 nmol Cd per plant part). Means and s.d. of four plants are shown.

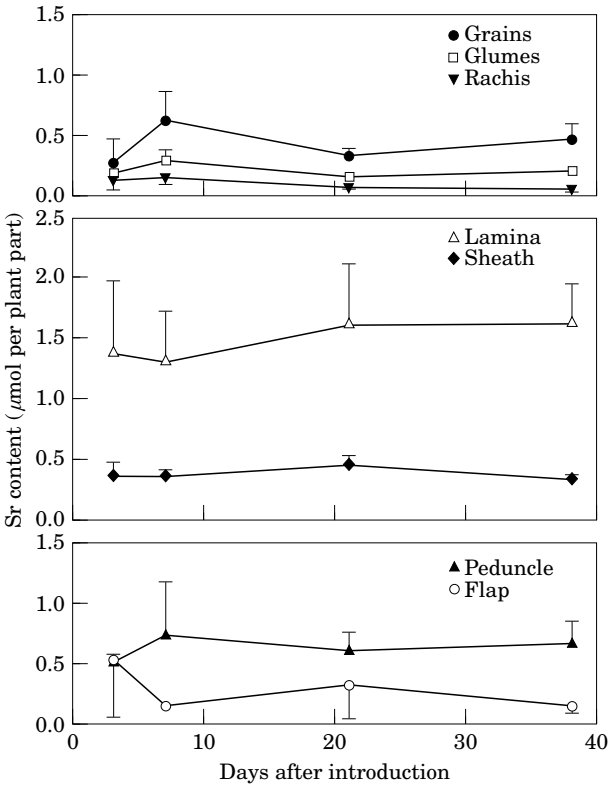

FIG. 2. Strontium contents in different parts of wheat shoots 3, 7, 21 and 38 d after the introduction of cadmium, strontium and rubidium. The solution (1 ml) containing 0·1 μmol $CdCl_2$, 5 μmol $SrCl_2$ and 5 μmol RbCl was introduced into intact plants via a flap cut in the stem below the flag leaf node. The strontium content before cutting the flap (day 0) was below 0·05 μmol Sr per plant part. Means and s.d. of four plants are shown.

spectively. The experiment was started on a sunny day before noon to ensure high transpiration in the treated plants. To minimize evaporation from the tube, the opening was covered with tape. For all plants, the introduction of the solution was completed within 36 h. The treated shoots (four replicates per treatment) were harvested 3, 7, 21 and 38 d after cutting the flap.

Cadmium redistribution in detached wheat shoots

Randomly selected shoots were cut in the field directly above the soil surface 2 weeks after anthesis. The basal part of the stem was submerged in deionized water and immediately recut below the second node from the top. These shoots were transported to the laboratory standing in water. The stems were submerged again and cut to their final length below the flag leaf node. The phloem of some shoots was interrupted below the ear by steam-girdling (Martin, 1982). This treatment kills all living cells in a 1 to 2 cm long section of the peduncle, while the xylem remains functional. In one experiment, the shoots (four replicates per treatment) were incubated for 6 d standing in solutions containing 1 mM $SrCl_2$, 1 mM RbCl and different concentrations of Cd (0, 0·1, 1, 10 μM $CdCl_2$). In another experiment, the shoots were incubated for 3 d standing in solutions containing 1 μM $CdCl_2$, 2 mM $SrCl_2$, 2 mM RbCl and a series of concentrations of $ZnSO_4$ (0, 5, 10, 20, 40, 80, 160, 320 μM). All the cut shoots were incubated in a culture room with a light/dark cycle of 14 h light (light intensity at ear level: 120 μmol m^{-2} s^{-1} from four Philips TL 40W/33 and two Osram Fluora fluorescent tubes; ambient temperature: 24–26 °C; relative humidity: 40–50 %) and 10 h darkness (ambient temperature: 21–23 °C; relative humidity: 70–80 %). At the end of the experiment the basal parts of the shoots were rinsed with deionized water.

Sample preparation and analyses

Each shoot was dried at 105 °C and divided into the following parts: grains, glumes (including rachillas and sterile grains), rachis, peduncle (including flag leaf node), flag leaf sheath and flag leaf lamina. These plant parts were heated separately in glass tubes with 1 ml 30 % H_2SO_4 at 95 °C. The plant material was then ashed at 550 °C. The ash was solubilized with 0·25 ml 10 M HCl, and 1·75 ml deionized water was added. For the measurement of elements by atomic absorption spectrophotometry the solutions were appropriately diluted with 1000 ppm CsCl in 0·1 M HCl (for Rb), 1000 ppm $LaCl_3$ in 0·1 M HCl with 50 mM EDTA (for Sr) and in 0·1 M HCl (for Cd and Zn).

Significant differences ($P = 0·05$) between steam-girdled and control shoots were identified with Student's *t*-test.

RESULTS

In intact plants 3 d after its introduction, cadmium was mainly detected in the lamina and in the peduncle (Fig. 1). Throughout the whole experimental period, the cadmium content in the peduncle decreased, while a slight accumulation was detected in the grains. In other shoot parts (flap, sheath, rachis and glumes), the cadmium contents remained at more or less the same level as for the initial distribution after 3 d. Strontium, added to the feeding solution as a marker for xylem transport, was detected in all shoot parts (Fig. 2). After 7 d, the strontium contents remained relatively constant. During the first 3 d, rubidium was strongly retained in the peduncle, the sheath and the lamina (Fig. 3). During later phases of maturation, the rubidium content of these plant parts decreased and rubidium accumulated in the grains.

Steam-girdling below the ear of detached shoots reduced the accumulation of cadmium in the grains and led to increased contents in the peduncle (Fig. 4). At the two lower concentrations (0·1 and 1 μM Cd), most of the cadmium was retained in the stem indicating a removal of this element from the transpiration stream. When cadmium was introduced at a concentration of 10 μM, the cadmium contents of glumes, rachis and lamina were higher in comparison to those of the grains. Rubidium supplied in the same solution (10 μM Cd), was almost completely retained in the peduncle (Fig. 5). In general, when the phloem was interrupted by steam-girdling, rubidium was no longer transported to the ear as observed in the control shoots. The distribution of rubidium in the detached wheat shoots was very similar at all cadmium concentrations in the solutions introduced.

The presence of 5 and 10 μM zinc in the cadmium-containing solution had no effect on the cadmium distribution in detached shoots (Fig. 6). When the phloem was interrupted below the ear by steam-girdling, the cadmium content of the grains was lower and more cadmium was retained in the peduncle. Zinc concentrations up to 80 μM reduced the cadmium content of the grains, both in control and steam-girdled shoots. The effect of steam-girdling on the cadmium contents of the grain was still obvious in the presence of these zinc concentrations (10–80 μM). At higher zinc concentrations (160 and 320 μM), steam-girdling no longer affected the grain cadmium content. At these zinc concentrations less cadmium was retained in the stem than at the lower zinc concentrations, but more cadmium was detected in the glumes and the leaf lamina.

The zinc content in the grains was not affected by concentrations up to 20 μM zinc in the solutions supplied (Fig. 7). At 40 and 80 μM zinc, the content in the grains of control shoots (but not in steam-girdled shoots) was slightly higher than at 0 to 20 μM zinc in the solution supplied. At zinc concentrations above 160 μM, steam-girdling no longer influenced the zinc content of the grains.

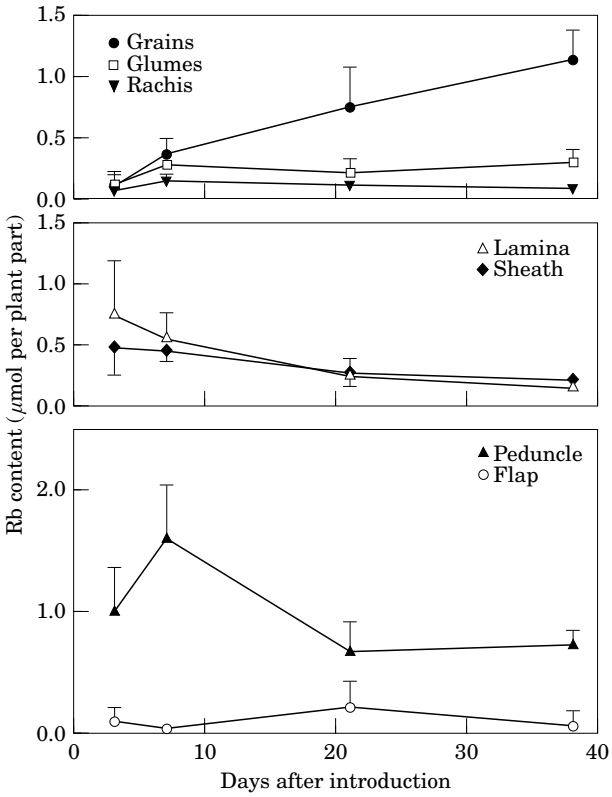

FIG. 3. Rubidium content in different parts of wheat shoots 3, 7, 21 and 38 d after the introduction of cadmium, strontium and rubidium. The solution (1 ml) containing 0·1 μmol $CdCl_2$, 5 μmol $SrCl_2$ and 5 μmol RbCl was introduced into intact plants via a flap cut in the stem below the flag leaf node. The rubidium content before cutting the flap (day 0) was below 0·02 μmol Rb per plant part. Means and s.d. of four plants are shown.

DISCUSSION

Strontium, introduced as a marker for the immobile calcium, was mainly transported to the organs with a high transpiration rate, i.e. the glumes and the leaf lamina (Fig. 2). Rubidium, a marker for the highly mobile potassium, was rapidly removed from the transpiration stream in intact plants (Fig. 3) and in detached shoots (Fig. 5). In intact plants, cadmium was slightly redistributed from vegetative parts to the grain (Fig. 1). In detached shoots, the highest cadmium contents were found in the peduncle, but considerable amounts were also detected in other organs (Fig. 4). A toxic effect of cadmium on the transport systems can be ruled out, since the rubidium distribution was very similar at all cadmium levels. Therefore, the relative mobility of cadmium in our system was also between that of rubidium and that of strontium, as stated previously (Kabata-Pendias and Pendias, 1992).

Essentially no rubidium was detected in grains of shoots that had been steam-girdled (Fig. 5). This confirms previous research by Feller (1989) that rubidium is transported to the grains almost exclusively in the phloem. In control shoots with an intact phloem, less cadmium was retained in the peduncle than in shoots with an interrupted phloem (Fig. 4). This may indicate that a part of the cadmium reached the ear (and especially the grains) via the phloem.

Analogous to field experiments (Mortvedt *et al.*, 1981; Davis, 1984; Grupe and Kuntze, 1987), higher cadmium concentrations in the feeding solutions led to an increase in

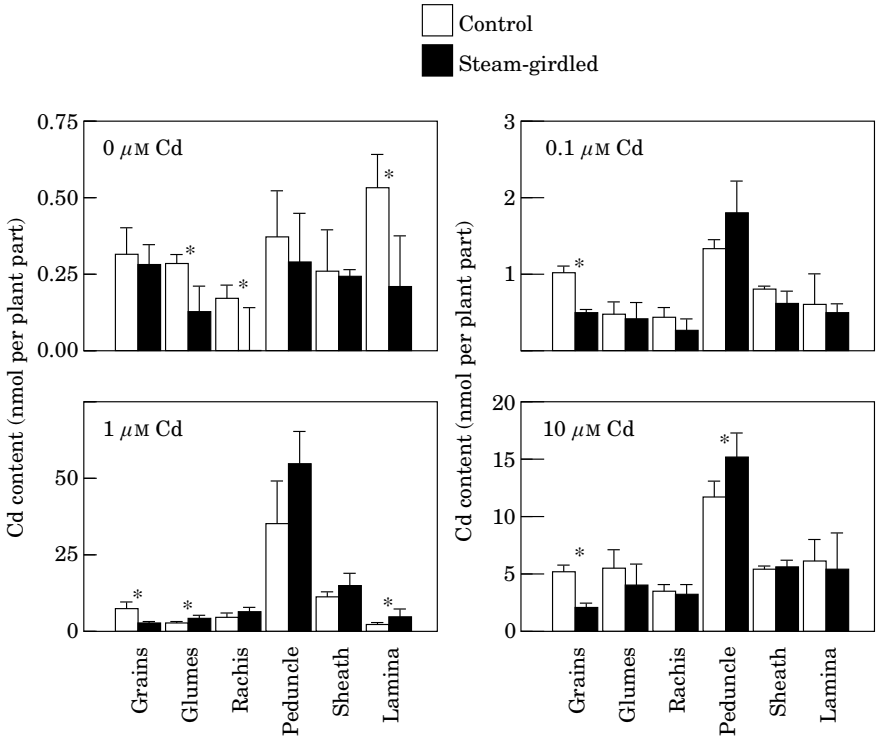

FIG. 4. Cadmium contents in shoots cut below the flag leaf node and supplied with solutions containing 1 mM $SrCl_2$, 1 mM RbCl and various Cd concentrations (0, 0·1, 1, 10 μM $CdCl_2$). Standing in the appropriate solution, the shoots were incubated for 6 d in a culture room with a 14/10 h light/dark cycle. The phloem of some shoots was interrupted directly below the ear by steam-girdling immediately before incubating the shoots in the solutions mentioned above. Means and s.d. of four replicates are shown. Significant differences ($P = 0{\cdot}05$) between steam-girdled and untreated shoots are indicated by an asterisk.

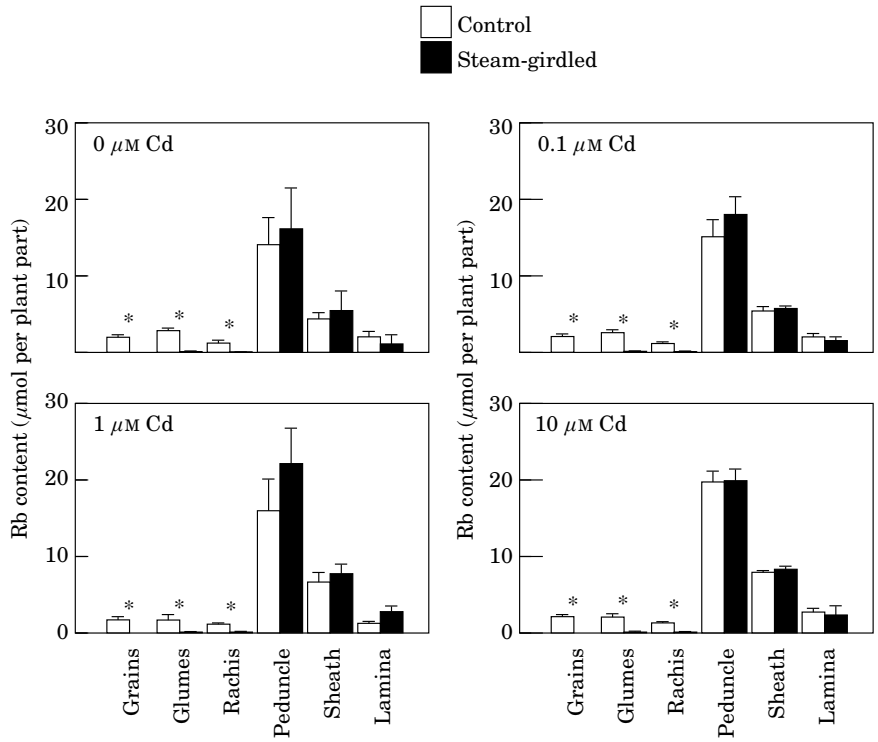

FIG. 5. Rubidium contents in shoots cut below the flag leaf node and supplied with solutions containing 1 mM $SrCl_2$, 1 mM RbCl and various Cd concentrations (0, 0·1, 1, 10 μM $CdCl_2$). Standing in the appropriate solution, the shoots were incubated for 6 d in a culture room with a 14/10 h light/dark cycle. The phloem of some shoots was interrupted directly below the ear by steam-girdling immediately before incubating the shoots in the solutions mentioned above. Means and s.d. of four replicates are shown. Significant differences ($P = 0{\cdot}05$) between steam-girdled and untreated shoots are indicated by an asterisk.

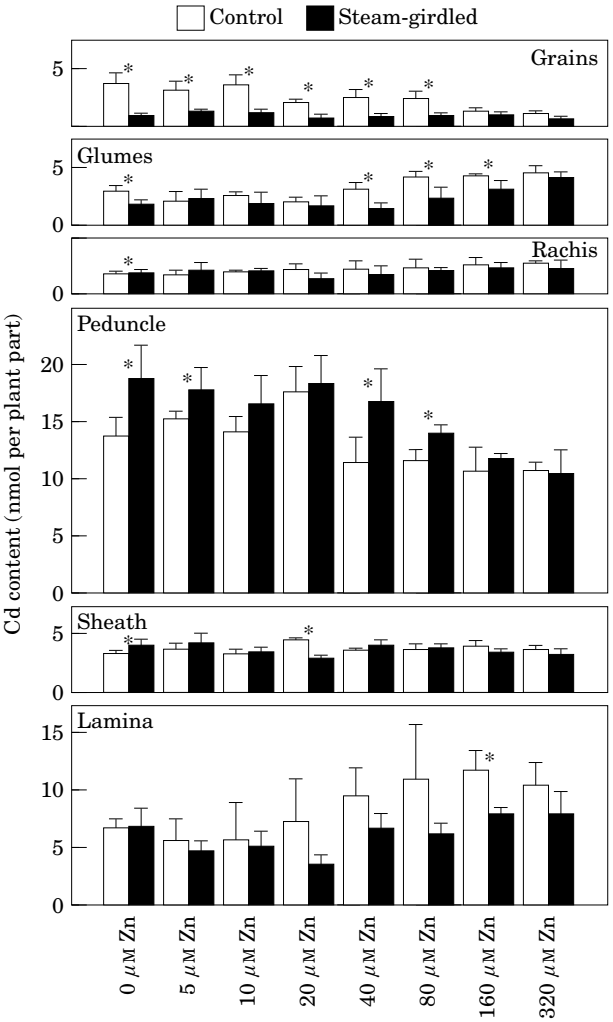

FIG. 6. Cadmium contents in shoots cut below the flag leaf node and supplied with solutions containing 2 mM $SrCl_2$, 2 mM RbCl, 1 μM $CdCl_2$ and various Zn concentrations (0, 5, 10, 20, 40, 80, 160 and 320 μM $ZnSO_4$). Standing in the appropriate solution, the shoots were incubated for 3 d in a culture room with a 14/10 h light/dark cycle. The phloem of some shoots was interrupted directly below the ear by steam-girdling immediately before incubating the shoots in the solutions mentioned above. Means and s.d. of four replicates are shown. Significant differences ($P = 0{\cdot}05$) between steam-girdled and untreated shoots are indicated by an asterisk.

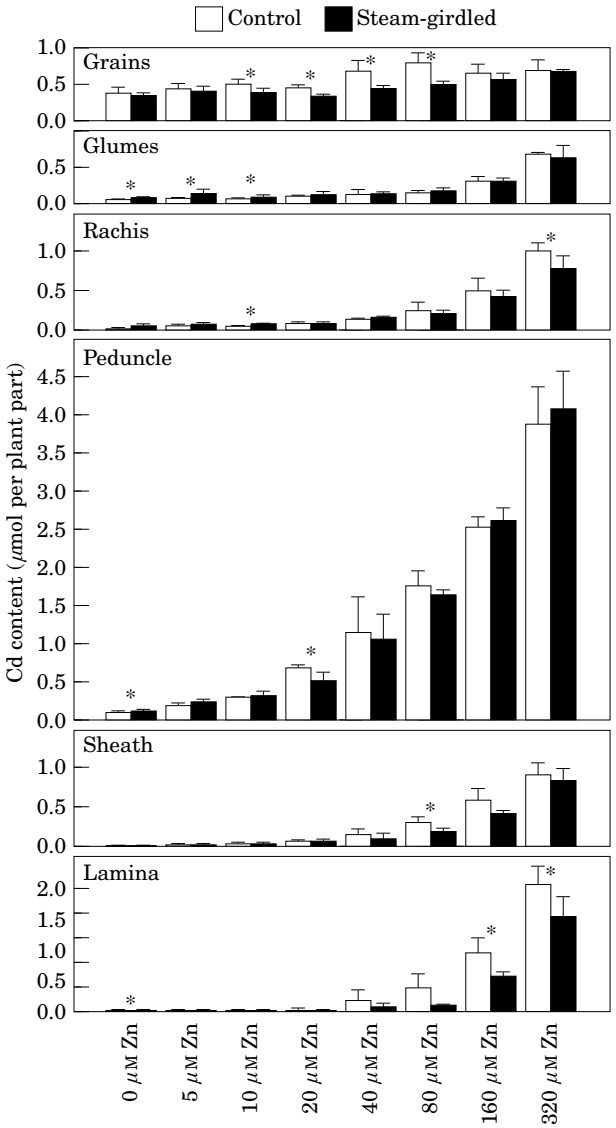

FIG. 7. Zinc contents in shoots cut below the flag leaf node and supplied with solutions containing 2 mM $SrCl_2$, 2 mM RbCl, 1 μM $CdCl_2$ and various Zn concentrations (0, 5, 10, 20, 40, 80, 160 and 320 μM $ZnSO_4$). Standing in the appropriate solution, the shoots were incubated for 3 d in a culture room with a 14/10 h light/dark cycle. The phloem of some shoots was interrupted directly below the ear by steam-girdling immediately before incubating the shoots in the solutions mentioned above. Means and s.d. of four replicates are shown. Significant differences ($P = 0{\cdot}05$) between steam-girdled and untreated shoots are indicated by an asterisk.

the grain cadmium contents (Fig. 4). At the highest cadmium concentration used (10 μM) not all the additional cadmium (as compared to the lower concentrations) could be removed from the xylem and proportionally more cadmium was flowing to transpiring organs. For zinc, which has similar chemical properties to cadmium, it was shown that higher zinc concentrations in feeding solutions caused only a minor increase in the zinc levels in the grains (Herren and Feller, 1994, 1996).

The cadmium content in grains of detached shoots incubated in a solution containing 1 μM cadmium was lower when more than 20 μM zinc were introduced (Fig. 6). In the range between 20 and 80 μM zinc, lowered cadmium contents were accompanied by slightly increased zinc contents of the grains (Fig. 7). Above 80 μM zinc, the grain contents were lower for cadmium and for zinc. This was probably due to a reduced phloem flux to the grains, which also resulted in drastically reduced rubidium contents of the grains at the highest zinc levels in the feeding solution (data not shown). It is known that increased zinc levels have a negative influence on phloem loading and transport (Rauser and Samarakoon, 1980; Herren and Feller, 1996).

The redistribution of cadmium within cereal shoots is highly relevant for the accumulation of this heavy metal in the grains. Cadmium may enter cereal plants via the roots (on contaminated soil) or via the shoot surface (after dry or

wet deposition of this heavy metal from the atmosphere). The relative mobility in the phloem and the xylem-to-phloem transfer in the peduncle or in lower internodes are important for the accumulation of cadmium in the maturing grains. The observed interferences with zinc indicate that the long-distance translocation of cadmium may depend on the availability of other elements. It remains a challenge for future workers to elucidate the effect of the developmental stage on the translocation of cadmium and on its accumulation in the grains and to further characterize the physiological processes involved in the redistribution of this pollutant in major crops.

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