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Remobilization of cadmium in maturing shoots of near isogenic lines of durum wheat that differ in grain cadmium accumulation

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Abstract

Cadmium accumulation in grain of durum wheat (*Triticum turgidum* L. var. *durum*) represents a concern to consumers. In an effort to understand the regulation of Cd accumulation in maturing grain, the remobilization of ^{109}Cd applied to stem and flag leaves was examined in two near-isogenic lines that differ in grain Cd accumulation. Absorbed ^{109}Cd was primarily retained in the labelling flap (50–54% and 65–80% for stem and flag leaves, respectively). Cadmium exported from the stem flap initially (3 d) accumulated in the stem in a declining gradient towards the head. Subsequent remobilization of Cd deposited in the stem was associated with Cd accumulation in the grain. Cadmium exported from the flag leaf flap was primarily directed to the grain. Little (<1%) Cd accumulated in the glumes or rachis, and transport of Cd to shoot tissues below the flag leaf node was low (<1%). On average, 9% and 17% of absorbed ^{109}Cd accumulated in the grain 14 d after labelling the stem and flag leaf, respectively. Irrespective of labelling position, the low Cd-accumulating isoline averaged 1.5–2-fold lower Cd accumulation per grain and Cd concentration in the grain than the high Cd-accumulating isoline. Cadmium accumulation in the grain was inversely correlated with Cd retention in the stem (stem labelled) and labelling flap (flag leaf labelled) for both isolines. Cadmium translocation to the grain was not inhibited by Zn when both were applied simultaneously (50 pM ^{109}Cd; 0.5 μM ^{65}Zn) to the flag leaf. These results show that elevated remobilization of Cd from the leaves and stem to the maturing grain may be partially responsible for the high accumulation of Cd in durum wheat grain.

Key words: Cadmium, durum wheat, flag leaf, redistribution, zinc.

Introduction

Increasing international concern about the risks associated with long-term consumption of crops contaminated with cadmium (Cd) (McLaughlin *et al*., 1999) has led the international food standards organization, Codex Alimentarius Commission, to propose a 0.1 mg Cd kg^{-1} limit for cereals, pulses and legumes (Codex Alimentarius Commission, 2000). Compared to bread wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* L. var. *durum*) has a genetic propensity to accumulate Cd in grain (Meyer *et al*., 1982) to levels often exceeding the proposed limit for cereal grains (Wolnik *et al*., 1983; Li *et al*., 1997; McLaughlin *et al*., 1999). Because trade of durum wheat could be affected if the proposed limit was adopted, recent scientific effort has focused on understanding the processes leading to high accumulation (Grant *et al*., 1998) and to breeding low Cd-accumulating cultivars (McLaughlin *et al*., 1999). While there has been progress in breeding low Cd-accumulating cultivars (Clarke *et al*., 1997), the physiological basis for high grain-Cd accumulation remains poorly understood.

Accumulation of Cd in the grain of durum wheat may be regulated by several physiological processes, including Cd uptake from the soil solution, xylem translocation from root to shoot, and phloem movement into the grain during maturation (Hart *et al*., 1998). Cadmium uptake and root–shoot translocation have been characterized in a wide variety of species (Grant *et al*., 1998), including wheat (Smeyers-Verbeke *et al*., 1978; Jalil *et al*., 1994), although only recently have these

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processes been explored as possible explanations for high Cd-accumulation in durum wheat grain (Hart *et al*., 1998; Archambault *et al*., 2001). It has been shown that Cd uptake and root to shoot translocation were higher in bread wheat seedlings than in durum wheat seedlings (Hart *et al*., 1998), suggesting that these factors do not contribute to high Cd-accumulation in grain. More recent evidence, however, suggests restricted root–shoot translocation is an important regulatory step (Archambault *et al*., 2001). Hart *et al*. speculated that durum wheat may have a greater capacity to transfer Cd to grain via the phloem (Hart *et al*., 1998).

Evidence from linseed (Becher *et al*., 1997), peanut (Popelka *et al*., 1996) and wheat (Herren and Feller, 1997) suggests Cd movement into developing seeds occurs predominately via the phloem. However, it remains unclear as to whether differences in phloem transport can account for excess Cd accumulation in grain of durum wheat. Recently, Cakmak *et al*. compared uptake and retranslocation of leaf-applied ^{109}Cd in diploid, tetraploid and hexaploid wheat seedlings (Cakmak *et al*., 2000*b*). Most of the ^{109}Cd applied (95%) was retained in the treated leaf and retranslocation to other tissues was unrelated to wheat ploidy. In fact, the proportion of ^{109}Cd translocated from treated leaves to roots was 2-fold higher in *T. aestivum* genotypes than in durum wheat genotypes. This and other studies (Welch *et al*., 1999; Cakmak *et al*., 2000*a*) provide valuable information on the phloem mobility of Cd, but little insight into movement of Cd into grain. Source–sink relationships can dramatically change during the switch from vegetative to reproductive growth (Marschner, 1995), as can the relative mobility of micronutrients such as Zn and Mn (Pearson and Rengel, 1995*a*, *b*).

Because of their similar chemical properties, Cd and zinc (Zn) may be taken up and translocated within the plant via similar pathways (Grant *et al*., 1998). Evidence for a common pathway of remobilization in wheat shoots, as shown by competitive inhibition of Cd movement by Zn, is contradictory. Application of Zn to the soil in field studies has been shown to inhibit Cd uptake and accumulation in wheat grain (Choudhary *et al*., 1995), although some studies have failed to demonstrate any relationship (Oliver *et al*., 1997). Similarly, foliar application of Zn inhibited (Oliver *et al*., 1997) or did not affect (Choudhary *et al*., 1995) Cd accumulation in wheat grain. Recently, Cakmak *et al*. demonstrated that remobilization of ^{109}Cd from the treated leaf to other parts of the shoot and the roots in durum wheat seedlings was reduced by up to 4-fold by increasing the Zn status of the plant (alleviating Zn deficiency) (Cakmak *et al*., 2000*a*). The only study to directly measure Zn interference of Cd transport to wheat grain showed that Zn inhibited phloem transport of Cd to the grain in detached shoots, but only at high (>10 μM) Zn concentrations (Herren and Feller, 1997). Inhibition of Cd accumulation in wheat grain by Zn (at physiological relevant concentrations) may be restricted to conditions where application of Zn alleviates Zn stress.

The objective of this study was to determine whether remobilization of ^{109}Cd applied to the stem and flag leaf correlated with the propensity of two near-isogenic durum wheat lines to accumulate Cd in grain. The remobilization of ^{65}Zn applied to the flag leaf was also studied to determine if differences between isolines in ^{109}Cd translocation were specific to Cd. The data show that remobilization of ^{109}Cd from the flag leaf to the grain and, to a lesser extent from the stem, correlated with capacity of the isolines to accumulate Cd in grain under field conditions. These results suggest that elevated remobilization of Cd from leaves to maturing grain may be responsible for the high Cd-accumulation in durum wheat grain, although the relative contribution of leaf Cd-stores, compared to stem or root stores, to grain Cd content remains undetermined.

Materials and methods

Plant growth

A pair of near-isogenic durum wheat (*Triticum turgidum* L. var *durum*) lines, high (8982-TL-H; TL04) and low (8982-TL-L; TL05) for grain Cd accumulation (Clarke *et al*., 1997), were used in these experiments. Field studies of five high and low isoline pairs (including 8982-TL) showed that high lines accumulate 2.5-fold higher concentrations of Cd in their mature grain than low lines (Clarke *et al*., 1997; McLaughlin *et al*., 1999). Four plants per pot were grown in 10 l of a 4:1:1:1 (by vol.) mixture of topsoil (pH 7.3), sand, vermiculite, and peat. The pots were placed in a growth chamber with a photon flux density of 250 μmol m^{-2} s^{-1} at the top of the plants immediately prior to stem elongation and day/night temperatures of 21/16 °C (16/8 h). Pots were watered daily, and a complete fertilizer solution (20–20–20 All Purpose Fertilizer; Plant Products Co., Brampton, ON) was applied once a week at the recommended rate for cereal crops. Auxiliary tillers were removed, and the ears were de-awned after emergence. Plants were labelled with ^{109}Cd at 7 d post anthesis, with the treatment beginning 4 h after the start of the light period.

Time-course of redistribution of ^{109}Cd applied to the stem

The leaves (lamina and sheath) originating from below the fourth node (from the top) exhibited varying levels of senescence and were therefore removed 24 h prior to treatment. A stem flap was cut 5 mm below the third node and inserted into a small vial containing 1 ml of 250 pM $^{109}CdCl_2$ (1590 Bq ^{109}Cd). The flap (45 mm long) included one-quarter of the stem circumference and was cut through to the pith. The vial was attached with tape to the stem and remained fixed until harvest. The labelling solution was taken up within 2–3 h. An additional 1 ml of deionized water was added 24 h following treatment. Plants were harvested 3, 7 and 14 d after treatment, and the shoots were divided into grain, glumes (including glumes, lemma, palea, rachillas), rachis, peduncle, labelling flap, flag leaf lamina, flag leaf sheath, lamina (second and third

leaves), sheaths (second and third leaves), stems (second, third and fourth internodes), and the fourth leaf (lamina + sheath).

Time-course of redistribution of ^{109}Cd applied to the flag leaf

A rectangular flap (5 mm wide, 50 mm long) was cut in the middle of the flag leaf lamina (according to Schenk and Feller, 1990), and inserted into a small vial containing 1 ml of 50 pM $^{109}CdCl_2$ (155 Bq ^{109}Cd). The flag leaf and vial were attached vertically to a stake with tape, and the vial remained fixed until harvest. An additional 0.5 ml of deionized water was added 2 d following treatment, when approximately 75% of the labelling solution had been absorbed. Plants were harvested 3, 7 and 14 d after treatment. The shoots were divided into grain, glumes (including glumes, lemma, palea, rachillas, and sterile grains), rachis, peduncle, labelling flap, flag leaf lamina apical, middle (including labelling flap window), and basal thirds, flag leaf sheath, and the lamina, sheath and stem of the second and third leaves.

Redistribution of ^{109}Cd and ^{65}Zn applied to the flag leaf

Cadmium (1 ml of 50 pM $^{109}CdCl_2$; 322 Bq ^{109}Cd) and zinc (1 ml of 0.5 μM $^{65}ZnCl_2$; 415 Bq ^{65}Zn) were applied singularly and in combination to the flag leaf using the leaf flap labelling technique described above. Plants were harvested 7 d after treatment and partitioned as described above.

Analyses

Harvested plant parts were dried at 70 °C for 7 d and ^{109}Cd and ^{65}Zn activities were determined using a gamma counter (Beckman Gamma 4000). The distribution of ^{109}Cd and ^{65}Zn within the shoot was calculated as a percentage of total activity absorbed by the labelling flap. A small percentage of the total applied ^{109}Cd (<5%) and ^{65}Zn (<2%) activity remained in the vial. There were no significant ($P > 0.05$) differences between isolines in the activity remaining in the vials. Significant differences between isolines in ^{109}Cd and ^{65}Zn accumulation were identified with Student's *t*-test.

Results

Most of the ^{109}Cd applied to the stem below the third node was retained in the labelling flap (Fig. 1, insets). Between 18% and 23% of the ^{109}Cd absorbed by the labelling flap was redistributed to other parts of the shoot. A consistently high (25–28%) fraction of absorbed ^{109}Cd remained unaccounted for and probably represents transport to the roots. Three days after introduction, the ^{109}Cd transported up the shoot was mostly detected in the stem and grain (Fig. 1A). As the grain matured, ^{109}Cd accumulation increased in the grain and decreased in the stem (Fig. 1B, C). The two isolines accumulated similar total amounts of ^{109}Cd in the grain, except for day 7 where TL-H was 2-fold higher than TL-L. Very little ^{109}Cd accumulated in the grain support structures (glumes and rachis). The ^{109}Cd accumulation per grain increased over time (Fig. 2A) whereas the grain ^{109}Cd concentration remained more or less constant over time (Fig. 2B). Significantly higher ^{109}Cd accumulation per grain and grain ^{109}Cd concentration was observed in TL-H at two harvests.

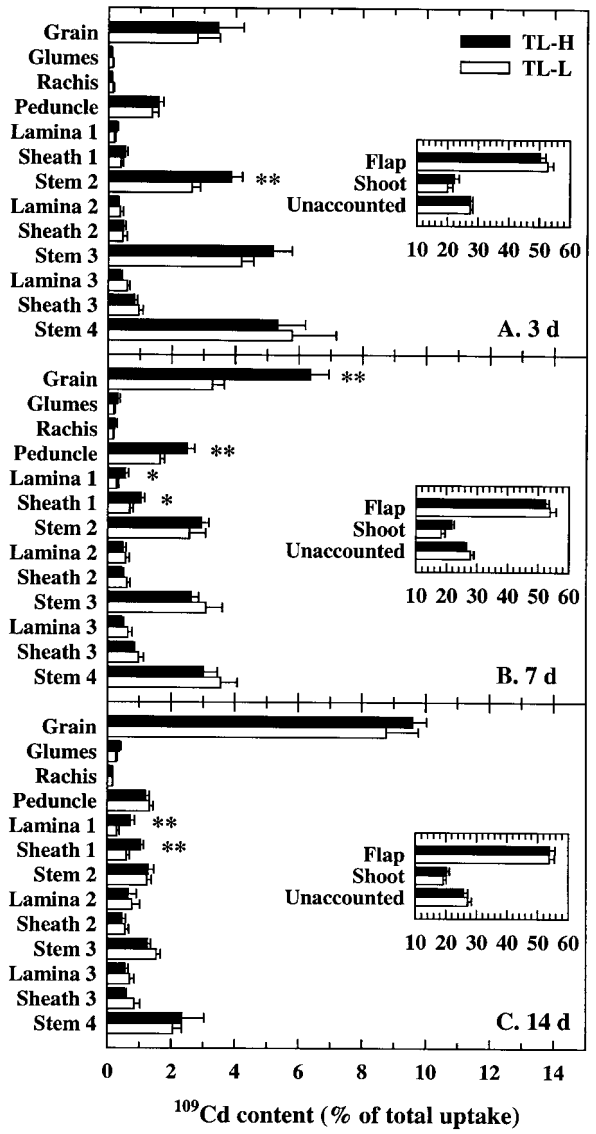

Fig. 1. Time-course of ^{109}Cd redistribution from below the third node to other shoot parts of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat. ^{109}Cd (1 ml of 250 pM $^{109}CdCl_2$) was fed 7 d post-anthesis through a flap in the stem below the third node (from the top), and the shoots were harvested 3 (A), 7 (B) and 14 (C) d after treatment. Lamina, sheaths and stems are numbered from the top. Accumulation of ^{109}Cd in the fourth leaf (lamina and sheath combined) was less than 0.1%, and was constant over time and not different between isolines (data not shown). Insets show percentage retention of ^{109}Cd in the labelling flap, percentage export of ^{109}Cd to other parts of the harvested shoot and percentage of ^{109}Cd uptake remaining unaccounted. Means and standard errors of eight replicates are plotted. Significant differences between isolines for each plant part are indicated by * ($P \leq 0.05$) and ** ($P \leq 0.01$).

Accumulation of ^{109}Cd in the grain of stem-labelled plants was significantly correlated with loss of ^{109}Cd from stem 2, 3 and 4 in both isolines (Table 1), but showed no relationship to ^{109}Cd retention in the labelling flap or

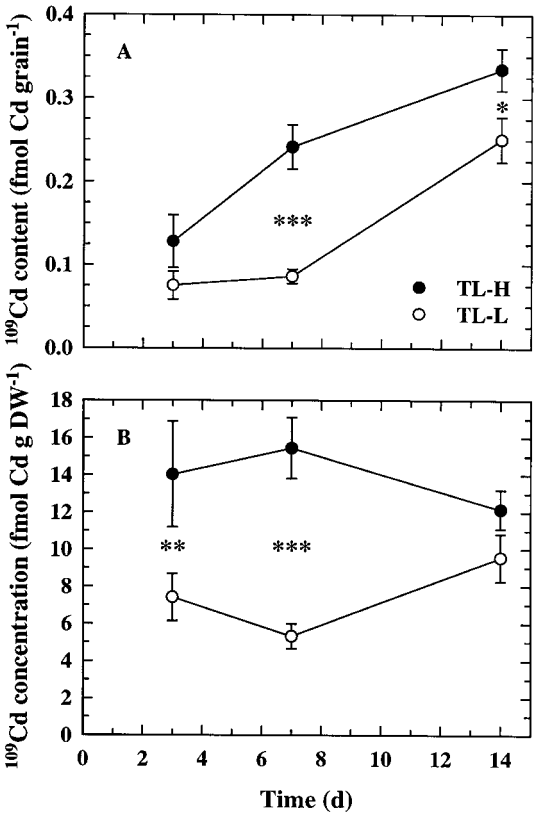

Fig. 2. Time-course of ^{109}Cd accumulation per grain (A) and grain ^{109}Cd-concentration (B) following labelling of a stem-flap of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat. ^{109}Cd (1 ml of 250 pM $^{109}CdCl_2$) was fed 7 d post-anthesis through a flap in the stem below the third node, and the shoots were harvested 3, 7 and 14 d after treatment. Means and standard errors of eight replicates are plotted. Significant differences between isolines for each time point are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

Table 1. *Correlation between grain ^{109}Cd accumulation and ^{109}Cd accumulation in other parts of the plant of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat fed with ^{109}Cd (1 ml of 250 pM $^{109}CdCl_2$) through a flap into the stem below the third node*

Correlation coefficients calculated from data in Fig. 1, pooled over three harvests ($n = 24$). Significant correlation coefficients are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P = 0.001$).

Variate	Pearson correlation coefficient	
	TL-L	TL-H
Glumes	0.398	0.530**
Rachis	0.099	0.122
Peduncle	−0.049	−0.298
Lamina 1	0.098	0.638***
Sheath 1	0.117	0.545**
Stem 2	−0.438*	−0.723***
Lamina 2	0.187	0.419*
Sheath 2	0.134	0.117
Stem 3	−0.566**	–0.684***
Lamina 3	0.217	0.362
Sheath 3	−0.087	−0.389
Stem 4	–0.457*	−0.472*
Leaf 4	−0.094	−0.333
Flap	−0.116	0.061
Unaccounted	−0.140	−0.186

export from the shoot (unaccounted). In TL-H, accumulation of ^{109}Cd in the grain was also positively correlated with ^{109}Cd accumulation in the glumes, lamina 1, sheath 1 and lamina 2 (Table 1).

Most of the ^{109}Cd applied to the flag leaf was also retained in the labelling flap, decreasing from nearly 80% on day 3 to between 65% and 70% on day 14 (Fig. 3, insets). Redistribution of ^{109}Cd exported from the labelling flap was strongly directed to the grain. The time-course of increase in grain ^{109}Cd accumulation was correlated with loss of ^{109}Cd from the labelling flap in TL-H ($r = -0.862$; $P \leq 0.001$) and TL-L ($r = -0.787$; $P \leq 0.001$). Grain ^{109}Cd accumulation was also correlated with the unaccounted fraction ($r = 0.500$; $P \leq 0.05$) in TL-H and with ^{109}Cd accumulation in lamina 2 ($r = -0.406$; $P \leq 0.05$) in TL-L. Very low accumulation of ^{109}Cd in the lamina, sheaths and stems of nodes 2 and 3 suggests that the unaccounted fraction was unlikely to have been in leaves and stems of lower nodes. At all harvests ^{109}Cd accumulation per grain and grain ^{109}Cd concentration was significantly higher in TL-H than in TL-L (Fig. 4). Grain ^{109}Cd concentrations were constant between harvests at 3 d and 7 d (Fig. 4B), but declined by day 14 as the rates of ^{109}Cd import per grain (Fig. 4A) declined. TL-L consistently produced larger heads (grains spike^{-1}) than TL-H (20 grain average difference), although both produced similar sized grain (Fig. 5).

Simultaneous application of ^{65}Zn to the flag leaf lamina of TL-H significantly decreased ^{109}Cd retention in the labelling flap, and significantly increased ^{109}Cd transfer to the grain, glumes, rachis, and peduncle (Fig. 6A, B). In contrast, simultaneous application of ^{65}Zn to TL-L had little effect on ^{109}Cd redistribution, other than a small but significant increase in accumulation in sheath 1. Grain ^{109}Cd accumulation was higher in TL-H than in TL-L when applied alone or jointly with ^{65}Zn (Fig. 6A, B). In contrast to ^{109}Cd, ^{65}Zn was readily exported from the labelling flap (Fig. 6C), with between 64% (TL-L) and 71% (TL-H) of total ^{65}Zn uptake accumulating in the grain 7 d following labelling (Fig. 6C). Significantly higher ^{65}Zn accumulation in the grain of the high Cd-accumulating isoline (TL-H) was associated with significantly lower retention of ^{65}Zn in the labelling flap and lower ^{65}Zn remaining unaccounted. Although still a small component, ^{65}Zn was more readily transported than ^{109}Cd to shoot tissues, other than the grain. ^{109}Cd accumulation per grain and grain ^{109}Cd concentration were 1.5–2 times higher in TL-H than in TL-L (Fig. 7A, C). Similarly, ^{65}Zn accumulation per grain and grain ^{65}Zn concentration were 1.2–1.4 times

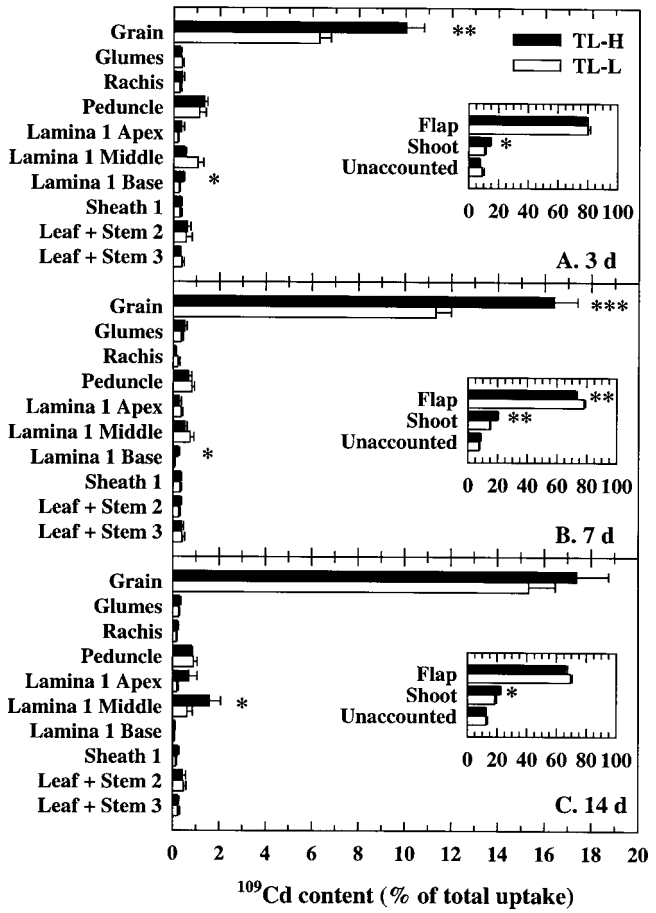

Fig. 3. Time-course of ^{109}Cd redistribution from the flag leaf (lamina 1) to other shoot parts of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat. ^{109}Cd (1 ml of 50 pM $^{109}CdCl_2$) was fed 7 d post-anthesis through a flap in the lamina of the flag leaf, and the shoots were harvested 3 (A), 7 (B) and 14 (C) d after treatment. Lamina, sheaths and stems are numbered from the top. The flag leaf (lamina 1) was analysed as three parts of equal length, with the middle section containing the labelling flap prior to harvest. Accumulation of ^{109}Cd in second and third lamina, sheaths and stems was low, constant over time, and not different between isolines (data combined for brevity). Insets show percentage retention of ^{109}Cd in the labelling flap, percentage export of ^{109}Cd to other parts of the harvested shoot and percentage of ^{109}Cd uptake remaining unaccounted. Means and standard errors of eight replicates are plotted. Significant differences between isolines for each plant part are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

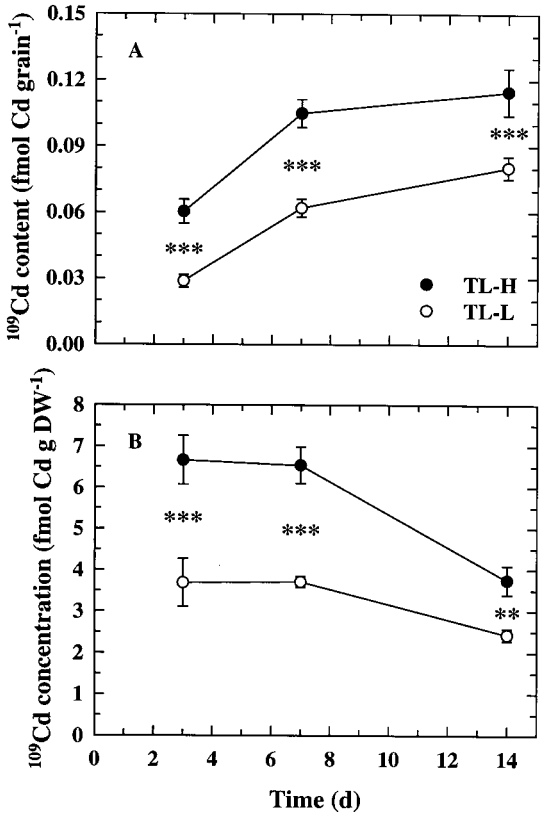

Fig. 4. Time-course of ^{109}Cd accumulation per grain (A) and grain ^{109}Cd-concentration (B) following labelling of the flag leaf of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat. ^{109}Cd (1 ml of 50 pM $^{109}CdCl_2$) was fed 7 d post-anthesis through a flap in the lamina of the flag leaf, and the shoots were harvested 3, 7 and 14 d after treatment. Means and standard errors of eight replicates are plotted. Significant differences between isolines for each plant part are indicated by ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

higher in TL-H than in TL-L (Fig. 7B, D). Compared to when applied singularly (^{109}Cd alone), simultaneous application of ^{65}Zn and ^{109}Cd had no significant ($P > 0.05$) effect on accumulation per grain or grain concentration of ^{109}Cd.

Discussion

The results of this study provide the first experimental evidence for a positive correlation between elevated remobilization of Cd in durum wheat shoots and high

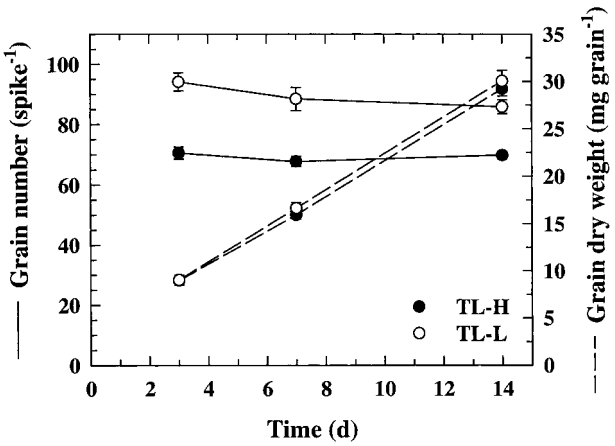

Fig. 5. Grain number and weight of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat harvested 3, 7 and 14 d after labelling the stem or flag leaf. Means and standard errors of 16 replicates are plotted (combined data from stem and flag leaf labelling experiments).

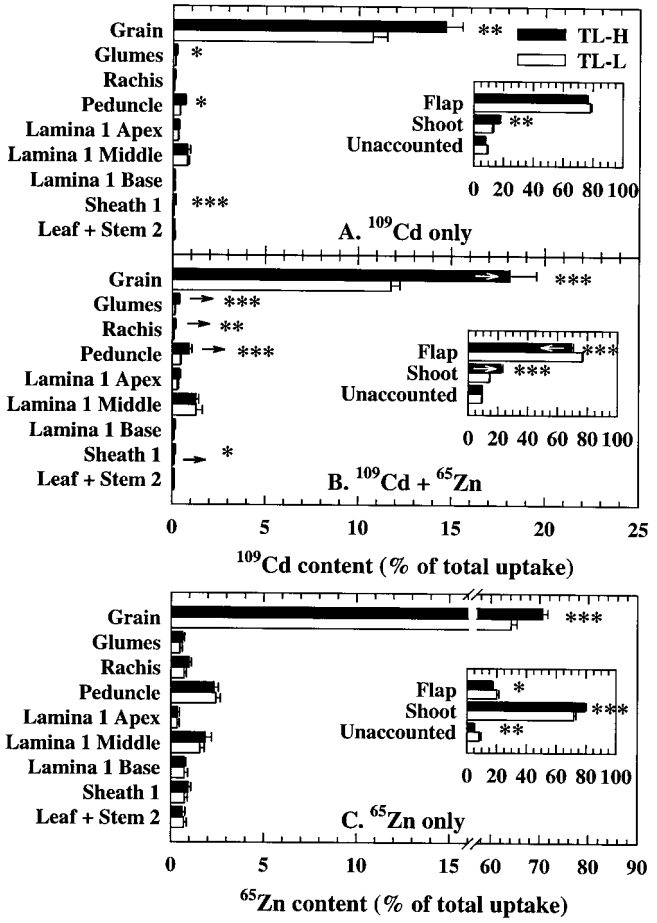

Fig. 6. Redistribution of ^{109}Cd (A, B) and ^{65}Zn (C) from the flag leaf (lamina 1) to other shoot parts of high (TL-H) and low (TL-L) Cd-accumulating isolines of durum wheat. ^{109}Cd (1 ml of 50 pM $^{109}CdCl_2$) and ^{65}Zn (1 ml of 0.5 μM $^{65}ZnCl_2$) were applied singularly (A and C, respectively) and in combination (B) to a flap in the flag leaf lamina, and the shoots were harvested 7 d following treatment. ^{109}Cd had negligible impact on the redistribution of ^{65}Zn (data not shown). Lamina, sheaths and stems are numbered from the top. The flag leaf (lamina 1) was analysed as three parts of equal length, with the middle section containing the labelling flap prior to harvest. Accumulation of ^{109}Cd and ^{65}Zn in second lamina, sheath and stem was low, constant over time, and not different between isolines (data combined for brevity). Insets show percentage retention of ^{109}Cd or ^{65}Zn in the labelling flap, percentage export of ^{109}Cd or ^{65}Zn to other parts of the harvested shoot and percentage of ^{109}Cd or ^{65}Zn uptake remaining unaccounted. Means and standard errors of 10 replicates are plotted. Arrows within or above bars indicate significant ($P \leq 0.05$) changes in ^{109}Cd distribution after addition of ^{65}Zn. Significant differences between isolines for each plant part are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

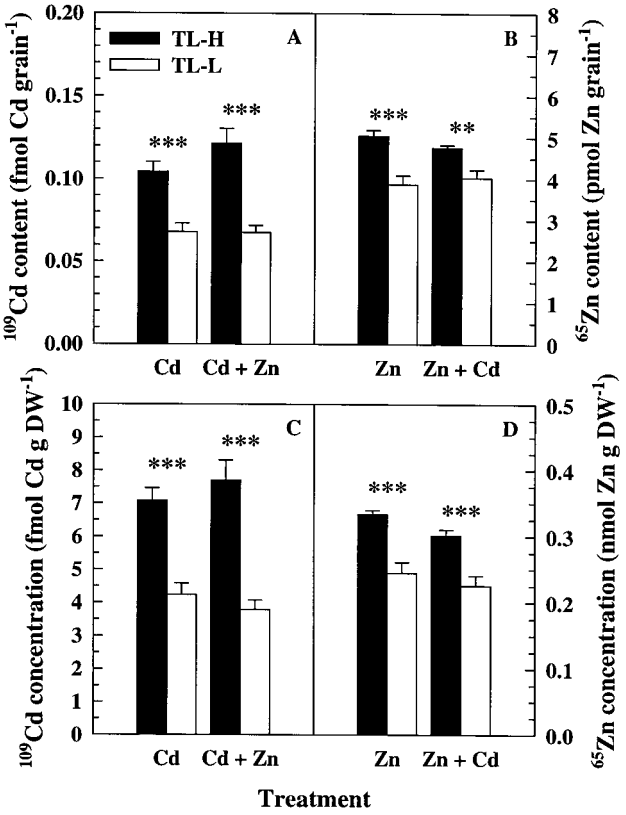

Fig. 7. Effects of dual labelling the flag leaf (lamina 1) with ^{109}Cd and ^{65}Zn on the accumulation per grain (A, B) and grain concentration (C, D) of ^{109}Cd (A, C) and ^{65}Zn (B, D). ^{109}Cd (1 ml of 50 pM $^{109}CdCl_2$) and ^{65}Zn (1 ml of 0.5 μM $^{65}ZnCl_2$) were applied singly and in combination to a flap in the flag leaf lamina, and the shoots were harvested 7 d following treatment. Means and standard errors of 10 replicates are plotted. Significant differences between isolines for each treatment are indicated by ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

Cd-accumulation in grain. Compared to the high Cd-accumulating isoline (TL-H), the low Cd-accumulating isoline (TL-L) averaged 1.5–2-fold lower Cd accumulation per grain and Cd concentration in the grain for flag leaf (Figs 4, 7) and stem (Fig. 2) labelled plants. Under field conditions, the Cd concentration of mature grain for TL-L (0.088 mg Cd kg^{-1}) is 2.7-fold lower than for TL-H (0.235 mg Cd kg^{-1}) (McLaughlin *et al.*, 1999). Although the magnitude of these differences can be partially attributed to the large head size of TL-L (Fig. 5), the low Cd-accumulating isoline still accumulated significantly lower total amounts of Cd in the grain in 4 out of 5 harvests for flag leaf-labelled plants (Figs 3, 6). The differences between isolines in total grain Cd accumulation were less pronounced for stem labelled plants (Fig. 1).

Export of Cd from the flag leaf was strongly directed to the grain (Fig. 3). Very little Cd accumulated in the glumes or rachis, and transport of Cd to shoot tissues below the flag leaf node was low (<1%). The specific targeting of Cd transported from the flag leaf to the grain is similar to that observed for Co, Ni, Rb, and Zn (Schenk and Feller, 1990; Herren and Feller, 1996; Zeller and Feller, 1999), and likely represents remobilization via the phloem. Developing grain represents major phloem sinks in the shoots (Marschner, 1995), and markers of xylem transport (such as Sr) are poorly transported out of the flag leaf (Schenk and Feller, 1990). The absence of Cd accumulation in the glumes also supports the concept of phloem providing the pathway

for Cd transported from the flag leaf. Micronutrients transported to the grain via xylem tend to accumulate in the glumes, as shown for Mn (Pearson and Rengel, 1994, 1995*b*; Pearson *et al*., 1996).

Between 10% and 15% of ^{109}Cd applied to the flag leaf remained unaccounted for and may have represented transport of Cd to the roots. Thus, Cd transport to the grain could have occurred by secondary remobilization from the roots. However, this appears unlikely given the low (<1%) accumulation of Cd in the leaves and especially the stem below the flag leaf node. As shown here and by others (Herren and Feller, 1997; Archambault *et al*., 2001), Cd is readily removed from the transpiration stream and accumulates in the stem of wheat.

Cadmium applied directly to the transpiration stream via a stem flap was initially redistributed up the stem in a declining gradient towards the head (Fig. 1). Subsequent accumulation of Cd in the grain at days 7 and 14 was associated with remobilization of Cd deposited in the stem (Table 1). These results are similar to those of others (Herren and Feller, 1997). They applied Cd to intact *T. aestivum* plants via a stem flap cut below the flag leaf node and found that after a short initial period of Cd accumulation in the peduncle, Cd accumulation in the grain was matched by remobilization of Cd from the peduncle. Temporary storage in the stem of wheat has also been observed for Zn and Mn (Pearson and Rengel, 1994).

In contrast to this study's results, Herren and Feller recorded a high accumulation of Cd in the flag leaf lamina of plants labelled immediately below the flag leaf node (Herren and Feller, 1997). The capacity of wheat stems to remove Cd from the transpiration stream appears to be concentration-dependent (Herren and Feller, 1997). Therefore, the low (50–250 pM) concentrations of Cd used in the present study could account for the low translocation of Cd to the lamina and sheaths. Furthermore, removal of Cd from the transpiration stream suggests that Cd may have been transported to the grain primarily by the phloem. The time-course of redistribution of Cd applied to intact wheat stems to the grain was similar to that of phloem-mobile Rb, but not to that of phloem-immobile Sr (Herren and Feller, 1997). In addition, steam girdling the peduncle of detached wheat shoots fed with Cd below the flag leaf node reduced Cd accumulation in the grain by approximately 50–75% (Herren and Feller, 1997), and xylem to phloem transfer of Cd is probably more efficient at the nodes, as shown for Rb (Feller, 1989) and Zn (Herren and Feller, 1994). However, positive correlations between Cd accumulation in the grain and transpiring organs (glumes, lamina 1 and 2, and sheath 1) in the high Cd-accumulating isoline (Table 1) suggests that Cd transport via the xylem may have partially contributed to Cd accumulation in the grain, and the relative contribution of the xylem may have been reduced by removing the awns (Pearson *et al*., 1996). It appears, at least at low concentrations, that Cd remobilized from the roots can be transferred from the xylem to the phloem in the stem of durum wheat and be transported to the grain via the phloem without passing through the leaves.

Cadmium was poorly remobilized from the flag leaf to the grain, compared to Zn (Fig. 6). Seven days after labelling the flag leaf, total Cd accumulation in the grain was 4–6-fold lower than for Zn. In other studies with durum wheat seedlings, Cd remobilization from the leaves to the roots was 10-fold lower than for Rb (Cakmak *et al*., 2000*a*), and 2-fold lower than Zn when transported between split-root compartments (Welch *et al*., 1999). It was shown that the grain-to-straw Cd-concentration ratio in mature wheat (in field studies) was 8-fold lower than for Zn, suggesting lower Cd remobilization in the shoot (Puschenreiter and Horak, 2000). Although the phloem mobility of Zn depends on the Zn status of the plant (Welch, 1995), it is generally considered to have intermediate mobility (Marschner, 1995). Therefore, the phloem mobility of Cd in durum wheat leaves should be considered low to intermediate.

Despite large differences in the relative mobility of Cd and Zn, the high Cd-accumulating isoline accumulated significantly higher amounts of Cd and Zn in the grain (Figs 6, 7). Because Zn can competitively inhibit Cd uptake by roots (Smeyers-Verbeke *et al*., 1978; Cataldo *et al*., 1983; McKenna *et al*., 1993), it is possible that Cd is transported into wheat grain in a manner similar to that of Zn, although given the large differences in relative mobility of Cd and Zn, remobilization of Cd and Zn from the leaves may be regulated by different processes. In either case, selection for the low Cd-accumulating trait in this isoline pair (Clarke *et al*., 1997) may have also inadvertently selected for low remobilization of Zn from the leaves. This would be undesirable given the well-documented Zn inefficiency exhibited by durum wheat (Graham *et al*., 1992; Cakmak *et al*., 1997), and the possibility of improving micronutrient levels in human diets by increasing the micronutrient density of grain (Rengel *et al*., 1999).

The absence of competitive inhibition of Cd transport to the grain by Zn suggests that Cd and Zn remobilization may be regulated by different processes. However, the low concentration of Zn used in this study (0.5 μM) may have been insufficient to demonstrate a competitive interaction between Cd and Zn. In field studies, foliar applications of Zn are either ineffective (Choudhary *et al*., 1995) or only effective at high application rates (Oliver *et al*., 1997) at decreasing the Cd concentration of wheat grain. Similarly, phloem transport of Cd to the grain in detached wheat shoots was only inhibited by Zn at high (>10 μM) concentrations (Herren and Feller,

1997). The effectiveness of Zn inhibition of Cd phloem transport is also related to the Zn status of the plant; inhibition appears most effective in Zn-deficient plants. For example, translocation of ^{109}Cd between physically separated roots in durum wheat seedlings was greatly reduced by alleviating severe Zn deficiency, but further additions of Zn to the growth medium had no additional effect on Cd translocation (Welch *et al*., 1999).

It remains to be determined to what extent remobilization of Cd via the phloem from the leaves to the grain contributes to overall Cd accumulation by the grain. As discussed above, remobilization of Cd from the flag leaf is low (compared to Zn), and Cd applied to the stem is transported to the grain without passing through the leaves. Furthermore, compared to the flag leaf, translocation of micronutrients and heavy metals from lower leaves to the grain is low (Zeller and Feller, 1999), even for highly phloem-mobile Rb (Schenk and Feller, 1990). More importantly, the stem and roots represent large pools of absorbed Cd in wheat plants (Choudhary *et al*., 1995; Grant *et al*., 1998), and may greatly contribute to grain Cd accumulation. Restricted root–shoot Cd translocation may directly regulate Cd accumulation in the grain, given that the differences between isolines in grain Cd-accumulation were small for Cd applied to the stem transpiration stream. In fact, recent studies with several pairs of high and low Cd-accumulating durum wheat isolines, including the TL pair, showed that root–shoot Cd translocation in seedlings was a good predictor of grain Cd concentration at maturity (Archambault *et al*., 2001). Attempts to measure fluxes in plant Cd pools of durum wheat grown in soil have proved inconclusive. Cieśliński *et al*. showed that Cd accumulated in the leaves and stems of durum wheat at heading stage, but very little Cd accumulated in the newly emerged spikes (Cieśliński *et al*., 1996). They suggested high accumulation of Cd in the grain at maturity probably reflected redistribution of Cd via the phloem pathway. However, grain Cd accumulation at maturity was also inversely related to Cd retention in the roots (Cieśliński *et al*., 1996). In a field trial, Cd accumulation in durum wheat grain during maturation was associated with a 3-fold decline in Cd concentration of the leaves and a 2-fold increase in Cd concentration of the stems (Chizzola, 1997). The relative contribution of Cd pools in the leaves, stems and roots to Cd accumulation in the grain may vary over time and with plant Cd content, as shown for Zn and Mn (Pearson and Rengel, 1994).

In conclusion, these results demonstrate that elevated remobilization of Cd from the leaves, and to a lesser extent from the stem, to maturing grain may be responsible for high accumulation of Cd in durum wheat grain. Future research will concentrate on determining the relative contribution of leaf, stem and root pools of Cd to grain Cd accumulation.

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