Mechanisms of cadmium accumulation by potato tubers (cont'd PT620)

Mike McLaughlin
CSIRO Land and Water

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Uptake and Partitioning of Cadmium in Two Cultivars of Potato (*Solanum tuberosum* L.)

A final report to Horticulture Australia Ltd.

Project No. PT 96020 (completed 28/02/2000)

M.J. McLaughlin *et al*.

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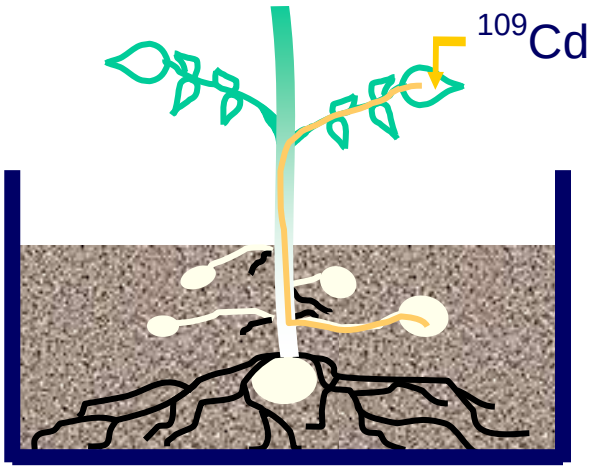

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Purpose:
This report describes the investigations conducted by CSIRO Australia and the University of Adelaide into the uptake and partitioning of cadmium in cultivars of potato (*Solanum tuberosum* L.), for which funding was supplied by Horticulture Australia Ltd. (project no. PT620). This original research examined physical and biological processes in potato plants that were previously uncharacterized, and the results have important implications in terms of food quality and safety, nutrition and human health.

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June 2007

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Media Summary

Cadmium (Cd) is a naturally occurring element in the environment, but it is often present in soils at elevated concentrations due to pollution. Cadmium is readily transferred from soils to plants, thus we risk Cd exposure through the foods we eat. This poses a danger because prolonged consumption of food materials high in Cd can cause a variety of adverse health effects. Potatoes have been identified as the key source of Cd in the Australian diet; therefore it is vital to keep the Cd content of potatoes as low as possible to maintain food safety.

We conducted a research program to determine whether variations in potato tuber Cd concentrations observed between different potato cultivars were due to differences in Cd absorption from soil or to differences in the distribution of the element within the plant once absorbed. We also examined the pathways through which Cd is transported to tubers, and how Cd moves around the plant within the vascular tissues. Finally, we investigated how Cd and zinc (Zn) interactions affect tuber Cd concentrations at harvest.

For the two cultivars we examined (Wilwash and Kennebec), we found that Cd uptake was similar but that tuber concentrations were very different. Therefore, the differences were due to differences in Cd partitioning within the plant, which is important information for plant breeders attempting to develop low Cd accumulating varieties. We also determined that the major route of Cd movement to tubers is transport from the basal roots to the leaves, followed by translocation to the tubers. This movement was shown to be rapid, with applied Cd reaching the tubers within as little as 8 hours. Therefore future research should focus on identifying the precise mechanisms of Cd absorption into roots and the chemical form of Cd transported around the plant, as this may lead to options for blocking Cd deposition in potato tubers.

The interactions between Zn and Cd were found to be complex, going beyond simple competition for uptake. Depending on environmental conditions and the cultivar examined, we found Zn could either decrease or increase Cd absorption into plants. Therefore, we could not identify how Zn can reduce Cd uptake by potato tubers as has been found in field agronomic experiments.

Technical Summary

Industrial pollution and use of fertilizers containing contaminants have resulted in elevated Cd concentrations in many soils. This poses a risk to the food chain because Cd is readily transferred from soils to plants to humans, and can cause adverse health effects with prolonged exposure. Potatoes are recognised as the key source of Cd in the Australian diet, and under certain growing conditions, potato crops can exceed the maximum permissible Cd concentration for food products. Therefore, to maintain safe food supplies and to preserve our ‘clean and green’ image, it is vital to minimize potato tuber Cd concentrations. Different cultivars have been shown to produce tubers with different Cd concentrations, but the reasons for the differences have not yet been identified. This research investigated the uptake and distribution of Cd in two cultivars of potato (*Solanum tuberosum* L.) known to display such differences.

An initial glasshouse trial sought to determine whether these differences resulted from variation in uptake from soil or Cd partitioning within the plant. Analysis of plant tissues revealed that total Cd uptake did not differ between cultivars, nor did the yield of tubers. However, there were marked differences in Cd distribution within the plant. We therefore concluded that tuber Cd

concentration differences between the two cultivars were due to differences in internal partitioning of Cd.

We then examined pathways of Cd uptake and movement within the plant using a split-pot method that separated basal roots from stolons and tubers. We grew plants for 13 weeks in soil labeled with the radioisotope ^{109}Cd and subsequently determined the activity in various plant parts. The results revealed basal roots were the dominant source of Cd for all tissues and accounted for approximately 85% of tuber Cd. The remaining 15% of Cd was sourced directly from stolons. However, there was no evidence of a direct link between the main (basal) root system and the stolons. Rather, the Cd pathway was roots to shoots via the xylem followed by shoots to tubers via the phloem. Although Cd did accumulate to some degree in the periderm, penetration into tubers across the periderm did not occur.

Further isotopic studies were conducted to examine short-term redistribution of Cd applied directly to leaves or to soil containing basal roots. Cadmium was found to be highly mobile in both the xylem and phloem, with added Cd being rapidly assimilated into all tissues following both root and foliar application. We found Cd applied to leaves could reach tubers in as little as 8 hours. Absorbed Cd was also rapidly sequestered by the stems, suggesting that stems may act as a transitional storage pool for nutrients and contaminants.

In addition, we investigated the effects of interactions between Zn and Cd on uptake of the two elements, as it has been suggested that competition between them for plant uptake may lead to lower tuber Cd concentrations. If this were proven correct then applying Zn to potato growing soils would be a potential agronomic option for reducing Cd in potatoes. We exposed potato plants to nutrient solutions with varying Zn: Cd ratios for 7 days and examined the elemental concentrations in plant parts. The results showed interactions between Zn and Cd to be complex, with both synergistic and antagonistic effects being observed for plant uptake depending on the cultivar examined and the external solution Zn: Cd ratio.

Our recommendations are therefore that future research should focus on identifying the mechanisms by which the low tuber Cd accumulator (cv. Wilwash) distributes Cd within the plant. This may pinpoint how lower concentrations are maintained in this cultivar and thus has importance for plant breeding and development of low Cd varieties. The form of Cd transported via the plant's vascular systems should also be investigated, as this may lead to options for blocking Cd deposition in tubers. Similarly, the physiological mechanisms driving Cd transport within the plant need to be investigated, because we found that simple concentration gradients alone cannot account for the movements of Cd observed. Such investigations may provide further indications of mechanisms for limiting Cd entry into tubers. Due to the complexity of interactions observed here, future research must also focus on determining the exact mechanisms of Cd uptake by potato roots and how they are affected by Zn, as such knowledge may provide further scope for minimising tuber Cd concentrations through limiting Cd assimilation into the plant at the point of entry.

1. Introduction

Cadmium is a heavy metal that occurs naturally in the environment. However, elevated concentrations of Cd can arise in soils due to various sources of pollution, including atmospheric fallout generated from fossil fuel combustion and mining and smelting operations, Cd contaminants applied to land in phosphatic fertilizers, and impurities in manures and composts applied to soils in farming areas (Tiller *et al.* 1997; Alloway 1995; Chang *et al.* 1987). Accumulation of Cd in soils creates potential risks for human health due to the possibility of food chain contamination, because higher levels in soils often result in greater concentrations in plants (Williams and David 1977). Ingestion of food materials containing elevated Cd concentrations can result in accumulation of the metal in the liver and kidneys, which over a prolonged period may cause severe damage to these organs. Of particular concern is the concentration of Cd in root and leafy vegetable food crops, because Cd appears to accumulate mostly in the roots, storage organs and leaves of plants (Page *et al.* 1981). In Australia, particular attention has been focused on the levels of Cd in potatoes, as they contribute over 50% of the dietary intake of Cd for the typical Australian adult (Stenhouse 1991). Currently, the maximum permitted concentration (MPC) of Cd in vegetables is 0.1 mg/kg fresh weight (ANZFA 1997). With a significant proportion of Australia's potato crop being near to or exceeding this limit (McLaughlin *et al.* 1997), attention has been focused on ways to limit Cd accumulation in the tubers. However, in order to achieve this it is necessary to first understand the mechanisms by which Cd is taken up by, and distributed within, the potato plant.

Previous research has shown that potato tuber concentrations of Cd can vary 2-3 fold depending on the cultivar being examined (McLaughlin *et al.* 1997), suggesting that uptake and distribution mechanisms may vary between cultivars. It is not known whether such differences are due to variations in Cd uptake from the soil, or to differences in partitioning within plant tissues once Cd is absorbed. Differences in uptake could possibly occur due to variations in root morphology, root exudate chemistry or plant-microbe symbiotic relationships, all of which can affect nutrient access in the rhizosphere (Welch 1995; Uren and Reisenauer 1988; Mench and Martin 1991), Differences in internal partitioning could arise through variations in translocation processes and characteristics of phloem solutions. Phloem solution chemistry is important in this respect, as evidence suggests that phloem vessels not only supply the photosynthate necessary for tuber bulking, but also the majority of the water entering tubers (Kratzke and Palta 1985). Phloem vessels may therefore also deliver the great majority of nutrients and contaminants (including Cd) to tubers. As yet this tenet has not been adequately verified and other potential entry mechanisms, such as direct periderm uptake, or supply via stolon and tuber roots have also been speculated. Supply by stolon and tuber roots has been demonstrated to occur for calcium (Wiersum 1966, Krauss and Marschner 1971, Kratzke and Palta 1986).

An understanding of Cd uptake and distribution processes, and the likely causes of the variations observed in potato tuber Cd concentrations, is vital if methods to limit Cd accumulation in potatoes are to be developed in order to safeguard food supplies. The aims of this research were therefore to analyse the pattern of Cd accumulation in potato tubers, to examine the pathways of Cd movement through potato plants and to determine the factors that lead to differences in Cd accumulation between different cultivars.

An examination of the effects of interactions between Cd and Zn on potato tuber Cd concentrations was also part of the investigation, as previous research has provided conflicting evidence regarding the effects of Zn on plant Cd uptake. Williams and David (1976) measured a positive effect Zn on Cd uptake, while a number of authors have also found decreases in Cd uptake in the presence of Zn (Moraghan 1993; Grant and Bailey 1997; McKenna *et al*. 1993; Choudhary *et al*. 1994; Oliver *et al*. 1994) or no effect (Cunningham *et al*. 1975; White and Chaney 1980).
Grotz *et al*. (1998) and Hart *et al*. (2002) have postulated that a competitive interaction between Cd and Zn may occur during plant uptake processes due to the possibility of the two elements sharing a common transport system across the root cell plasma membrane.The mechanisms potentially responsible for such a process remain unknown. McLaughlin *et al*. (1995) demonstrated that the addition of Zn to Zn deficient soils can reduce the concentration of Cd in potato tubers at the time of harvest. This result suggests that addition of Zn to soils may be a viable agronomic option for restricting tuber Cd concentrations. Due to the conflicting evidence in the literature it is vital to determine how potential Zn-Cd interactions may affect final potato tuber Cd concentrations before any such management practices are adopted.

The knowledge gained through this research will benefit the horticultural industry, as it identifies management techniques and strategies for developing potato cultivars that may lead to lower accumulations of Cd in potato tubers.

2. Methods

A number of experimental procedures were conducted during this research project, each designed to investigate a specific aspect of the study.

2.1 Glasshouse pot trial

The specific objective of this pot trial was to examine the distribution of Cd in two cultivars of potato during growth. The focus of this experiment was to determine whether any differences in tuber Cd concentration between the two cultivars could be related to total uptake of Cd from the soil or to partitioning of Cd within the plant. Two cultivars, cv. Wilwash and cv. Kennebec, were selected for the trial due to their contrasting capacities for Cd accumulation. In previous field trials McLaughlin *et al.* (1997) identified Kennebec as a relatively high Cd accumulator, with an average concentration of 48 µg Cd kg^{-1} fresh weight. In contrast, Wilwash tended to accumulate lower Cd levels, with an average concentration of 34 µg kg^{-1} fresh weight.

Plants were grown in a glasshouse in free draining pots containing 13 kg of washed sand and watered daily with 1 L of nutrient solution. Planting was carried out by placing pre-sprouted seed tubers 10 cm below the surface of the sand. An additional 2 kg of sand was mounded around each plant 4 weeks after emergence to ensure developing tubers did not break the surface. Cadmium was introduced as part of the nutrient solution, and was added as $CdCl_2$ at a concentration of 10 nM to represent the concentration of Cd in the soil solution of a non-polluted soil (McKenna *et al.* 1993).

Plants were harvested once per week for a total of ten weeks, with the first plant harvested four weeks after planting. Plants were de-topped by cutting at the base of the stem with a scalpel. The tops of each plant were divided into three sections; new leaves (the first 5 leaves from the

growing apex), old leaves (all remaining leaves), and stems. The pots, still containing the roots and tubers, were up-ended into a large stainless steel sink. All sand was carefully washed away with tap water and the remaining roots and tubers were separated from the underground stem and seed piece. To remove extracellular Cd, roots were desorbed in a solution containing 5 mM $CaCl_2$ and 1 µM $LaCl_3$ for 30 minutes. They were then rinsed in distilled water and blotted dry. The fresh weights of all individual plant parts were recorded.

Tubers were brushed gently with a nylon scrubbing brush to remove any adhering sand particles. Tubers were rinsed again in tap water before removing the skin with a potato peeler. A representative sample of tuber tissue was obtained from each individual tuber by taking a 1 cm longitudinal slice, from the bud end to the stem end. Slices were washed in distilled water and cut into smaller pieces for drying. Prior to chemical analysis by atomic absorption spectroscopy, all plant material (tops, roots and tubers) was dried, ground and digested in hot, concentrated nitric acid (HNO_3). The Cd concentrations in plant parts of the two cultivars were then compared statistically using ANOVA and least significance difference (*lsd*).

2.2 Examination of tuber Cd uptake pathways

The specific aims of this group of investigations were to determine the origin of the Cd found in potato tubers at harvest and to examine the possible pathways of Cd uptake. Three main uptake routes have been hypothesized; a) xylem transport from the basal roots to the leaves, followed by translocation via the phloem to the tubers, b) uptake from small roots on the stolon with transport directly to the tuber via the xylem, and c) direct uptake across the periderm of the tuber. A pot experiment using cv. Kennebec was designed to distinguish between pathways a) and b). Plants were grown in horizontally split pots, thereby dividing the root system so that the basal roots were separated from the stolons (and, therefore, the stolon roots) and tubers. Soil labelled with radioactive ^{109}Cd was added to either the compartment housing the basal roots or the stolons and tubers. At harvest plants were divided into sections and the ^{109}Cd in each part measured. The existence of pathway c) was determined by applying ^{109}Cd directly to the periderm of intact tubers and measuring any subsequent uptake into the tuber after 7 days.

2.2.1 Split- pot study

The split-pots had two main compartments that allowed separation of the roots from the tubers (Figure 1). Both compartments had a peat-moss/sand mixture (pH 5.58) as the growth medium. The ‘root compartment’ was made from a 27 L plastic container with holes in the base to be free draining. It contained 10 kg (L) of the peat-moss mixture. The ‘tuber compartment’ was formed by placing a 12 L bucket with a 2.5 cm hole in the centre of the base on top of the root compartment. The plant (pre-grown to the one and two leaf stage prior to planting in the lower section of the pot) was threaded through the hole in the base so the pot sat flush with the root compartment. This hole was then sealed so that no leaching could occur from one section of the pot to the other. To do this a sheet of non-toxic latex with a 1 cm hole in the centre was threaded over the plant and placed at the base between the seed tuber and any developing stolons. The top compartment was filled with 2.5 kg of the peat-moss mix. Three weeks after emergence a further 2.5 kg of the mix was used for mounding. The growth conditions set for the plants were a 12-hour day/night cycle with light irradiance of 275 µmol s^{-1} m^{-1}. Day temperature was set at 25°C while the night temperature was 20°C.

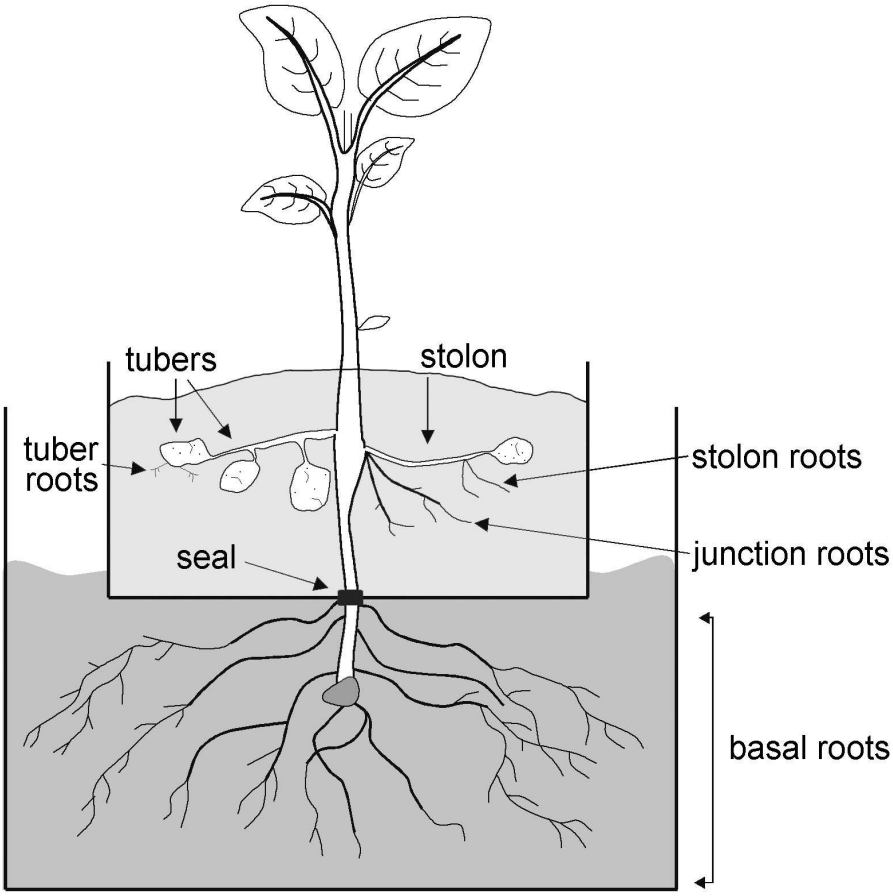

Figure 1: General anatomy of a mature potato plant and the arrangement of compartments in the split-pot experiment (Dunbar 2003).

The treatments imposed during the split-pot experiment were:

Basal Root Zone	labeled soil added to the bottom section of the pot, supplying ^{109}Cd to the basal roots.
Tuber Zone	labeled soil added to the top section of the pot, supplying ^{109}Cd to the stolon and tuber roots.
Whole Root System	labeled soil added to both sections of the pot, supplying ^{109}Cd to all roots.

Isotopic labeling of the growth medium was performed two weeks prior to planting, where 120 kg of the peat-moss/sand mix (the 'soil') was labelled with carrier free ^{109}Cd to provide an activity of 4 kBq kg^{-1}. Labeling was performed by diluting 1.26 µL of ^{109}Cd solution, with a specific activity of 381 kBq µL^{-1}, into 6 L of H_2O, which was then applied to the soil. The radioactive solution was applied in 6 batches, where for each 20 kg of soil was mixed with 1 L of solution in a cement mixer for 5 min. The solution was applied with a spray atomiser to ensure maximum distribution within the soil. The batches of labeled soil were combined in a large container and hand mixed. Random samples were analysed to ensure that the distribution of ^{109}Cd within the soil was uniform.

Plants were harvested 10 weeks after emergence. The new leaves, old leaves and stems were harvested from each plant as described in Section 2.1 above. Tubers were carefully removed from the top section of the pots and the soil removed with a nylon brush, before being rinsed, peeled and dried as before. No tubers were found to be growing in the bottom section of any of the pots. All plant material was dried, ground and digested in hot, concentrated nitric acid (HNO_3). The activity of ^{109}Cd in each digested sample was measured using a gamma counter, and all activities decay corrected.

2.2.2 Direct Cd uptake across the periderm

Another pot trial was conducted to determine whether Cd could be taken up directly by the tuber. Three plants each of four cultivars, Kennebec, Wilwash, Desiree and King Edward, were grown in the unlabelled peat-moss soil mix described above, under the same growth conditions as those described previously. Split-pots were once again used to ensure that tubers would be readily accessible without damaging the main root system. At 10 weeks after emergence one tuber from each plant (a total of three tubers per cultivar) was gently exposed and, while still attached to the stolon, suspended in a beaker containing 1 L of aerated nutrient solution containing 10 nM $^{109}CdCl_2$ for 7 days. A plastic lid was fitted to the top sections of the pots to ensure that the tubers were kept in the dark. The nutrient solution was monitored for depletion of ^{109}Cd at 1 h, 6 h, 12 h, 24 h, and each subsequent 24 h period until the end of the experiment. If the concentration of ^{109}Cd in the solution was found to have decreased by more than 10%, more ^{109}Cd was added.

After 7 days the tubers were removed from the nutrient solution and desorbed in a 1 L solution containing 5 mM $CaCl_2$ and 1 µM $LaCl_3$ for 30 min. Tubers were then removed from the plants, rinsed in distilled water and blotted dry. A 1 cm longitudinal core was taken using a stainless steel cork borer from two tubers of each cultivar. The skin was carefully removed from each end with a scalpel and the remainder of the core was divided into 1 cm sections. Each individual section (including the skin from each end) was accurately weighed and placed in a 6 mL vial for radioactivity analysis on the gamma counter. In order to determine whether any Cd had moved away from the tubers via the stolon, the stolons attached to tubers in the ^{109}Cd solution were cut into 1 cm sections and the their activity measured in the same manner.

2.3 Long distance transport of Cd within potato plants: The role of xylem and phloem

We used ^{109}Cd as an isotopic tracer to examine the role of xylem and phloem in long-distance transport of Cd to the tubers of potatoes. Movement of solutes in the xylem is unidirectional, driven by a water potential gradient, from the root cells, to the xylem sap, to the leaf cells, to the atmosphere. Consequently, any translocation of an isotopic tracer applied to a leaf must take place in the phloem (Marschner 1995). Conversely, movement of an element applied to the soil from the root cells to the leaves will occur in the xylem sap. ^{109}Cd was applied to either the leaves, to monitor phloem movement, or the roots, to monitor xylem movement. When ^{109}Cd was applied to the leaves, plants were harvested at 24, 32 and 54 h after application. When supplied to the roots, plants were harvested 30 and 48 h after application. Two cultivars were utilised and each treatment was replicated three times. Therefore, this experiment examined three factors; cultivar type, site of application and time allowed before harvesting (Table 1).

Table 1: Harvest times (hours after application) for two potato cultivars receiving ^{109}Cd applications to leaves and roots

Application zone	Wilwash	Kennebec
Leaves	24	24
	32	32
	54	54
Roots	30	30
	48	48

Seed tubers of cultivars Wilwash and Kennebec were planted in 15 L free draining pots containing 13 kg of the peat-moss soil described above and were grown under glasshouse conditions. Seedlings were thinned within the first week after emergence to ensure the growth of only one stem per plant. Four weeks after emergence the plants were mounded with a further 2 kg of soil. Application of ^{109}Cd to the plants occurred 64 days after planting, a time when the rate of tuber bulking would be at, or reaching, a maximum. For leaf application, approximately 0.5 mL of a solution containing 2 MBq mL^{-1} of ^{109}Cd was applied directly to the youngest fully expanded leaf (source leaf) using a small paintbrush. The exact amount of ^{109}Cd applied per leaf was determined by mass difference of the solution vessel and the paintbrush before and after application. For root application treatments, ^{109}Cd was applied to the root system of each plant via application to the soil at the base of the pots, where the root activity was expected to be greatest. One L of solution containing 1 MBq of ^{109}Cd was placed in a saucer beneath each pot and allowed to be drawn up into the soil. After 30 min the saucers were removed and carefully rinsed, using 250 mL of distilled water onto the surface of the pot. This ensured that any solution remaining in the saucers was added to the soil.

At the time of harvest, plants that had ^{109}Cd applied to the leaf had the source leaf carefully removed and the plants de-topped by cutting at the base of the stem with a scalpel. The remainder of the above ground material was divided into sections. The new leaves (all leaves above the source leaf) were separated from the main stem, which was then cut into four 20 cm sections, as measured downwards, starting from the point of attachment of the source leaf. Any additional length of stem was incorporated into the 4th section such that the stem sections were 0-20, 20-40, 40-60 or 60-80+ cm from the source leaf. The old leaves attached to each stem section were removed and grouped according to their vertical position on the stem (i.e. leaves 1, 2, 3 or 4). Plants that had ^{109}Cd applied to the roots were divided in the same manner except that the stem sections were measured in 20 cm intervals upwards from the base of the stem. As there was no source leaf, the new leaves were defined as the leaves directly above the youngest fully expanded leaf. The fresh weights of all sections were recorded immediately after harvest.

Roots were harvested by taking ten cores, 2 cm in diameter and 15 cm in length, from random positions within the pot, using a stainless steel cork borer. These cores were combined to make one root sample per pot. Soil was removed from the roots by washing in distilled water. The roots were then desorbed in a solution containing 5 mM $CaCl_2$ and 1 µM $LaCl_3$ for 30 minutes, and rinsed again in distilled water. The remainder of the roots were collected and weighed to obtain a total root mass for each plant.

Tubers were carefully excavated from each pot and the excess soil gently removed with a nylon brush, before being rinsed, peeled and sliced as described in section 2.1. All plant material was dried, ground and digested as described previously. One mL of each plant digest sample was added to 2 mL of distilled water in a 6 mL plastic tube and the activity of ^{109}Cd in the sample was measured using a gamma counter.

2.4 Interactions between Zn and Cd

The specific aim of this experiment was to determine how interactions between Cd and Zn may affect tuber Cd concentrations. Treatments were designed to investigate the short-term effects of differing concentrations of external zinc on the uptake of Cd and, conversely, the effects of differing concentrations of external Cd on the uptake of Zn. This was achieved by supplying plants, grown in nutrient solution, with varying ratios of radiolabeled ^{65}Zn and ^{109}Cd, so that the final treatments were a combination of high and low Cd, and high and low Zn.

Seed tubers of cultivars Wilwash and Kennebec were planted in approximately 4 cm of perlite, misted with distilled water until moist and placed in a growth cabinet where they were left to sprout at room temperature under a 24 h light regime for 3 weeks. Care was taken to ensure that they did not dry out at any time by regular misting with distilled water. At the end of the 3 weeks the seedlings were approximately 10 cm tall. Seedlings of similar size and growth habit were chosen for the experiment and were carefully removed and rinsed in water to remove all traces of perlite from the roots. The remaining seed tuber was also carefully removed. Plants were transplanted into individual 1 L containers containing aerated nutrient solution and allowed to grow for a further 7 days. Containers were covered with aluminium foil to ensure no light entered the nutrient solution. After completion of the 7-day pre-growth period, plants were placed in treatment pots containing 1 L of fresh nutrient solution (which had no Cd or Zn). Each pot was supplied with ^{65}Zn and ^{109}Cd in differing ratios so that the final treatments consisted of 4 combinations of Cd and Zn concentrations (Table 2), with 3 replicates of each treatment. Plants were grown in the treatment solution for 7 days, during which time they were checked daily and the nutrient solution topped up to 1 L with the Cd- and Zn-free solution as required. The low Cd-low Zn treatment solution had concentrations consistent with those used in the previous pot experiment, being 0.01 μM and 1 μM for Cd and Zn respectively. It should be noted that the term 'low' used here does not imply deficient.

Table 2: Cadmium and zinc concentrations in nutrient solutions

Treatment	Cd/Zn Ratio	Cd (μM)	Zn (μM)
low Cd-low Zn	1:100	0.01	1
high Cd-low Zn	10:100	0.1	1
low Cd-high Zn	1:1000	0.01	10
high Cd-high Zn	10:1000	0.1	10

Plants were harvested after 7 days in the treatment solutions. The shoot of each plant was cut above the lid without removing the plant from the pot to ensure no ^{109}Cd had come into contact with the outside of the stem or leaves. The fresh weight of each of the shoots was recorded immediately after harvest. Roots were rinsed in distilled water and then desorbed in a solution containing 5 mM $CaCl_2$ and 1 μM $LaCl_3$ at 5 minute intervals for a total of 30 min. The roots

were rinsed again in distilled water, blotted dry and the fresh weight recorded. All plant material was dried, ground and digested using the methods described previously. One mL of each plant digest sample was added to 2 mL of distilled water in a 6 mL plastic tube and the activity of ^{109}Cd and ^{65}Zn in the sample was measured using a dual channel gamma counter. Means of concentrations were analysed for significant statistical difference ($p<0.05$) using a two sample t-test assuming equal variances.

3. Results

3.1 Glasshouse pot trial

At the time of the first harvest, which was four weeks after planting, much of the final above ground biomass was already in place and root development was well advanced. Stolons were very short and some tubers had started to develop. The main changes in subsequent harvests were increases in stolons and in masses of tubers. Both varieties showed similarities in growth habit and no significant difference was noted between the two in terms of plant growth. This included both the rate of biomass production and the biomass at final harvest (Table 3). There were also no significant differences between total plant Cd concentrations for the two cultivars, however, the Cd concentration in tubers was significantly different ($p < 0.001$) (Table 3). It was found that the pattern of Cd accumulation varied between the two cultivars, with Kennebec showing net increases in tuber Cd concentrations after 8-9 weeks (start of tuber bulking) while no further increases were observed in its other tissues after this time. By contrast, Wilwash accumulated Cd approximately equally between tubers and roots during the same phase of growth (Figure 2). Therefore, due to the growth rates, biomass production and total plant Cd concentrations all being similar between the two cultivars (Table 3), the difference in tuber Cd concentrations must be due to differences in Cd partitioning within the plant, and not to differences in Cd uptake. Examination of the distribution of Cd within the plant confirms this, with Kennebec having 75% of total plant Cd in the tubers, whereas Wilwash had only 43% (Figure 3).

Table 3: Comparison of growth, total plant Cd and concentrations of Cd in tubers and whole plant tissue of potato cultivars Kennebec and Wilwash. Values are the mean ± SE of 4 plants harvested 13 weeks after planting

	Wilwash	Kennebec	Significance level
Total dry weight (g)	156 ± 15	159 ± 10	n.s.*
Total plant Cd (µg)	47.6 ± 4.3	48.9 ± 3.1	n.s.
Plant Cd concentration (µg g^{-1}DW)	0.308 ± 0.024	0.282 ± 0.003	n.s.
Tuber yield (g DW $plant^{-1}$)	143 ± 14	173 ± 10	n.s.
Tuber Cd concentration (µg g^{-1}DW)	0.143 ± 0.13	0.236 ± 0.01	$p < 0.001$

* n.s. indicates no significant difference between the two cultivars.

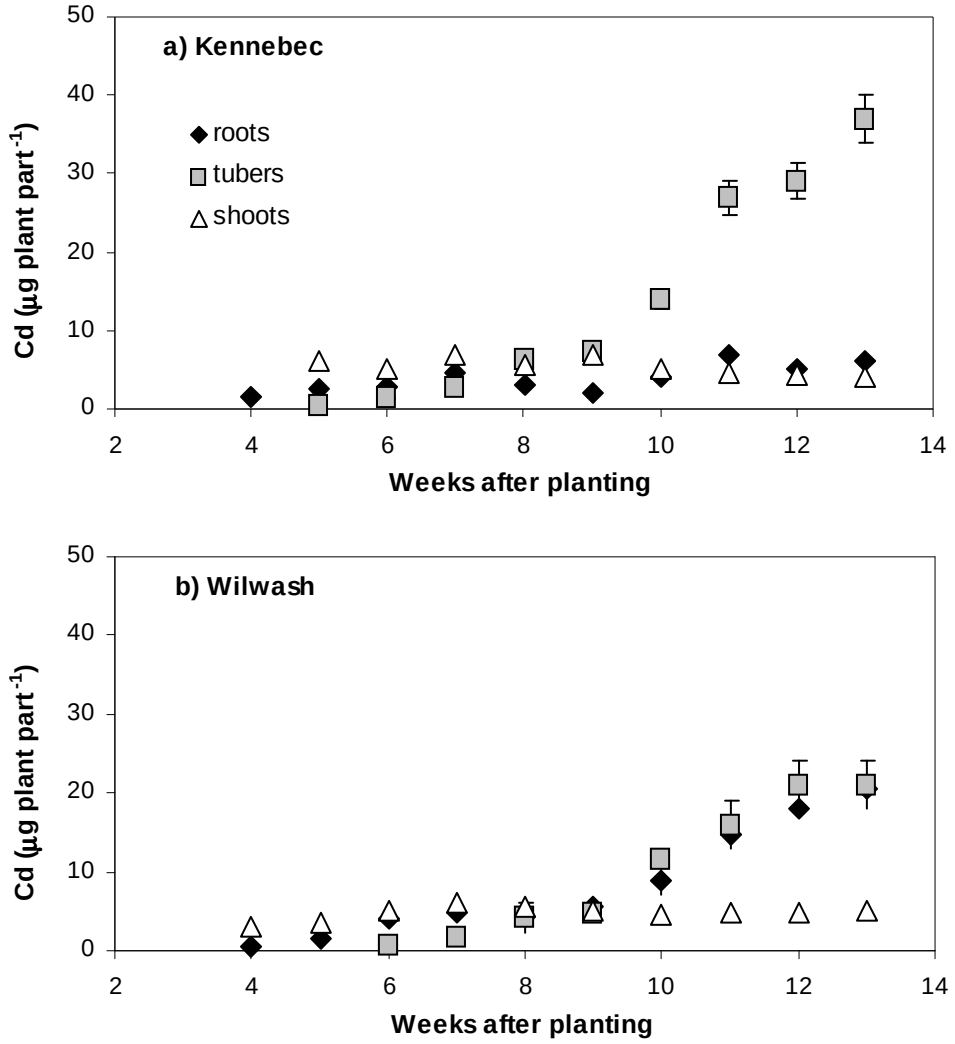

Figure 2: Distribution of Cd during growth of a) Kennebec and b) Wilwash. Each point represents the mean ± standard error of 4 replicates.

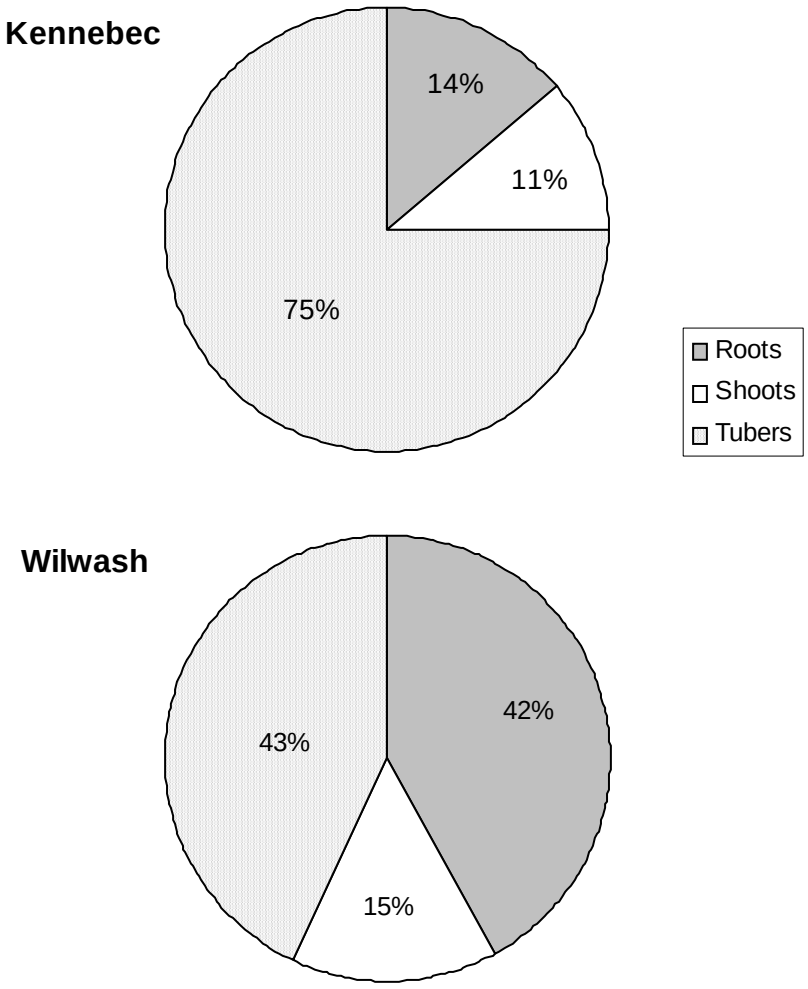

Figure 3: Distribution of Cd in tissues of potato plant cultivars Kennebec and Wilwash after 13 weeks growth. Values represent means of 4 plants.

3.2 Examination of tuber Cd uptake pathways

3.2.1 Split-pot study

The different root systems were found to vary in their role of supplying Cd to different sections of the plant (Figure 4). In each instance the basal roots were found to be the main source of the ^{109}Cd found in the plant tissues at harvest, including the tubers where 85% of the Cd present was supplied via this path. Stolon roots growing in the tuber compartment, although not quantified, were found to be extensive. Uptake of ^{109}Cd from these roots was less than that supplied via the basal roots, however this pathway was still important as it accounted for 15–20% of tuber Cd. By contrast, the importance of stolon roots in supplying Cd to the leaves and stems was minimal, although the presence of a small amount of ^{109}Cd in the leaves after application to the tuber zone does indicate a continuous xylem connection between these sections. No tuber roots were apparent on any of the tubers, thus this potential Cd pathway was not assessable.

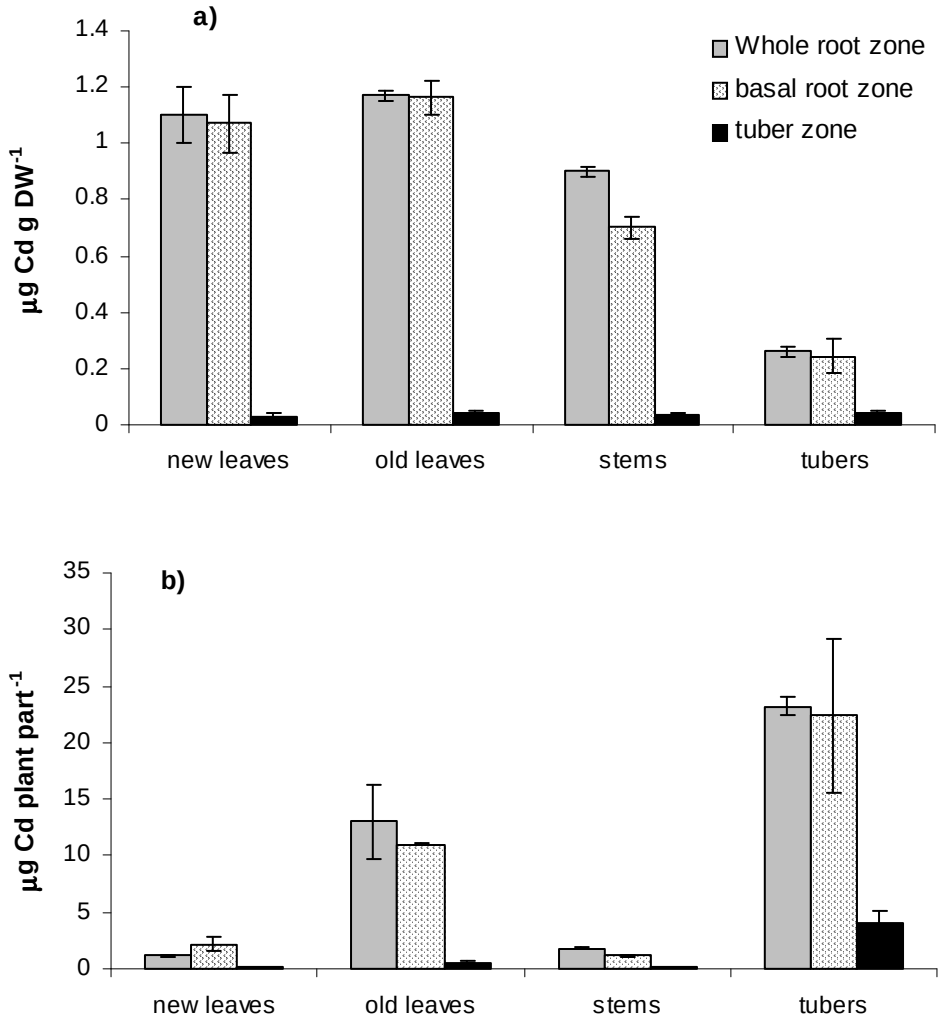

Figure 4: a) Concentrations in potato tissues of ^{109}Cd applied to root and tuber compartments in the split-pot experiment after 10 weeks, and b) total Cd content in the various tissues. Error bars indicate standard errors about the mean for 4 plants (cv. Kennebec).

3.2.2 Direct Cd uptake across the periderm

Depletion of Cd from the nutrient solution was less than 10%, indicating that tubers absorbed only minimal amounts of Cd. However, after 7 days in the nutrient solution ^{109}Cd was found in the periderm of most of the tubers. The highest activity was found in the periderm of cv. Wilwash, while the lowest activity was observed in the periderm of cv. Kennebec (Table 4). The presence of ^{109}Cd was found in the internal tissue of only one tuber, being in the first 1 cm of a tuber core from cv. King Edward. The presence of the radiotracer here was most likely a result of leakage due to deterioration of the periderm in that particular tuber. Therefore, uptake across the periderm is unlikely to be a significant source of tuber Cd. There was no evidence of ^{109}Cd activity in any section of the stolon above where it was attached to the tubers and was not in contact with the radiolabeled solution.

Table 4: Activity of ^{109}Cd (counts per minute) in sections of longitudinal cores of tubers submerged in radio-labelled (^{109}Cd) nutrient solution for 7 days

Section	Kennebec	King Edward	Desiree	Wilwash
Skin	17.6	24.3	11.3*	52.0
1 cm	16.0*	21.6	10.7*	16.3*
2 cm	9.6*	10.3*	10.3*	11.3*
3 cm	11.6*	12.0*	10.5*	16.3*
4 cm	10.9*	9.3*	15.2*	14.9*
5 cm	10.6*	12.4*	10.7*	9.0*
6 cm	9.0*	9.2*	12.4*	10.2*
7 cm	10.0*	13.3*	13.5*	15.3*

* Numbers followed by an asterisk indicate activities not significantly (>50%) above background levels.

3.3 Long distance transport of Cd within potato plants: The role of xylem and phloem

Uptake of soil-applied ^{109}Cd by the roots and subsequent distribution throughout the plant was rapid, with Cd being detected in all parts of the plant after 30 h. After 48 h more than 85% of the total plant-associated Cd was found in non-root tissues (Figure 5). In Kennebec, the majority of the absorbed Cd was present in the stems, followed by the tubers and roots, with smaller amounts being detected in both the young and old leaves. In Wilwash the majority of absorbed Cd was in the tubers, followed by the stems and roots. After 48 h the amount of Cd absorbed by Kennebec was 23.4 ± 1.3 ng per plant compared to only 11.0 ± 0.7 in Wilwash. In terms of Cd concentrations of plant tissues, the highest values were found in the stems, being much higher than in the leaves which in turn were higher than in the tubers (Figure 6 – root concentrations not shown because they were approximately 100 fold those of other tissues and therefore would obscure other values). Although the movement of solutes through the plant will be driven primarily by mass flow, the large differences in concentration means a concentration gradient will exist at the site of loading or unloading into individual tissues. Therefore, the low Cd concentration in tubers (caused by their high proportional mass) may contribute to further Cd accumulation in tuber tissues (i.e. there is a concentration gradient established from roots to stems to leaves to tubers).

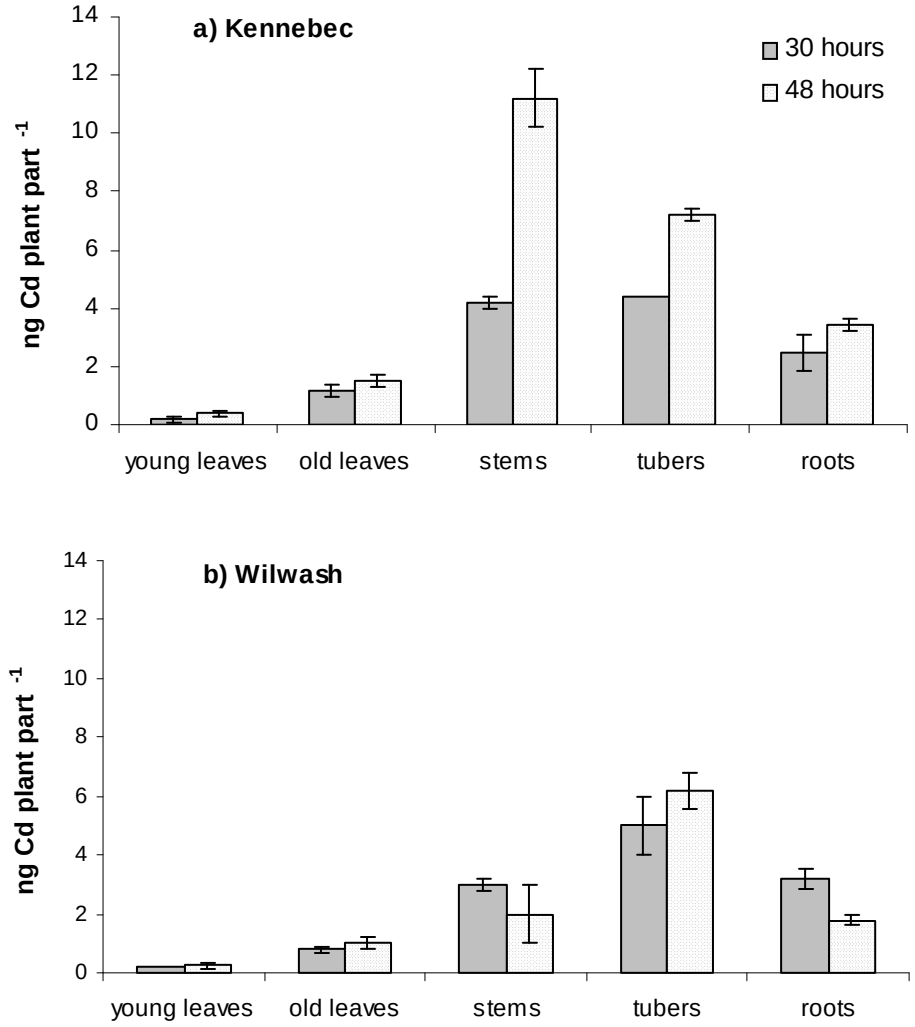

Figure 5: Distribution of ^{109}Cd in tissues of cv. Kennebec and Wilwash grown in soil labeled with ^{109}Cd. Error bars indicate standard errors about the mean (n = 3).

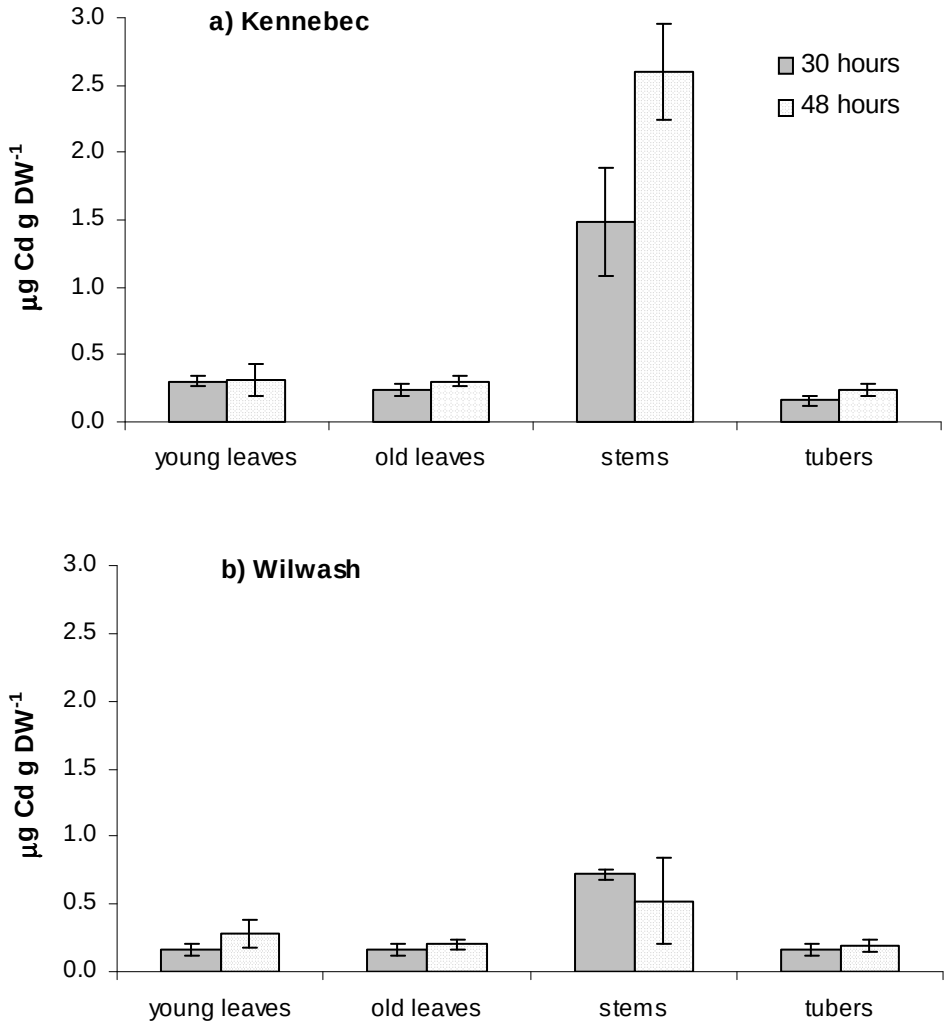

Figure 6: Concentrations of ^{109}Cd in tissues of a) Kennebec and b) Wilwash grown in soil labeled with ^{109}Cd. Error bars indicate standard errors about the mean (n = 3).

The application of ^{109}Cd to the leaves of the potato plants was successful in terms of detectable amounts being absorbed by the leaf tissue and transported throughout the plant. After 24 h ^{109}Cd was found in all sections of the plant, with the total amount absorbed increasing with each harvest time (Figure 7). The great majority of Cd absorbed from the source leaf was present in the tubers, with significant amounts also found in the stems and leaves. Cadmium had also been transported to the roots at each time interval, but this represented only a small fraction of the total amount absorbed. Both cultivars, Wilwash and Kennebec, showed similar foliar uptake. After 54 h no appreciable differences between cultivars were noted in the amounts of Cd absorbed from the source leaf, and the distribution within the plants was broadly similar (Figure 7). Despite the higher overall allocation of foliar-absorbed Cd to the tubers, the highest concentration in terms of mass Cd per unit dry weight was found in the stems of cultivar Kennebec (Figure 8), as occurred when Cd was absorbed from the soil (Figure 6). It is notable that the Wilwash cultivar had a greater concentration of the added Cd in tubers than Kennebec after 54 h (with the 'added Cd'

referring to the labelled ^{109}Cd, which is additional to any unlabelled Cd already present) (Figure 8). The results indicate that Cd transport via the phloem can be rapid, as significant increases in the concentration of added Cd were observed in most plant tissues between 24 and 54 h (Figures 7 – 8). Extrapolation of the change in concentration of added Cd in the tubers of cultivar Wilwash with time suggests that added Cd could have reached the tuber in as little as 8 hours (Figure 9).

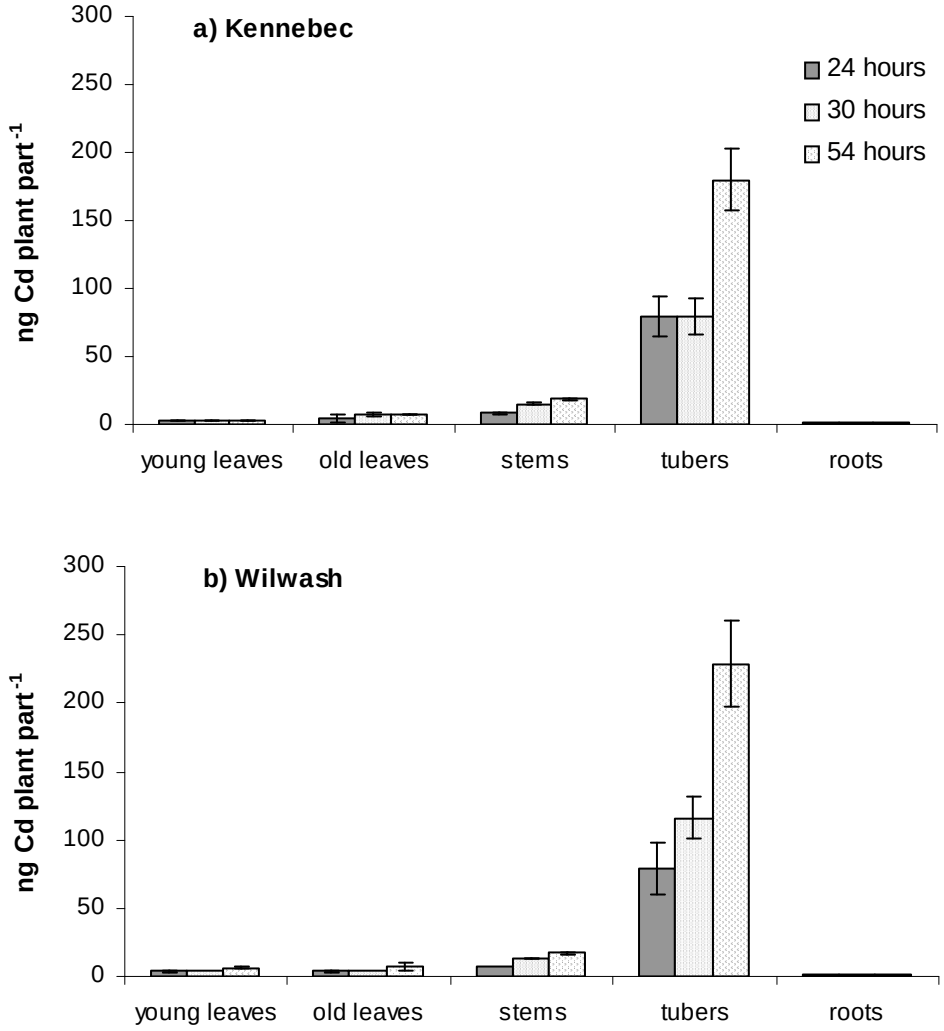

Figure 7: Cd content of various tissues of a) Kennebec and b) Wilwash 24, 30 and 54 hours after application of ^{109}Cd to a single leaf. Error bars indicate standard errors about the mean (n = 4).

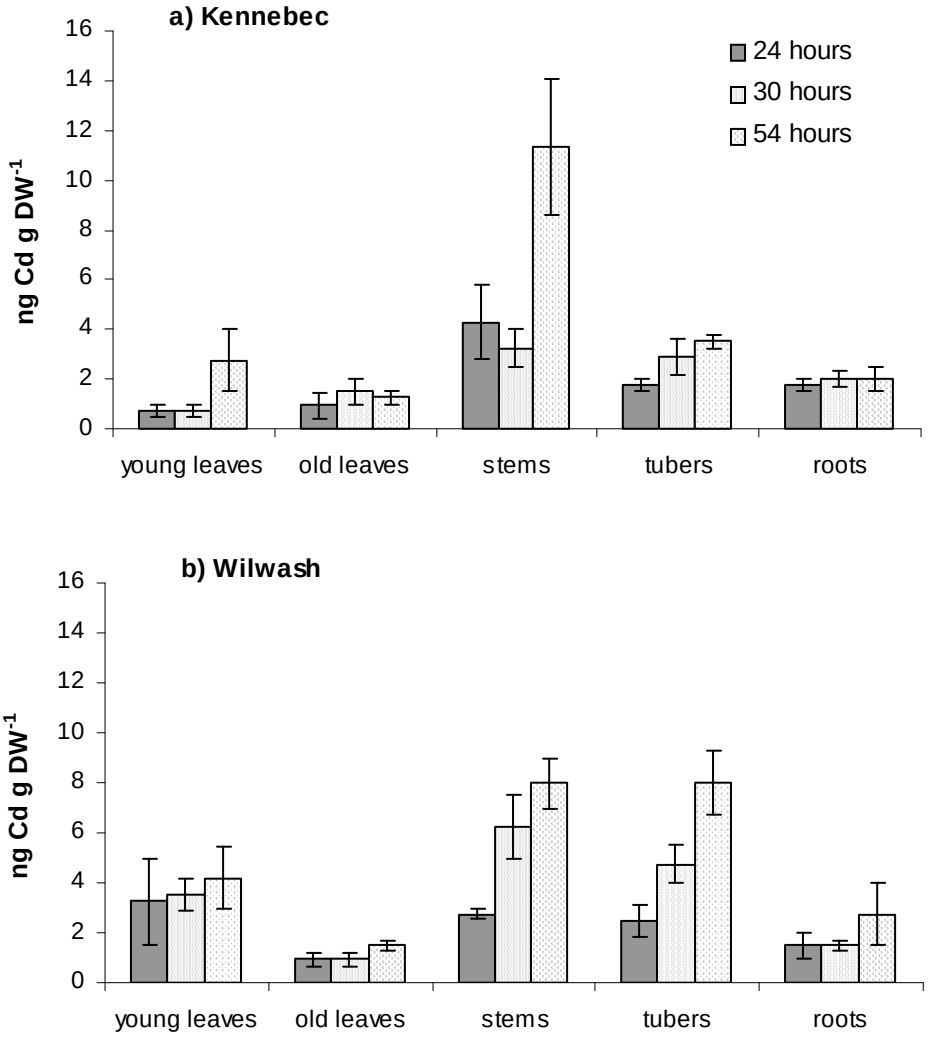

Figure 8: Cd concentration in various tissues of a) Kennebec and b) Wilwash 24, 30 and 54 hours after application of ^{109}Cd to a single leaf. Error bars indicate standard errors about the mean (n = 4).

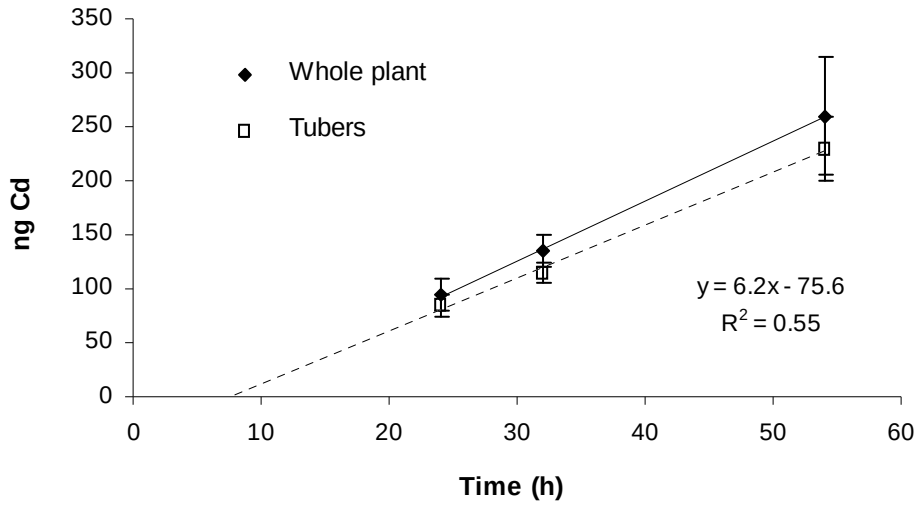

Figure 9: Cd content of tubers and whole plants of cv. Wilwash following application of ^{109}Cd to a single leaf. Data points indicate means of 3 plants ± standard error. The linear regression line for tuber contents was calculated using individual data points, rather than the means displayed here. When extrapolated, the line indicates added Cd may have reached the tubers within 8 hours of foliar application.

3.4 Interactions between Zn and Cd

3.4.1 Separate plant parts (roots and shoots)

Kennebec accumulated higher Cd concentrations in roots than did Wilwash (Figure 10b), with the difference being 2-fold or greater at both low and high solution Cd levels. In terms of root Cd concentration, no evidence for Zn-Cd uptake competition was observed in the case of Wilwash, as Cd concentrations were not significantly affected by solution Zn concentrations (Figure 10a). However, evidence for uptake competition was observed in the case of Kennebec, where, at the higher solution Cd concentration, increased solution Zn concentration resulted in lower root Cd concentrations (Figure 10a).

Despite the greater root Cd concentration in cv. Kennebec, the amount of Cd translocated to shoots was similar between the two cultivars (Figure 10b). No evidence was observed for an effect of Zn on Cd translocation to shoots in the lower solution Cd treatment. However, at the higher solution Cd level elevated Zn concentrations resulted in decreased Cd concentrations in shoots (Figure 10b).

As with Cd, root Zn concentrations were greater in Kennebec than in Wilwash for both high and low Zn treatments (Figure 11a). For Kennebec, at the higher solution Zn level increased Cd concentration resulted in decreased Zn accumulation in the roots (Figure 11a). Together with the results of Cd uptake discussed above, this result indicates that a reciprocal root uptake competition exists between Cd and Zn for this cultivar when both metals are in high concentration in solution (i.e. they are mutually antagonistic). For Wilwash, Cd inhibited Zn uptake into the root only at low solution Zn concentration, whereas at high Zn concentration Cd appeared to enhance Zn accumulation (Figure 11a).

The effect of solution Cd concentration on the translocation of Zn from roots to shoots appeared to be minor, with some inhibition observed in the high Zn treatment for Kennebec (Figure 11b). As was observed for root uptake of Zn, the concentration of Zn in the shoots of Wilwash was only negatively affected by increased Cd in the low Zn treatment (at the higher solution Zn level variations in Cd concentration did not significantly alter shoot Zn, Figure 11b).

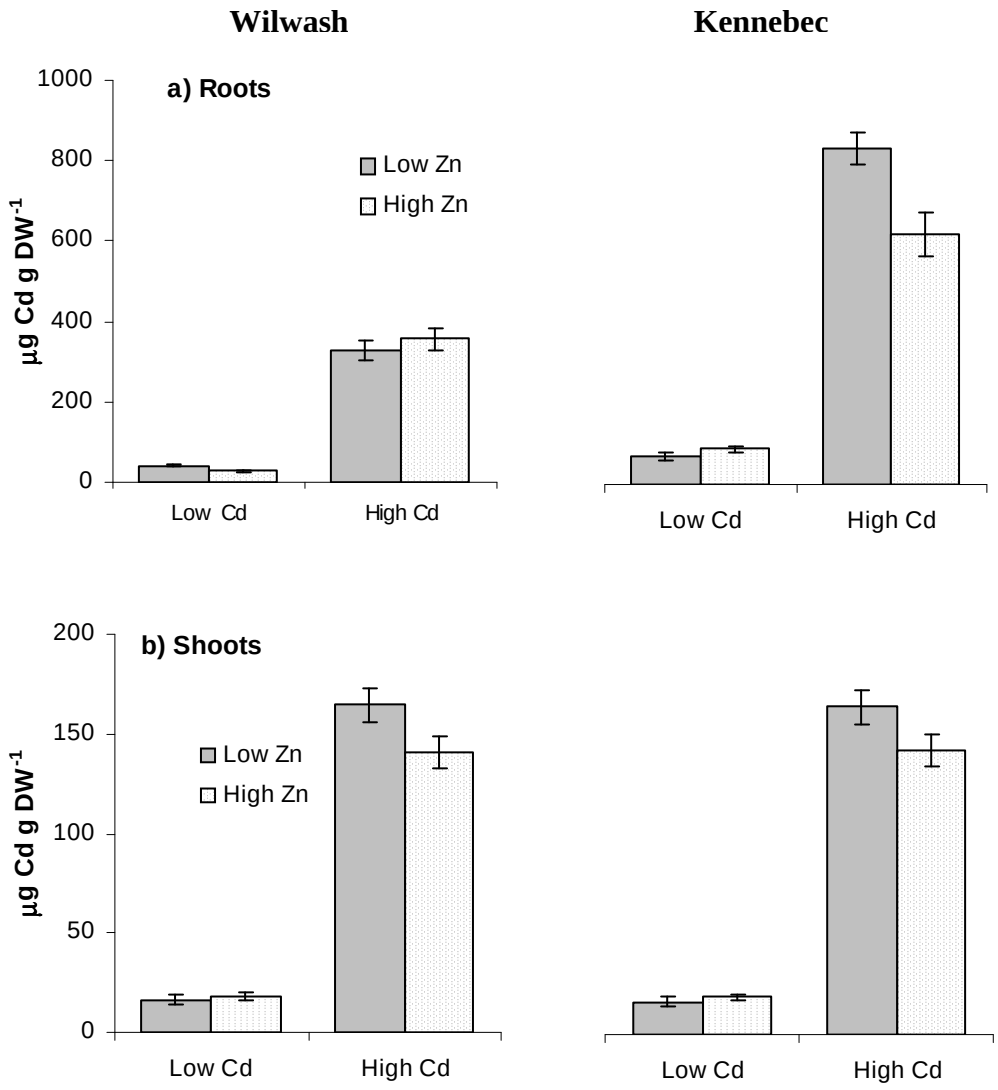

Figure 10: ^{109}Cd concentrations in a) roots and b) shoots of cultivars Wilwash and Kennebec as a function of Zn concentration in nutrient solution (7-day exposure time). Error bars indicate standard error about the mean (n = 3).

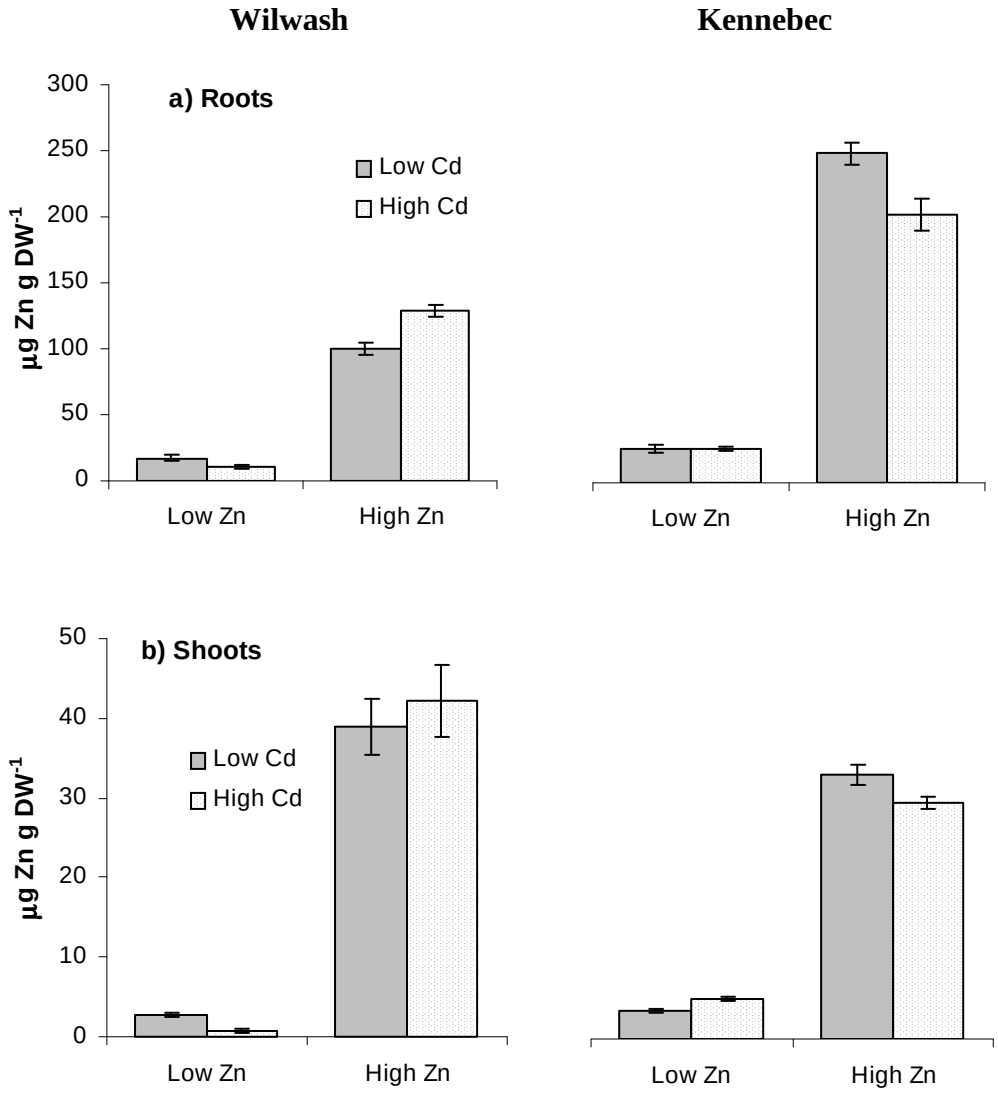

Figure 11: ^{65}Zn concentrations in a) roots and b) shoots of cultivars Wilwash and Kennebec as a function of Cd concentration in nutrient solution (7-day exposure time). Error bars indicate standard error about the mean (n = 3).

3.4.2 Whole plant basis

When whole plant uptake of Cd and Zn was examined on the basis of root dry mass (i.e. total plant uptake per unit mass of root tissue), the interactions between the two metals appeared variable and complex. At low concentrations of external Cd, high Zn resulted in a significant decrease in Cd uptake in Wilwash, however, the same treatment for Kennebec resulted in a significant increase in Cd uptake (Figure 12a). Similarly, when solution Cd concentration was high, significant inhibition of Cd uptake was noted for Kennebec in the high Zn treatment, whereas a significant increase in Cd uptake was observed for Wilwash (Figure 12a). The effects of Cd on Zn uptake essentially mirrored those of Zn on Cd uptake, with the exception that there was no significant affect of Cd concentration on Zn uptake in Wilwash at high solution Zn concentrations (Figure 12b).

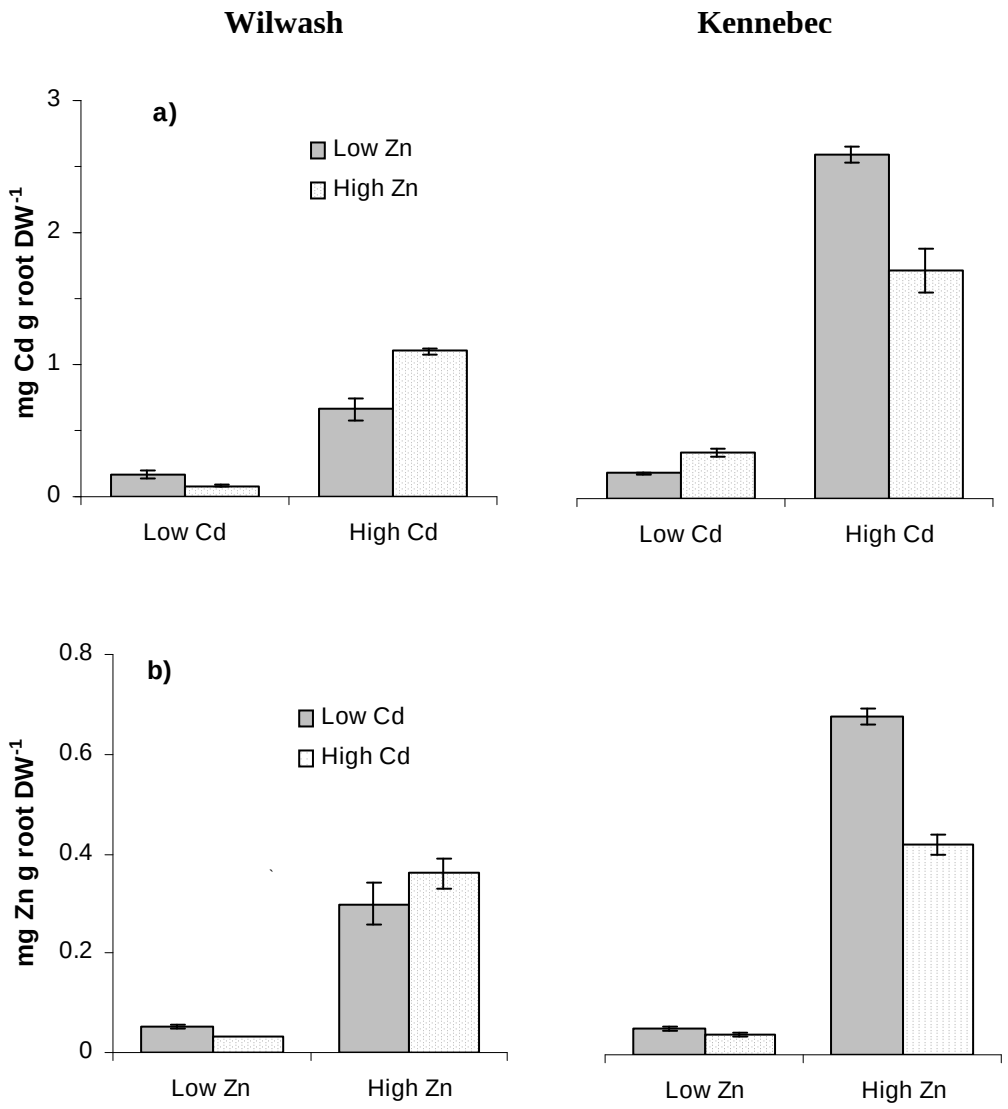

Figure 12: Effects of a) solution Zn concentration on uptake of ^{109}Cd and b) solution Cd concentration on uptake of ^{65}Zn. Uptake is expressed as the total amount of Cd or Zn in the plant divided by the dry root mass. Plants were exposed to nutrient solutions for 7 days. Error bars indicate standard errors about the mean (n = 3).

4. Discussion

The overall aims of this research were to analyse the pattern of Cd accumulation in potato tubers, to examine the pathways of Cd movement through potato plants and to determine the factors that lead to differences in Cd accumulations between different cultivars. The various investigations conducted have successfully addressed these aims.

4.1 Glasshouse pot trial

The glass house trial revealed that differences in tuber Cd concentrations observed between different potato cultivars are due to differences in internal partitioning of Cd and not to differences in total Cd uptake. The results suggest that chemical sequestration occurs in the vegetative tissues of cv. Wilwash, leading to a relatively restricted transport rate in xylem and

phloem vessels which in turn leads to lower concentrations in tubers. This outcome will be of great interest to plant breeders and potato growers, as the information will contribute to the development of potato varieties capable of accumulating less Cd in tubers.

4.2 Examination of tuber Cd uptake pathways

The initial split-pot experiment revealed that the major pathway of Cd movement to tubers occurred through xylem transport from the basal roots to the leaves, followed by translocation via the phloem to the tubers. The alternative process hypothesised, that of direct transfer from roots to tubers in the xylem, was not supported by the data and is therefore considered to be non-significant. Experiments examining possible uptake via the periderm also demonstrated that this uptake pathway does not significantly contribute to tuber Cd concentrations. The benefit of acquiring this knowledge is that future research aimed at restricting Cd accumulation in potato tubers can now be focused on the key transport pathway identified here.

4.3 Long distance transport of Cd within potato plants: The role of xylem and phloem

Examination of long-distance Cd transport mechanisms confirmed the high mobility of Cd in both the xylem and phloem, with added Cd being rapidly assimilated into all tissues following both root and foliar application. These findings are in agreement with those of other authors, who demonstrated phloem transport of leaf-applied Cd in wheat (Welch *et al.* 1999, Cakmak *et al.* 2000) and soil-applied Cd in peanuts (McLaughlin *et al.* 2000). In the short-term experiment conducted here newly absorbed Cd was rapidly sequestered by the stems when applied to either the soil or to the source leaf. This provides valuable information on the movement of added Cd during tuber bulking and may suggest that the stems act as a transitional storage pool when rapid turnover of nutrients and other mineral elements is required for phloem loading into the tuber. Thus the stem appears to be a site for removal of Cd from both xylem and phloem, and so is potentially a major transfer point between the two vascular systems. The research indicates that mechanisms in addition to simple concentration gradients govern the movement of Cd once loaded into xylem and phloem vessels, particularly if it exits and re-enters these vascular tissues at a number of points (i.e. in the stem). Therefore future research into Cd mobility within potato plants should focus on identifying these mechanisms. Similarly, if the stems of plants are indeed acting as an intermediary Cd storage pool, future research should investigate whether structural or physiological differences exist between cultivars which may further explain differences in tuber Cd concentrations.

4.4 Interactions between Zn and Cd

The interactions between Zn and Cd were shown to be complex, with both synergistic and antagonistic effects being observed for plant uptake. The results indicated that, in the case of cv. Wilwash, addition of high levels of Zn would reduce Cd uptake under conditions where the Cd concentration in the growth medium was low. However, with high Cd concentrations in the growth medium the addition of Zn would actually increase the amount of Cd assimilated into plant tissues. By contrast, under high Cd conditions Zn was able to reduce Cd uptake in cv. Kennebec, whereas under low Cd concentrations additions of Zn appeared to stimulate Cd assimilation. The complexity of the interactions between Cd and Zn in potato bear some similarities to those described for lettuce and spinach (McKenna *et al.* 1993) and for wheat (Welch *et al.* 1999), but contrast with the simple patterns of apparent competition for uptake seen in some other studies (e.g. Hart *et al.* 2002). The results suggest the mechanisms for Zn and Cd

absorption into roots are more complicated than a simple shared ion transporter process. Importantly, in terms of potato growing techniques, the results indicate that addition of Zn to soil would not be a wise practice to adopt in attempts to limit tuber Cd concentrations. On the contrary, depending on the prevailing soil conditions and the cultivar being produced, addition of Zn may well lead to increased tuber Cd concentrations.

5. Technology Transfer

The research described in this report formed part of a PhD program conducted by Ms Kelly Dunbar (now Dr. Kelly Dunbar) of the University of Adelaide. The full PhD thesis is available through the university library and through the School of Earth and Environmental Sciences, University of Adelaide. The thesis was entitled "Uptake and Partitioning of Cadmium in Two Cultivars of Potato (*Solanum tuberosum* L.)".

The research reported here has also been published in two articles in internationally refereed journals:

Dunbar KR, McLaughlin MJ, Reid RJ (2003). The uptake and partitioning of cadmium in two cultivars of potato (*Solanum tuberosum* L.). *Journal of Experimental Botany* **54**, 349-354.

Reid RJ, Dunbar KR, McLaughlin MJ (2003). Cadmium loading into potato tubers: the roles of the periderm, xylem and phloem. *Plant Cell and Environment* **26**, 201-206.

In addition, selected parts of this research have been presented at national and international conferences:

Dunbar KR, McLaughlin MJ, Reid RJ (2000). The relationship between zinc and cadmium distribution in two cultivars of potato. In: 'Potatoes 2000: Proceedings of the Australian Potato Research, Development and Technology Transfer Conference, Adelaide, South Australia' (Eds CM Williams, LJ Walters) pp. 221-222.

Dunbar KR, McLaughlin MJ, Reid RJ (1999). The role of basal roots in the supply of cadmium to the tuber of potato (*Solanum tuberosum* L.). In: 'Proceedings of the 5th International Conference on the Biogeochemistry of Trace Elements in the Environment' (Eds WW Wenzel, DC Adriano, B Alloway, HE Doner, C Keller, NW Lepp, M Mench, R Naidu, GM Pierzynski) pp. 510-511. International Society for Trace Element Research, Vienna, Austria.

Dunbar KR, McLaughlin MJ, Reid RJ (1998). Cadmium uptake and distribution in two cultivars of potato (*Solanum tuberosum* L.). In: 'Combined Conference Abstracts of the 42nd Annual ASBMB – 38th Annual ASPP and 20th Annual NZSPP Conferences' pp. 247. Australian Society for Biochemistry and Molecular Biology, Adelaide, South Australia.

6. Recommendations

This study sought to provide insight into the uptake, translocation and partitioning of Cd within the potato plant, with particular focus on cultivar differences in tuber Cd concentration at maturity. The results revealed that differences observed between tuber Cd concentrations of the two cultivars examined were due to differences in internal plant distribution of Cd, rather than to differences in Cd uptake or growth patterns. Therefore recommendations for future research include focusing on the mechanisms by which the low tuber Cd accumulator (cv. Wilwash) distributes Cd within the plant, as this may pinpoint how lower tuber Cd concentrations are maintained in this cultivar (and thus has importance for plant breeding and development of low Cd varieties). The ratio of root Cd to tuber Cd may be a useful screening tool for identifying cultivars with the potential for producing tubers of low Cd concentrations, as this research program found a large ratio indicative of a cultivar that distributes Cd more equally among its tissues rather than directing the majority to the tubers (cv. Wilwash had a ratio of 61, compared to 11 for Kennebec). Research should be conducted to verify the validity of such a screening tool.

This project has also revealed that potato plants are capable of rapidly transporting Cd to various tissues via the vascular system (xylem and phloem vessels), with significant proportions being transferred to tubers and, perhaps temporarily, to plant stems. Therefore, the form of Cd transported via the plant's vascular systems is another avenue of recommended research, as this may lead to methods of blocking Cd deposition in tubers. Similarly, the physiological mechanisms driving Cd transport within the plant need to be investigated, because the current project has indicated that simple concentration gradients alone cannot account for the movements of Cd observed. Such investigations may provide further leads on limiting Cd entry into tubers.

In addition, this research has indicated that Cd-Zn interactions are complex and variable and certainly do not exhibit a simple case of competition for uptake. The research revealed that, depending on the environmental conditions and cultivars involved, interactions between Cd and Zn may lead to either reduced or enhanced uptake of Cd into potato plants. Therefore, future research must also focus on determining the exact mechanisms of Cd uptake by potato roots and how they are affected by Zn, as such knowledge may provide further scope for minimising tuber Cd concentrations through limiting Cd assimilation into the plant at the point of entry.

7. Acknowledgements

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