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

A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy at the University of Adelaide

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November 2004

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Abstract

This thesis presents the results of an investigation into the uptake and distribution of cadmium (Cd) in two cultivars of potato (*Solanum tuberosum* L.) shown to contain different concentrations of Cd in the tuber at maturity.

An initial glasshouse trial sought to determine whether differences in tuber Cd between these two cultivars resulted from differences in uptake from the soil, or were due to differences in the allocation of Cd to the various tissues within the plant. Total uptake of Cd from the soil did not differ between cultivars, nor did the yield of tubers. However, there were marked differences in Cd distribution within the plant. Most of the differences in tuber Cd concentration could be accounted for by a large (3-fold) retention of Cd in the roots of cultivar Wilwash. The concentration of Cd in the shoots of Wilwash was also higher than of Kennebec, although to a lesser extent than the roots.

Further studies were conducted to trace the pathways of Cd uptake and movement within the plant. A split-pot trial, involving long-term growth of potatoes in ^{109}Cd-labelled soil, was undertaken to determine the overall pattern of Cd distribution and the importance of the root system in supplying Cd to the tubers. The root system of the potato plant is different to many plants, in that the main root system (basal roots) is augmented after tuber initiation by roots extending from the stolon and from the tuber itself. The basal roots were found to be the dominant source of Cd to all tissues and accounted for approximately 85% of tuber Cd. The remaining tuber Cd was sourced directly from the stolon and tuber roots. However, there was no evidence of a direct link between the main (basal) root system and the stolons. Although Cd was found to accumulate in the periderm of the tubers, there was no uptake into the tuber tissue itself.

Isotopic studies were undertaken to investigate the short-term movement of newly absorbed Cd in the xylem and the phloem. 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. Newly absorbed Cd was rapidly sequestered by the stems when applied to either the soil or to a source leaf, suggesting that the stems may act as a transitional storage pool when rapid turnover of nutrients and other mineral elements is required during tuber bulking.

Inhibition of Cd uptake by zinc (Zn), has been proposed as a method for reducing the concentration of Cd in various agricultural crops, including potatoes. The ability of Zn to reduce Cd uptake was found to be highly dependent upon cultivar and on the concentration of Cd in the external medium. Although competition between Zn and Cd was found for cultivar Wilwash when the external concentration of Cd was low, when the concentration of Cd in the external media was high, increasing Zn served to increase Cd uptake. Both synergistic and competitive responses were also noted for cultivar Kennebec. However, the patterns of response were opposite to those evident in Wilwash. The complexity of these interactions highlighted the possible shortcomings in using soil applied Zn to limit Cd uptake by potatoes.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to the librarian of the Barr-Smith Library, University of Adelaide, or his/her appointed agent to make this thesis freely available for photocopy or loan.

Kelly R. Dunbar
November 2004

Acknowledgments

First and foremost I would like to acknowledge and thank my supervisors Dr Mike McLaughlin and Dr Rob Reid for offering their support, guidance and wealth of knowledge throughout this project. Even more so, I would like to thank them both for never giving up. Without their unending support and encouragement this thesis would have never been completed.

This research has been undertaken with the support of the Horticultural Research and Development Corporation and is gratefully acknowledged.

I would also like to acknowledge the former Departments of Soil & Water and Environmental Biology, and CSIRO Land and Water. The excellent departmental support including the provision of resources, technical and academic input, and friendship of staff and fellow students made my time as PhD student an immensely rewarding and pain-free experience.

Thank you especially to Debbie, John, Colin, Tracey, Jill and Michelle for your technical and administrative support and for generally making life a little easier.

Finally, thank you to all of my family, friends and loved ones for their encouragement, support and endless nagging, for always being there when I need them, and for helping me get here in the first place.

Chapter 1

Literature Review

1.1 Introduction

Cadmium (Cd) is a heavy metal that is of increasing environmental concern. It occurs naturally in the earth's crust and, as a result, is found in varying concentrations in virtually all components of freshwater, marine and terrestrial ecosystems. Cadmium reaches the environment naturally due to the weathering of rocks and volcanic activity. These processes release otherwise unavailable Cd from the earth's crust into the environment. More recently, however, the Cd input into the environment has increased as a result of mining and agricultural practices.

In the earth's crust, Cd is largely found associated with ores of other metals such as zinc (Zn), lead (Pb) and copper (Cu) (Elinder 1992). The mining and processing of these ores, along with the combustion of fossil fuels, releases Cd into the atmosphere as gases and contaminates natural waters through the disposal of waste water and liquid effluents (Alloway 1995). These sources can result in soil contamination due to atmospheric fallout and waste-water run off. In addition, direct application of Cd-containing phosphatic fertilisers and sewage sludge to agricultural land has significantly increased soil Cd levels (Chang *et al*. 1987). Prolonged application results in accumulation of Cd in the top soil due to the high retention capacity of most soils for heavy metals (McGrath 1987).

The accumulation of Cd in soils has raised concerns for human health due to the possibility of contamination of the food chain. Food ingestion and smoking by humans are believed to lead to accumulation of Cd in the liver and kidneys with the possibility that prolonged

exposure may cause damage to these organs. As a result, many countries, including Australia, have legislated to limit the amount of Cd in foods for human consumption.

Although all plants contain at least trace levels of Cd (Page *et al*. 1981), the accumulation of Cd in soils leads to increased concentrations in plants (Williams and David 1977). Of particular concern, is the accumulation of Cd in food crops such as root and leafy vegetables, as 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 wt (ANZFA 1997). With a significant proportion of Australia's potato crop near or exceeding this limit (McLaughlin *et al*. 1997), attention has been focused on ways to limit Cd accumulation in the tubers. In order to achieve this, it is necessary to understand the mechanisms by which Cd is taken up by, and distributed within, the potato plant.

1.2 Cadmium availability at the root surface

The uptake of nutrients and metals by plant roots is affected by a complex interaction of soil and plant processes. Soil factors such as total soil Cd, pH, soil salinity, soil nutrient status and transport of ions to the root surface affect the availability of ions to the root, and hence their uptake (Chaney and Hornick 1978). Ion uptake is also determined by plant processes, particularly, root architecture, function, biological modification of the rhizosphere and the interaction between the plant and micro-organisms, such as mycorrhiza.

1.2.1 Soil factors

As with all mineral ions, Cd is present in both the solid phase and liquid phase of the soil. In the soil solid phase, Cd is bound to the negatively charged surfaces of silicate clays,

oxides and organic particles. Cadmium can also be present in the solid phase as precipitates of sulphate, carbonate and phosphate, although this is believed important only in highly polluted soils. Cadmium in the soil solid phase is not available for plant uptake and must first be dissolved in the soil solution. The fraction of total soil Cd present in the soil solution is dependent upon many factors with pH considered dominant as it affects the adsorption mechanisms of Cd to soil particles (Alloway 1995). Decreasing soil pH increases the fraction of Cd in the soil solution, increasing the plant available Cd. H^+ ions have a higher attraction to the negatively charged surfaces of soil particles and displace Cd and other positive ions (Alloway 1995). In many studies, soil pH has been found to be negatively correlated with plant Cd concentrations (Andersson and Nilsson 1974, Miller *et al*. 1976, Reddy and Patrick 1977, Chaney and Hornick 1978, Page *et al*. 1981, Eriksson 1989, Jackson and Alloway 1991, Oliver *et al*. 1994, McLaughlin *et al*. 1994). Plants grown in nutrient solution, however, show an opposite response. Competition between H^+ and Cd^{2+} for uptake results in plant Cd concentrations increasing with increasing pH (Tyler and McBride 1982, Florijn and Beusichem 1993).

Alloway *et al*. (1989) found that the total concentration of Cd in the soil was more important in determining concentrations of Cd in four different crops grown on both polluted and non-polluted soils. Significant positive correlations between total soil Cd concentrations and plant Cd concentrations have also been found for lettuce and cabbages (Chumbley and Unwin 1982), wheat grain (Hornberg and Brummer 1986) and several other crop species (Lund *et al*. 1981).

The nutrient status of soils also affects the amount of Cd available to plants. The concentration of other metals such as Zn, Pb, Cu and nickel (Ni) in the soil have been shown to affect Cd uptake by plants (Abdel-Sabour *et al*. 1988, Cataldo *et al*. 1988). Oliver *et al*. (1994) showed that the Cd concentration in grains of wheat grown on Zn-deficient

soils could be reduced by the addition of Zn at planting. The authors suggest that low Zn concentrations in the soil may affect plant membrane integrity allowing increased uptake of Cd. It is also possible that Zn competes with Cd for uptake at the root surface. In addition to competition at the root surface, preferential binding of other metals, such as Pb, to soil surfaces can increase the concentration of Cd in the soil solution and, hence, uptake by the plant, although this has little practical significance in agricultural soils (Adriano 1986). Recently Florijn *et al*. (1992) reported on the effects of the form of nitrogen nutrition on the uptake of Cd in lettuce varieties grown in nutrient solution culture. Cadmium uptake was found to increase when nitrogen was supplied in the form of NH_4 in contrast to a decreased uptake when supplied as NO_3. Further work conducted by Willaert and Verloo (1992) supported these findings for soil grown plants, although the N effect in this study was attributed to the effect of N fertiliser on soil pH.

1.2.2 Plant factors

Plant root processes can alter the availability of both essential and non-essential elements at the root surface, differing between and within species and with environmental conditions (Foster 1978). By the release of root exudates, plants can alter the conditions in the rhizosphere in such a way as to increase nutrient availability. Root exudates can actively influence nutrient availability by acidification of the rhizosphere, chelation of ions, by the release of organic acids and other phytometallophores (Welch 1995), and by alteration of the redox potential by release of reducing agents such as phenolic compounds (Uren and Reisenauer 1988, Mench and Martin 1991). In addition, root exudates can indirectly affect microbial activity in the rhizosphere, rhizosphere physical properties and root growth (Mench and Martin 1991), all of which are important in nutrient availability.

Root exudates are believed to play an important role in the acquisition of heavy metals from the soil. Several studies have shown the ability of exudates to mobilise and/or bind

heavy metals (Mench *et al*. 1985, Merckx *et al*. 1986, Mench *et al*. 1987, Mench *et al*. 1988, Mench and Martin 1991, Rodecap *et al*. 1994). Specifically, exudates of maize (Zea mays L.) roots were found to mobilise Cd (Mench *et al*. 1985, Morel *et al*. 1986, Mench *et al*. 1987). Exudates of two species of tobacco (*Nicotiana* spp. L.) and maize enhanced the solubility of Cd, with the extraction of Cd by the root exudates being similar to the order of Cd bioavailability of the three species when grown on soil (Mench and Martin 1991).

1.3 Cadmium uptake by the root

1.3.1 Pathways of uptake

Once an ion reaches the root surface, it enters the root via the apoplast, comprising the cell wall continuum and intercellular spaces of the root cells. This movement is a non-metabolic, passive process driven by mass flow (Marschner 1995). The apoplastic space provides a site for the accumulation of positively charged ions, particularly divalent or polyvalent cations. The negatively charged carboxyl groups of the apoplast provide sites for cation exchange, increasing the concentration of cations in the vicinity of the active uptake sites of the plasma membrane (Marschner 1995, Kinraide 2001). The uptake of some ions, such as Ca^{2+} and K^{+} have been correlated with the cation exchange capacity (CEC) of the roots (Marschner 1995 after Haynes 1980) but the relationship between Cd uptake by plants and CEC is not clear (Florijn and Van Beusichem 1993). The binding capacity of fern root cell walls was not found to differ in plants grown on contaminated and uncontaminated soil, whereas total uptake of Cd increased in plants from the contaminated site (Nishizono *et al*. 1987). This suggests that the cell wall has a finite capacity for binding Cd. If the apoplastic binding of Cd is a very specific process, as has been suggested for other heavy metals, such as Cu (Marschner 1995), continued or increased exposure to Cd may result in the saturation of available binding sites. This

specificity of apoplastic binding of heavy metals may play an important role in the differential uptake of trace elements (Florijn and Van Beusichem 1993).

The apoplast provides a continuous pathway from the external solution, through the cortex of the root to the endodermis, allowing solutes/ions to reach the boundary of the vascular cylinder (i.e. the xylem and phloem) without having to enter a cell. Once at this boundary the apoplastic pathway is blocked by the presence of a suberised layer of cells in the endodermis, known as the Casparian strip. Thus, any further uptake of the ion or solute by the plant necessitates entry into the symplastic pathway via transport across the plasma membrane of a cortical cell.

In addition to suberisation of the endodermis, some plants have been identified as having another layer of suberised cells in the hypodermis. These 'hypodermal Casparian bands' have been shown to have similar structural and functional characteristics as endodermal Casparian bands (Perumalla *et al* 1990), providing a further barrier to apoplastic movement of water and ions. A histological survey of the roots of 181 angiosperm species identified hypodermal Casparian bands in 89% of species tested, including four species from the family Solanaceae (Perumalla *et al* 1990). The study used uptake of the fluorescent dye Cellufluor to examine roots in a region of mature primary growth, at least 60 mm from the root tip. Although the presence of a hypodermis was not tested for potatoes specifically, the study found suberisation of the hypodermal layer to be a very common feature and strongly correlated to taxa. Suggesting a potential for such a layer to exist in the root cells of potatoes.

The presence or absence of a hypodermal Casparian band in addition to an endodermal Casparian band may not necessarily affect the uptake of some ions. For example, the uptake of Ca has been shown to occur preferentially at the root apex (Harrison-Murray and Clarkson, 1973) where the hypodermal Casparian band has not yet matured (Perumalla *et*

al 1990) and the lack of a tight endodermal barrier allows direct apoplastic movement of Ca into the xylem, from where it is distributed to the rest of the plant (Harrison-Murray and Clarkson, 1973).

1.3.2 Principles of membrane transport

In plants three different mechanisms are recognised for transport across the plasma membrane; i) *simple diffusion*, passive movement down an electrochemical gradient directly through the bi-lipid layer of the plasma membrane; ii) *facilitated diffusion*, passive movement down an electrochemical gradient via a carrier protein or ion channel located in the plasma membrane and; iii) *active transport*, movement against an electrochemical gradient via a carrier protein, requiring the expenditure of metabolic energy (i.e. directly from ATP hydrolysis or indirectly by co-transport with another ion, usually H^+).

Direct diffusion of ions across the plasma membrane is limited due to the non-polar nature of the centre of the phospholipid bilayer. This layer is highly impermeable to charged molecules and large non-charged molecules, with impermeability increasing with both charge and size. Small non polar-molecules, such as O_2, diffuse through the membrane easily, with small polar molecules, such as CO_2 and H_2O, also having a high membrane permeability (Taiz and Zeiger 1990).

Due to the negative membrane electrical potential, there is a strong, inwardly directed driving force propelling divalent cations across the plasma membrane (Clarkson and Lüttge 1989), with the driving force for each individual cation depending on the difference in the free activity of that particular ion between the external solution and the cytoplasm, and the membrane potential difference (Welch and Norvell 1999).

1.3.3 Transport of Cd^{2+}

The mechanism(s) by which Cd is transported across the plasma membrane of higher plants is not known. The few kinetic studies that have been performed tend to show that Cd absorption by roots is bi-phasic, characterised by both saturable and linear components (Cataldo *et al*. 1983, Costa and Morel 1993, Welch and Norvell 1999, Hart *et al*. 1998). It was proposed that the saturable component represented transport through a membrane protein (carrier or ion channel) and that the linear component was due to either cell wall binding of Cd (Hart *et al*. 1998) or to leakage through non-selective cation channels (Welch and Norvell 1999).

There is a vast range of cation channels in plant plasma membranes, many of which allow the permeation of a range of metal cations (Demidchik *et al*. 2002, Véry and Sentenac 2002) and could serve as a leak pathway for Cd, more so at higher concentrations. The only divalent cation whose principal uptake is known to occur via ion channels is Ca (White 2000) which has many physical characteristics similar to Cd, most notably its size (ionic radii: Cd^{2+} - 97 pm, Ca^{2+} - 99 pm). It is possible that Cd^{2+} may enter the cell via a Ca^{2+} channel in place of Ca^{2+}. Indeed, this has been reported to be the case in certain bacteria. Nies (1995) described the uptake of Cd^{2+} into bacteria as 'leakage' through Ca^{2+} or Mg^{2+} transport channels. Uptake of Cd^{2+} in animal cells has also been shown to be via a Ca^{2+} channel (Hinkle *et al*. 1992). However, the importance of this leak pathway at low Cd concentrations (i.e. as in agricultural soils) is questionable. In contrast, the saturating systems described in the kinetic studies have Kms in the approximate range 20–80 nM (Cataldo *et al*. 1983, Costa and Morel 1993, Hart *et al*. 1998) which is realistic in terms of concentrations in uncontaminated soils (Mullins and Sommers 1986, McKenna *et al*. 1993).

The non-essentiality of Cd in the nutrition of higher plants makes it unlikely that a carrier protein specific to Cd^{2+} would exist. It is more likely that transport is opportunistic via another divalent cation carrier, such and Zn^{2+}, Cu^{2+} or Fe^{2+} (Welch and Norvell 1999) and it is therefore not surprising that Cd uptake is inhibited by a range of divalent metals (Cataldo *et al*. 1983, Costa and Morel 1993).

Recent molecular cloning of genes from Arabidopsis has revealed a number of families of high affinity metal transporters in the plasma membrane (for reviews of this topic see Curie *et al*. 2000, Guerinot 2000, Reid and Hayes 2003). The most important are ZIPs, which includes the Fe transporter IRT1, and Nramps. For each there appears to be a preferred substrate and several 'unintended' substrates. For example, several of the Nramp proteins that are upregulated by Fe deficiency are capable of transporting Cd. Over expression of one Nramp protein increased the sensitivity to Cd toxicity (Thomine *et al*. 2000).

An interesting insight into the structural aspects of transporters that influence selectivity was provided by Rogers *et al*. (2000) who manipulated the amino acid sequence of IRT1 in *Arabidopsis* and examined transport characteristics of the mutants in yeast. The wild type unmodified IRT1 transported Fe, Mn and Zn and conferred Cd sensitivity. Single amino acid substitutions generated mutations in which single or multiple transport activities were knocked out, but none of the substitutions generated a mutant capable of Fe transport alone. This work highlights the sensitivity of transporters to subtle changes in protein conformation, and also of the potential for increasing or reducing membrane transport of undesirable metals such as Cd.

The accumulation of Cd to plants is related to its net uptake rather than simply influx. Potentially toxicity could be ameliorated by pumping of Cd from cells. Nothing is known about the mechanism of Cd efflux in plants but it presumably occurs via an active process involving ATP-ases, as is the case with Ca (Sze *et al*. 2000). Clarkson and Lüttge (1989)

point out that efflux of Ca occurs from a cytoplasmic concentration of less than 1 µM and that in yeast at least, the Ca-ATPase has affinities for other divalent metals that are roughly compatible with their differing cytoplasmic concentrations.

1.3.4 Transport of Cd-complexes

The importance of ion pairs and Cd-complexes in the uptake of Cd has been suggested recently (Bingham *et al*. 1983, Bingham *et al*. 1984, Smolders and McLaughlin 1996a, Smolders and McLaughlin 1996b, McLaughlin *et al*. 1997a, McLaughlin *et al*. 1997b). The direct uptake of Cd-chloride complexes has been suggested to explain the increased uptake of Cd in the presence of Cl^- in solution (McLaughlin *et al*. 1997c). At high levels of Cl^- in the soil solution, Cd forms stable complexes of $CdCl_2$ and $CdCl^+$. These complexes have a decreased charge relative to the free ion and may therefore have an increased membrane permeability, allowing some direct diffusion across the plasma membrane. Alternatively, $CdCl^+$ may enter the cell opportunistically, via a monovalent cation transport channel (Welch and Norvell 1999). The transport of Cd into the cell as the ion pair $CdSO_4$ may also occur via simple diffusion, with McLaughlin *et al*. (1997a) reporting no difference in Cd uptake when the activity of Cd^{2+} was decreased and the activity of $CdSO_4$ increased. Direct uptake of Cd in chelated forms with synthetic chelates, such as EDTA, has also been suggested (McLaughlin *et al*. 1997b).

The mechanisms governing uptake of Cd in higher plants are still speculative, with much more research needed in this area. Evidence from animal and bacterial cells suggests the existence of more than one mechanism, with both channel and carrier transport proteins likely to play important roles in Cd uptake (Welch and Norvell 1999).

1.4 Intracellular complexation of cadmium

In the cytoplasm free ionic species, particularly divalent cations, are potentially toxic. Their high reactivity results in the formation of hydroxyl free radicals, powerful oxidants

which are potentially toxic to the plant (Welch 1995). Heavy metal ions, such as Cd also bind to essential sulphydryl groups of enzymes and structural proteins, interfering with the plant's metabolism (Vögeli-Lange and Wagner 1990). In addition, most divalent cations have phosphates of low solubility. Unrestricted accumulation of these species in the cytoplasm would result in precipitation of cytoplasmic P, causing cell death (Clarkson and Lüttge 1989). It is, therefore, necessary for the concentration of divalent cations in the cytoplasm to be tightly controlled and maintained at a tolerable level. Clarkson and Lüttge (1989) outline several processes involved in this regulation:

- Restriction of influx across the plasma membrane
- Rapid sequestration after entry into the cell, eg. into vacuoles or endoplasmic reticulum
- Complex formation or adsorption
- Precipitation
- Strong efflux pumping (against the diffusion gradient)

It is likely that more than one, and possibly all of these processes, are important in regulating the free cytoplasmic Cd activities, as has been shown for Ca (Clarkson and Lüttge 1989).

1.4.1 Sequestration

Studies of Cd accumulation and distribution within plants have shown the vacuole to be a major compartment for the sequestration of Cd and other heavy metals (Salt and Wagner 1993, Vögeli-Lange and Wagner 1990, Krotz *et al*. 1989). Indeed, Salt and Wagner (1993) report the active transport of Cd into oat root vesicles via a Cd^{2+}/H^{+} antiporter. The vacuole contains high concentrations of organic ligands, such as malate, citrate and phytate (Florijn and Van Beusichem 1993). These substances bind to the free Cd ions decreasing the activity of Cd^{2+} in the vacuole, thereby regulating (buffering) both the vacuolar and cytoplasmic Cd^{2+} activities. In addition to organic complexes, Cd can also form complexes

with phosphate, chloride and sulphide in the vacuole. Wagner and Krotz (1989) suggest the formation of these salts to be important in the accumulation of Cd in the leaves of tobacco at low levels.

1.4.2 Complex formation

Most cellular Cd is found in non-ionic forms (Clarkson and Lüttge 1989), and this is considered the major mechanism for heavy metal tolerance. Many forms of low molecular weight metal binding agents in the cytoplasm are believed to play important roles in the maintenance of metal homeostasis in the cytoplasm. Considered most important in Cd tolerance are a group of metal binding polypeptides (poly(γEC)nG), known as phytochelatins (Steffens 1990, Meuwly *et al.* 1995, Rauser 1995). These phytochelatins are cysteine-rich and have a high affinity for binding Cd, due to the presence of sulphydryl groups (Vögeli-Lange and Wagner 1990). The synthesis of phytochelatins is stimulated by the presence of trace elements, including Cd, but can also be inhibited by the presence of ligands, such as EDTA or metal-free phytochelatins possibly due to the operation of a feedback mechanism that regulates phytochelatin synthesis (Grill *et al*. 1987).

Phytochelatins are also believed to play a role in Cd transport to the vacuole. Vögeli-Lange and Wagner (1990) suggest that Cd is transported across the tonoplast as a phytochelatin complex. In their model they suggest that once in the vacuole, the Cd-phytochelatin complex dissociates, due to the more acidic conditions, leaving the Cd to bind with other ligands present, such as malate or oxalate. Indeed, their studies with tobacco have shown the concentration of organic acids in the vacuole of leaf cells to be sufficient to complex Cd. Recently, however Ernst *et al*. (1992, after Florijn and Van Beusichem 1993) have questioned the role of phytochelatins in the transport of Cd and other metals to the vacuole.

In the cytoplasm, free Cd^{2+} activity may be controlled by specific and non-specific binding to proteins as well as to phytometallophores such as nicotianamine, mugineic acid and

avenic acid (Welch and Norvell 1999). Senden *et al*. (1995) also suggest organic acids such as citrate and malate as having a role in the regulation of the cytoplasmic Cd^{2+} activity.

1.4.3 Precipitation

Little is known of the role of precipitation in regard to the control of cytoplasmic Cd^{2+} activity, but at least two studies have reported precipitation of cellular Cd. Sheet-like deposits of Cd have been found in cells of the alga *Lemna minor* (Van Stevenink *et al*. 1990) and Rauser and Ackerley (1987) found globular deposits of Cd in both the cytoplasm and the vacuole of root cells of *Agrostis gigantea*.

1.4.4 Strong efflux pumping

Clarkson and Lüttge (1989) proposed strong efflux pumping as one further process involved in the regulation of ion activity in the cytoplasm. The existence of efflux pumps for many nutrient ions, particularly cations such as Ca^{2+} and Na^{+}, is well established (Marschner 1995). The existence of such efflux pumps for Cd^{2+} in higher plants has been suggested but is only speculative (Costa and Morel 1993). Evidence, however has been found for the presence of a cation efflux system that excludes Cd from the bacteria *Alcaligenes eutrophus* (Nies and Silver 1989).

1.5 Cadmium transport within the plant

Very little is known about the processes regulating the transport of micronutrient and non-essential metals within plants (Welch 1995). This includes xylem sap loading, translocation, unloading, phloem sap loading, re-translocation and phloem unloading and deposition (Welch and Norvell 1999). In addition, little is known about the forms of metal elements in the xylem and phloem saps (Welch 1995).

1.5.1 Xylem transport

The mechanism of Cd transfer into the tracheids and vessels of the xylem is not known with any certainty. Transfer from the cortical cells to the xylem in roots may be a passive process as ions move down an electropotential gradient (Bowling 1981). Even though ions in the xylem are being held against an electrochemical gradient when compared to the external solution (i.e. the root apoplast), the electropotential in the xylem vessels may be still more negative than the cortical cells, resulting in passive loading of the xylem (Taiz and Zeiger 1990). Lüttge and Higinbotham (1979), however, suggest that the final step of xylem loading is an active process. Studies using root segments have shown that protein synthesis inhibitors, such as cycloheximide can inhibit xylem loading without affecting uptake into the cortex (Taiz and Zeiger (1990), indicating more than one process in the regulation of xylem loading.

The driving force for movement of solutes in the xylem sap is mass flow along the transpiration stream but the form in which Cd travels is not clear. Several authors suggest that Cd moves mainly as the free ion Cd^{2+} (Petit and Van de Geijn 1978, Hardiman and Jacoby 1984). Others suggest Cd forms complexes with other compounds, including organic acids, amino acids and phytochelatins (Cataldo *et al*. 1988, Senden *et al*. 1995, Welch 1995). Cataldo *et al*. (1988) found Cd in xylem exudates of rice seedlings associated with the amino acid/peptide fraction, suggesting that Cd may be complexed with these compounds. Cadmium transport within the xylem has also been associated with citric acid. After addition of citric acid to the roots of tomatoes, Senden *et al*. (1995) reported Cd uptake by the root to double, whereas transport of Cd to the shoot was increased by a factor of six.

The form in which Cd is transported in the xylem sap of plants needs to be ascertained as this plays an important role in the rate of Cd transport to the shoots (Hagemeyer *et al*. 1986).

1.5.2 Phloem transport

Even less is know about the processes of phloem loading, unloading and transport of micronutrients and non-essential trace elements than is known for xylem (Welch 1995). There have been no direct studies on the forms and mechanisms of loading and unloading of Cd in the phloem. Information about these processes is only speculative, based on the known properties of the phloem, such as pH and E_H (standard redox potential) and the transport of other metals such as Zn.

The pH of the phloem is much higher than the xylem, being typically >8 (Welch 1995). As a result, the free ionic activity of all cationic metals in the phloem would be virtually nil, instead they would be complexed with other compounds (Welch 1995). The high levels of phosphate in the phloem, typically 14 mM, can also present a problem at such a high pH, as metals would be likely to form precipitates such as oxides, hydroxides and phosphates (Welch 1995). For these elements to remain mobile in the phloem sap, compounds with a high affinity for binding metals such as Cd and Zn, must be present. Such compounds are likely to include phytochelatins, phytometallophores, cysteine and other sulphydryl-containing molecules (Welch and Norvell 1999). The phytometallophore, nicotianamine has been found to be essential in the phloem transport of Fe in tomato (Scholz 1989) and Taylor *et al*. (1988) suggest that complexes similar to phytochelatins are important in the phloem mobility of Zn in blight-affected citrus trees. It is possible that these complexes may play an important role in the transport of Cd in the phloem.

1.6 Physiology of the potato plant

1.6.1 Tuber structure and development

Potato tubers form as a result of tuberisation of the stolon tip, with the stolons being lateral shoots arising from the basal nodes of the stem below soil level (Cutter 1992). The stolon tip is hooked and tuberisation occurs just below this hook as a result of cell division and/or cell enlargement, although the precise mechanism is under debate. After stolon initiation, environmental factors, such as photoperiod, temperature and the presence of nutrients, play an important role in the further growth of the stolon and tuber (Cutter 1992). The interaction of these processes is such that factors which promote the growth of stolons, tend to suppress the formation of tubers. High temperatures and a long photoperiod (i.e. long days and short nights) act to increase stolon length and inhibit tuber formation (Cutter 1992). Conversely, tuber initiation occurs earlier when temperatures are lower and day length shorter (Cutter 1992). The production of particular plant hormones in response to environmental conditions dictates the course of stolon and tuber development. Plant hormones, such as cytokinins (CYT), gibberellins (GA) and abscisic acid (ABA) are produced in the leaves and translocated to the stolon tip (Burton *et al*. 1992). Generally CYT and GA enhance growth and developmental processes. Gibberellic acid is produced in response to long days (Taiz and Zeiger 1990) and high temperature and high GA levels in the plant promote stolon elongation and suppress tuber initiation (Krauss and Marschner 1976, Burton *et al*. 1992). In potato development, ABA is believed to be an antagonist of CYT and GA. Application of ABA to the stolon tip has been shown to promote tuber development under otherwise unfavourable conditions of long days and high temperature (El-Antably *et al*. 1967, Krauss and Marschner 1976). Whereas CYT specifically retards leaf senescence, ABA favours abscission of the leaves (Marschner 1995). Short day conditions appear to be favourable to the accumulation of ABA (Krauss 1985).

The level and continuity of nitrogen supplied to the potato plant also affects tuber development when combined with a particular photoperiod. Krauss and Marschner (1976) found that when plants were grown with a 12 h day length and supplied continuously with nitrogen, tuber development was completely inhibited. However, when the photoperiod was reduced to 8 h of daylight, tuber initiation was only delayed for 13 days. In addition, tuber development during long photoperiod could be promoted by discontinuing the nitrogen supply. Foliar application of nitrogen did not inhibit or delay tuberisation (Sattelmacher and Marschner 1979). Again, the response of the plant to nitrogen nutrition is related to the ratio of GA:ABA within the plant (Cutter 1992).

These responses highlight the importance of suitable environmental conditions and nutrient status, particularly when growing plants under glasshouse conditions, as unfavourable conditions could result in alterations to the normal growing cycle of the plants. Conversely, application of phytohormones could be used to tool to stimulate or inhibit the growth and development of tubers.

1.6.2 Root function and tuber nutrition

The potato has a highly branched, fibrous root system (Cutter 1992) which is made up of four different types of roots, as described by Kratzke and Palta (1985). The bulk of the root system is comprised of ‘basal’ roots which originate from the base of the stem. ‘Stem-stolon junction’ roots arise at the junction between stolons and the main stem, ‘stolon’ roots arise directly from nodes on the stolon and ‘tuber’ roots originate from the base of buds on the tuber. The characterisation of these different types of roots has led to speculation about the role of these roots in the mineral nutrition of the plant and tubers. As a result, many studies have investigated the function of the different parts of the root system. Kratzke and Palta (1985) used water soluble dyes to trace the pathway of water through Russet Burbank potatoes. It was found that both basal and junction roots supplied

water primarily to the aerial parts of the plant and were of little importance in the water supply to the tuber, even though evidence of a xylem connection between the junction roots and the stolon was found. Previous studies have shown that water transport to the tuber occurs at night due to the reduction in transpirative demand of the leaves (Baker and Moorby 1969, Gandar and Tanner 1976). However, even after a 24 hour period, Kratzke and Palta (1985) found no evidence for the direct transport of water from the basal or junction roots to the tuber. Stolon and tuber roots, however, were shown to provide water directly to the tuber, with dye being found in the xylem ring of the tuber two hours after application to these roots. The stolon roots also provided a path for water movement to the aerial parts of the plant.

Much attention has been focused on the role of different roots in the supply of nutrients, particularly Ca, to the tuber. Phloem-mobile nutrients, such as N, P, S and Cl, are taken up by the basal and junction roots and transported to the shoots and leaves via xylem flow. These nutrients are then transported, along with sugar, to the tuber via the phloem (Pan *et al*. 1990). Calcium has a low mobility in the phloem and is believed to travel exclusively in the xylem (Marschner 1995). The low levels of Ca in the tuber compared to the above ground tissues supports this assumption, as the concentrations of phloem-mobile nutrients, such as K and Mg, in the tuber are similar to those in the leaves (Nelson *et al*. 1990). As the basal and junction roots provide little or no water transport to the tuber it is likely that tuber Ca does not arise from these roots, but directly from stolon and junction roots. Split-pot experiments have been utilised in several studies to examine the source of Ca and other nutrients in the tuber (eg. Kratzke and Palta, Nelson *et al*. 1989, 1986, Pan *et al*. 1990). By physically separating the basal roots from the tuber and stolon regions Ca could be applied to the different root systems and its accumulation in the tuber measured. Application of Ca to the tuber region has been shown to result in a three-fold increase in tuber Ca

concentration, whereas addition to the basal roots had no effect on tuber Ca concentration (Kratzke and Palta 1986). In addition to uptake through stolon and tuber roots, early studies have shown that tubers have the ability to absorb Ca directly from the soil via transport across the periderm (Krauss and Marschner 1971, Davies and Millard 1985). The precise transport pathways to the tuber of nutrients with intermediate phloem mobility is unclear. Manganese, for example, is found in high concentrations in both the xylem and the phloem of the stolon, possibly a result of xylem to phloem transfer at several stages of transport (Nelson *et al*. 1990).

1.6.3 Cadmium accumulation by potatoes

Of the non-essential elements, Cd has received the most attention in terms of accumulation in potato tubers, due to their high contribution to the dietary intake of Cd. Most studies have focused on the external soil factors affecting Cd availability to potatoes. Soil factors such as pH (Sparrow *et al*. 1992, McLaughlin *et al*. 1994b), salinity, extractable-zinc concentration and soil type (McLaughlin *et al*. 1994a, McLaughlin *et al*. 1994b) have been found to affect the accumulation of Cd in tubers, as has fertiliser type (Sparrow *et al*. 1994) and irrigation water quality (McLaughlin *et al*. 1995). These factors act together to alter the amount of Cd available to the plant, such that the effects of one factor may enhance or override the effects of another. For example, Sparrow *et al*. (1994) found that tuber Cd concentration could be reduced by fertilisation with K_2SO_4 instead of KCl, whereas McLaughlin *et al*.(1995) reported that tuber Cd concentration was not affected by the chemical form of potassic fertiliser, due to the dominating effect of the irrigation water quality and the residual levels of Cd in the soil. The effects of salinity have also been shown to override the effects of pH, with high Cd concentrations found in tubers grown on neutral or alkaline soils (McLaughlin *et al*. 1994a).

Of all the factors found to affect tuber Cd concentration, the concentration of Cl in the soil appears to be dominant. Whether a result of planting on saline soils, irrigation with saline water or fertiliser type (in the absence of the former two factors), high levels of Cl in the soil result in increased tuber Cd concentrations (Sparrow *et al*. 1994, McLaughlin *et al*. 1994a, McLaughlin *et al*. 1995, McLaughlin 1997). Chloride reduces the sorption of Cd to soil particles and organic matter (O'Connor *et al*. 1984, Boekhold *et al*. 1994) by forming chloro-complexes, thereby increasing the total Cd in the soil solution (Garcia-Miragaya and Page 1976). McLaughlin *et al*. (1997) postulated that the formation of these chloro-complexes increases Cd uptake by either increasing the diffusion of Cd to the roots, or by the uptake of the chloro-complexes across the plasma membrane. The possibility that chloro-complexes may be taken up by the plant is supported by the finding that tuber Cd concentrations are related to the level of chloro-complexation, and not the activity of Cd^{2+}, in the soil solution (McLaughlin 1997).

As with many other plants, potato cultivars have been shown to differ in their ability to accumulate Cd, although the differences between cultivars are not consistent. Harris *et al*. (1981) found no differences in the tuber Cd concentration of six cultivars grown. McLaughlin *et al*. (1997), however, found that cultivars grown commercially in Australia exhibited significant differences in tuber Cd concentration, with a reduction of up to 50% between some cultivars. It must be noted, however, that differences between cultivars were affected by soil and environmental conditions.

1.7 Conclusions

Much of the research to date has focused on the soil and environmental factors affecting the accumulation of Cd in potatoes. As a result, various agronomic options have been suggested to reduce Cd concentrations in potato tubers, including improving irrigation

water quality, avoiding saline and acidic soil, reducing the level of Cd in fertilisers, and alleviating zinc deficiency.

However, the mechanisms controlling cellular uptake and the subsequent transport and allocation of Cd within the potato plant have been overlooked. Further effective strategies for reducing tuber Cd concentration may be developed through the understanding of these processes, particularly in respect to breeding and developing potato cultivars which accumulate low levels of Cd in the tuber.

It was the main purpose of this project to describe the basic features of Cd uptake and translocation, and to uncover the reasons behind genotypic differences in Cd accumulation in tubers.

Chapter 2

Plant Uptake and Partitioning

2.1 Introduction

Plants readily absorb Cd from the soil, but the extent of uptake and the distribution of Cd within plant tissues has been shown to vary between both species and cultivar. Differences in Cd accumulation between species have been widely reported. Bingham *et al*. (1975) compared the Cd concentration in the plant tissue of 22 different plant species grown on soil amended with Cd-enriched sewage sludge. The concentration of Cd ranged from less than 1 mg Cd kg^{-1} in paddy and upland rice, to 161 mg Cd kg^{-1} in spinach and turnip. Jarvis *et al*. (1976) showed similar differences in the Cd concentration of 23 species grown in flowing nutrient solution containing 9 nM $CdCl_2$.

Cultivar and genotype have also been shown to influence the concentration of Cd accumulated by the plant (Reed *et al*. 1999). Differences in Cd uptake between cultivar have been reported for many species including maize and lettuce (Florijn *et al*. 1993, Florijn and Van Beusichem 1993), wheat (Cieslinski *et al*. 1996a), strawberry (Cieslinski *et al*. 1996b), carrot (Harrison 1986) and sunflower (Li *et al*. 1994). Some of these cultivar differences can be explained in terms of differences in distribution within the plant. Florijn *et al*. (1993) compared the uptake and distribution of Cd within six inbred lines of maize and found that distribution, rather than uptake, was the factor most affecting genotypic differences in shoot Cd concentrations. Data from Reed *et al*. (1999) suggest that both total uptake and partitioning are important in explaining differences in the tissue Cd concentration of different cultivars of switchgrass.

Although different cultivars of field grown potatoes have been shown to accumulate different concentrations of Cd within the tubers (McLaughlin *et al*. 1994), it is not known how differences in tuber Cd arise, whether it is due to increased uptake form the soil, or to differences in partitioning of total Cd within the plant. Describing mechanistic differences between cultivars is important in terms of identifying whether uptake of Cd by the roots or translocation from the leaves is the most important factor in limiting tuber Cd concentrations.

To investigate these differences a glasshouse pot trial was undertake so that environmental conditions could be controlled. Although previous studies have shown differences between potato cultivars, variation in location of field trials has resulted in cultivar responses being confounded by soil and environmental conditions.

2.2 Objectives

The objective of this pot trial was to examine the distribution of Cd in two cultivars of potato during growth, with a focus on determining whether any differences in tuber Cd concentration between the two cultivars could be related to total uptake of Cd from the soil or partitioning of Cd within the plant.

2.3 Methods

2.3.1 Plant species

In previous field trials (McLaughlin *et al*. 1997) cultivars of field-grown potatoes were found to differ significantly in the concentration of Cd found in the tuber at harvest. Cultivars were categorised as high, medium or low accumulators based on the tuber Cd concentration (Table 2.1). Two cultivars, cv. Wilwash and cv. Kennebec, were chosen for the glasshouse pot trial based on this categorisation.

Cultivar Kennebec was found to accumulate a high concentration of Cd in the tuber, with field trials showing a mean concentration of 48 $\mu g\ kg^{-1}$ fresh weight. In contrast, cv.

Wilwash was classified as a low accumulator, having an average tuber Cd concentration of 34 µg kg^{-1} fresh weight.

Russet Burbank was disregarded for representation of a low accumulator in this trial, due to it being a late maturing variety. Both Kennebec and Wilwash are main season in maturity and were expected to have similar growth rates and habit.

Table 2.1 Tuber Cd concentration for different field grown potato cultivars. Modified from McLaughlin *et al*. (1994)

Tuber Cd Group	Cultivar	No. of Sites[A]	Tuber Cd (µg kg^{-1} FW)	
			Mean	s.d.
High	Toolangi Delight	4	50	2
(>45)	Kennebec	6	48	2
	Crystal	4	46	2
Medium	Wilcrisp	5	41	2
(35–45)	Sebago	6	40	2
	Nooksack	5	37	2
	Winlock	7	37	2
	Pontiac	8	36	2
	Atlantic	8	36	2
	Desiree	5	35	2
Low	Wilwash	4	34	5
(20–35)	Russet Burbank	4	34	2
	Lehmi Russet	4	29	4

[A]Number of sites at which each cultivar was present

2.3.2 Plant growth

Plants of cultivars Wilwash and Kennebec were grown in a glasshouse in free draining pots of sand and watered daily with one litre of nutrient solution. Pots were arranged in a randomised block design with four replicates per treatment. All plants were grown in 305 mm black plastic, free draining pots (Figure 2.1). A layer of black fibreglass flyscreen mesh was placed in the bottom of each pot to prevent any loss of sand from the system. This was followed by a layer of black plastic beads, to reduce the likelihood of algal

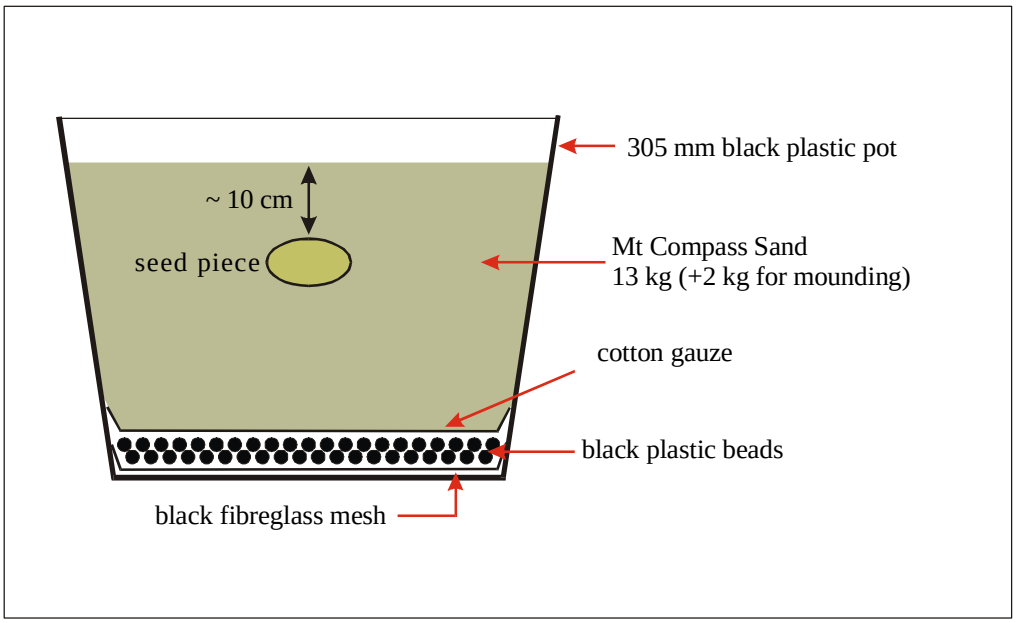
growth at the draining points at the base of the pots, and a layer of cotton gauze to separate the beads from the sand. Each pot was filled with 13 kg of washed Mt Compass sand (Table 2.2) and topped (after planting) with another layer of black plastic beads, again for the prevention of algal growth.

Pre-sprouted seed tubers were planted 10 cm below the surface of the sand, after first being weighed and inspected for deterioration of the seed piece. Each plant was 'mounded' with an extra 2 kg of sand four weeks after emergence. This was simply a process of adding extra sand around the base of the stem to ensure that developing tubers do not break through the surface.

Table 2.2 Chemical composition and physical characteristics of Mt Compass sand.

Nutrient	Conc. (mg kg^{-1})		
Nitrate N	2	Texture	1
Ammonium N	1	Colour	Grey-white
Phosphorus (Colwell)	2	pH ($CaCl_2$)	5.2
Potassium (Colwell)	8	pH (water)	6.1
Sulphur	1.1	Conductivity	0.007 dS m^{-1}
Copper DTPA	0.07	Organic Carbon	0.23%
Zinc DTPA	0.07		
Manganese DTPA	0.73		
Iron DTPA	5.51		
Reactive iron	26		
Boron (hot water)	0.3		

(a)



(b)

Figure 2.1 (a) Design of pots for growth experiment and (b) glasshouse layout.

2.3.3 Nutrient solution parameters

Plants were watered with nutrient solution. From planting to emergence the soil was kept just damp to prevent the seed piece from rotting. During the first two weeks after emergence one litre of nutrient solution was applied every second day. For the remainder of the experiment plants were watered daily with one litre of nutrient solution. The nutrient solution was modified from Ulrich and Fong (1970) and its composition is given in Table 2.3. Cadmium was added to the solution 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). Immediately prior to watering the pH of the solution was measured and adjusted to 5.5 with NaOH. GEOCHEM analysis of the nutrient solution indicated the free Cd^{2+} activity to be 4.72 nM, representing 78.2% of the total Cd in solution.

Table 2.3 Nutrient solution composition.

Macronutrients	Concentration (mM)	Micronutrients	Concentration (µM)
KNO_3	3.0	H_3BO_3	20.0
$MgSO_4.7H_2O$	1.0	$MnSO_4.H_2O$	20.0
NaCl	0.5	$ZnSO_4.7H_2O$	10.0
$Ca(NO_3)_2.4H_2O$	2.5	$CuSO_4.2H_2O$	1.0
K_2SO_4	0.5	$Na_2MoO_4.2H_2O$	0.5
KH_2PO_4	0.5	$FeCl_3$	25.0
		$CdCl_2$	10 nM
MES	1 mM	DTPA	25 µM

2.3.4 Plant harvest

Plants were harvested once per week for a total of ten weeks, with the first harvest occurring 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. Immediately after harvest the fresh weight of all plant material was recorded.

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, blotted dry and the fresh weight 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.

Although the tuber skins were not analysed for Cd concentration, it is assumed that they would only represent a very small amount of the total Cd in the whole plant. If it is assumed that the skin comprises 10 g of a 260 g tuber (fresh weight) and it has double the Cd concentration of the tuber flesh, then the skin would only comprises 7% of the total Cd in the tuber and a much smaller percentage of the total Cd in the whole plant. In addition, analysis of the skins could potentially lead to an over estimation of tuber Cd concentration due to likely adsorption of Cd to the skin from the external growing medium.

2.3.5 Plant analysis

Prior to chemical analysis all plant material was dried, ground and digested using the following procedure. Plants were oven dried at 75°C for two days in pre-weighed paper bags. Dry weight was recorded before all material was ground to less than 500 µm in a stainless steel mill. Approximately 0.5 g of each ground sample was accurately measured and placed in 100 ml borosilicate digestion tubes. Five ml of Mallinckrodt concentrated nitric acid (HNO_3) was added to each tube. Tubes were placed in a digestion block and

digested until the solution was clear and the volume in the tube was reduced to 1 ml. Table 2.4 shows the individual temperature steps for the acid digestion. The solution was allowed to cool, then made up to a volume of 20 ml with 0.016 M HNO_3, stirred with a vortex mixer and filtered through Whatman® 41 ashless filter papers. Digested samples were refrigerated at 4°C in 70 ml plastic containers prior to analysis.

The Cd concentration in each plant component was determined by atomic absorption spectroscopy with graphite furnace atomisation and deuterium background correction. Orthophosphoric acid was used as a modifier. The concentration of other elements was determined by Inductively Coupled Plasma Absorption Emission Spectroscopy (ICP-AES). Cadmium concentrations of each cultivar were compared using standard error about the mean values.

Table 2.4 Steps of acid digestion.

Step	Temperature (°C)	Digest Time (hours)
1	40	8
2	75	5
3	100	2
4	110	2
5	140	until volume reaches 1 ml

2.4 Results

2.4.1 Plant growth and cadmium accumulation

Plants were first harvested four weeks after planting, approximately six weeks after seed tubers had first sprouted. By this stage 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 in the increase in stolons and in the mass of the tubers. Both varieties showed similarities in

growth habit and no significant difference was noted in the plant growth of the two varieties. This includes both the rate of biomass production and the biomass at final harvest (Table 2.5).

Table 2.5 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	Sign. diff.
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 ± 0.009	$p < 0.001$

Figure 2.2a shows the growth, in terms of dry weight, of cultivars Wilwash and Kennebec over the course of the experiment. No significant differences in total Cd uptake or total plant Cd concentration were found between the two cultivars of potato (Figure 2.2b). Total Cd accumulation in the plant tissues occurred at a constant rate until the sixth harvest, nine weeks after planting. Further uptake also showed a linear response with time but at an increased rate. This seems to correspond with the major changes in plant growth.

During the initial growth period the shoots dominated the total plant dry weight, but by eight weeks after planting any increase in plant growth could be attributed to rapid expansion of the tubers (Figure 2.3). Similarly, during the first few weeks most of the Cd was found in the shoots but by eight weeks after planting (start of tuber bulking) net Cd input to shoots ceased and subsequent increases in Cd occurred only in the roots and in the tubers (Figure 2.4). In Kennebec almost all of the Cd in this latter growth phase was accounted for by tuber Cd whereas in Wilwash, Cd was allocated approximately evenly between roots and tubers (Figure 2.4).

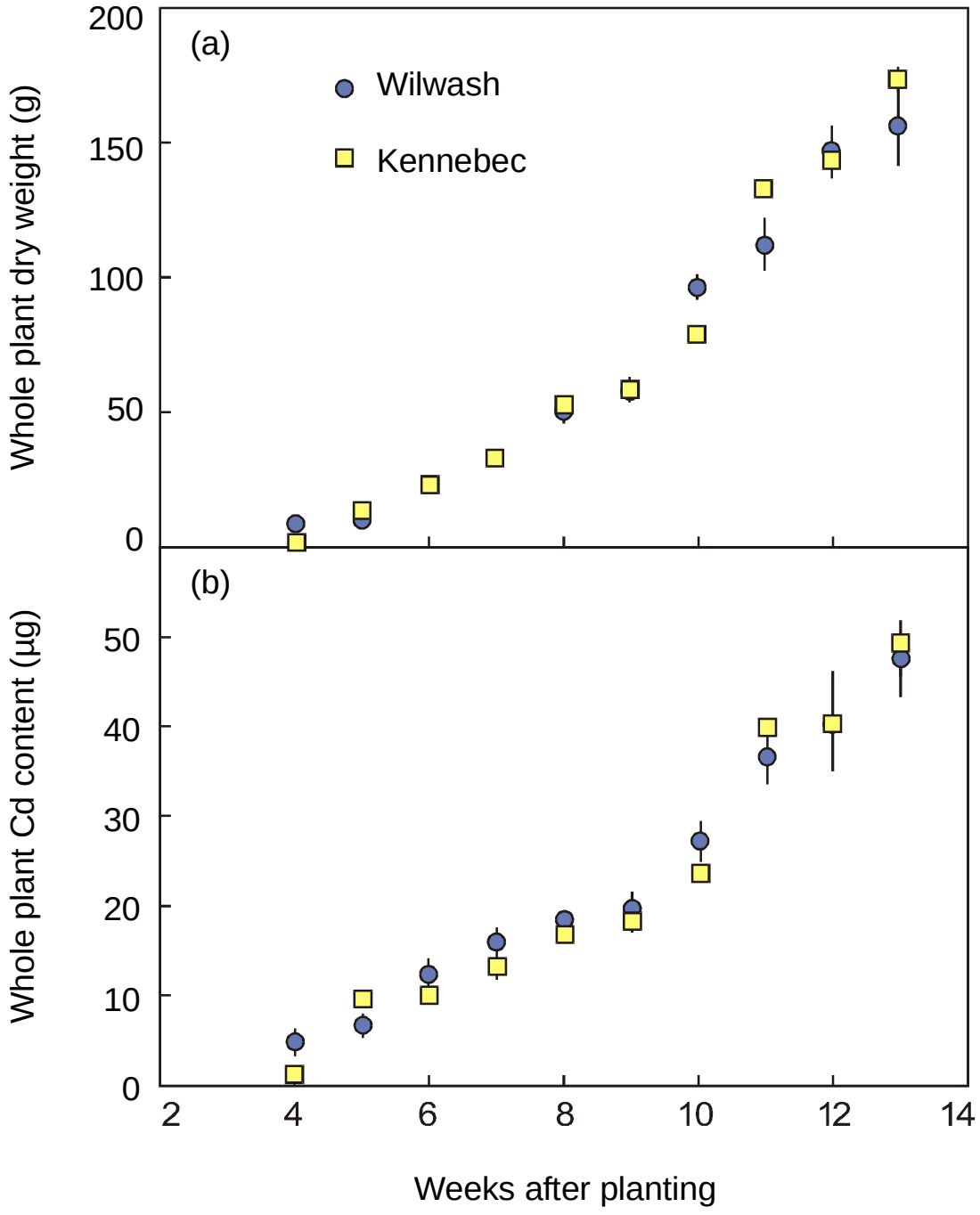


Figure 2.2 (a) Growth of potato varieties Wilwash and Kennebec and (b) whole plant Cd content. Each point is the mean ± SE of 4 plants.

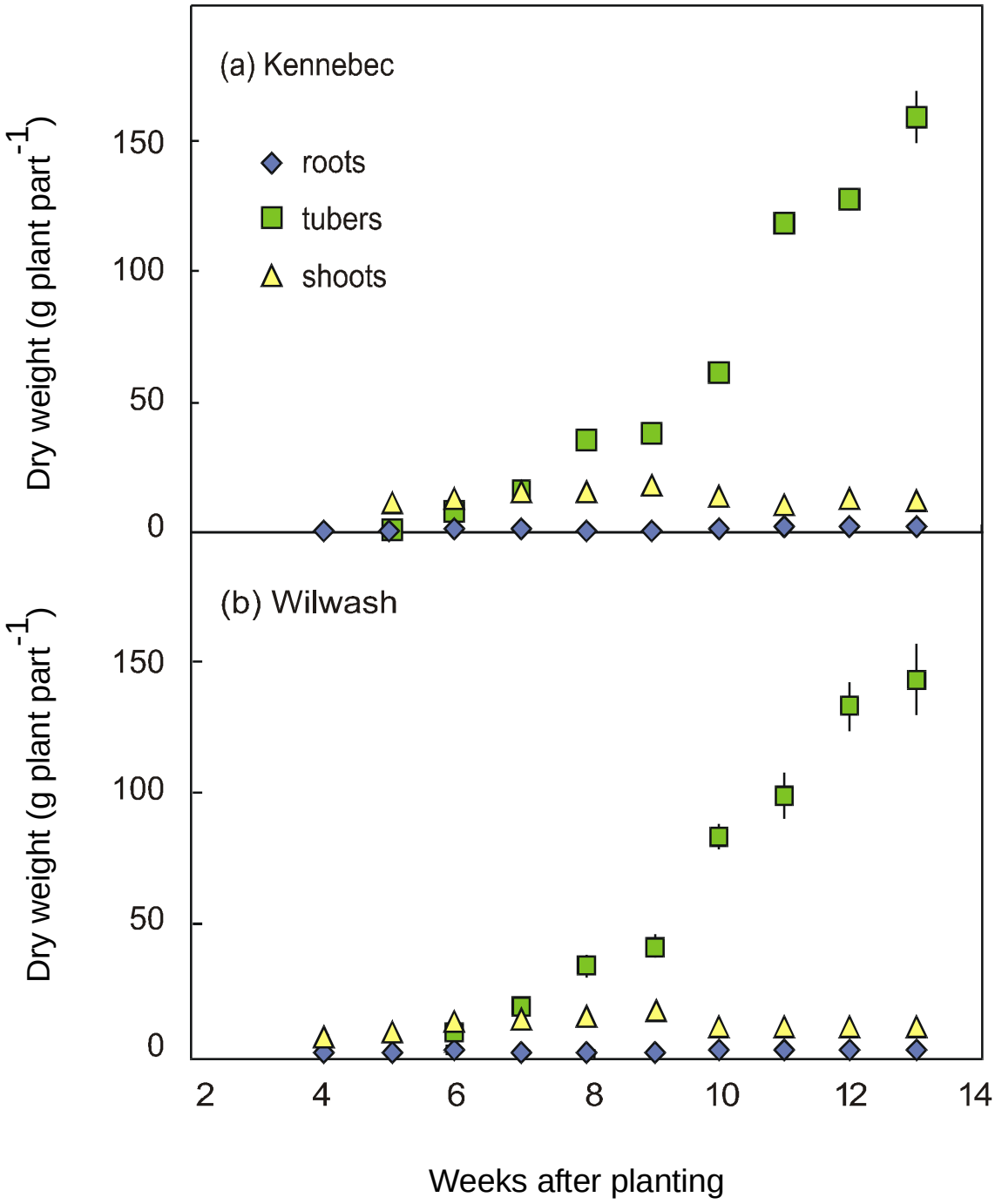


Figure 2.3 Distribution of dry weight during growth of (a) Kennebec and (b) Wilwash. Each point is the mean ± SE of 4 plants.

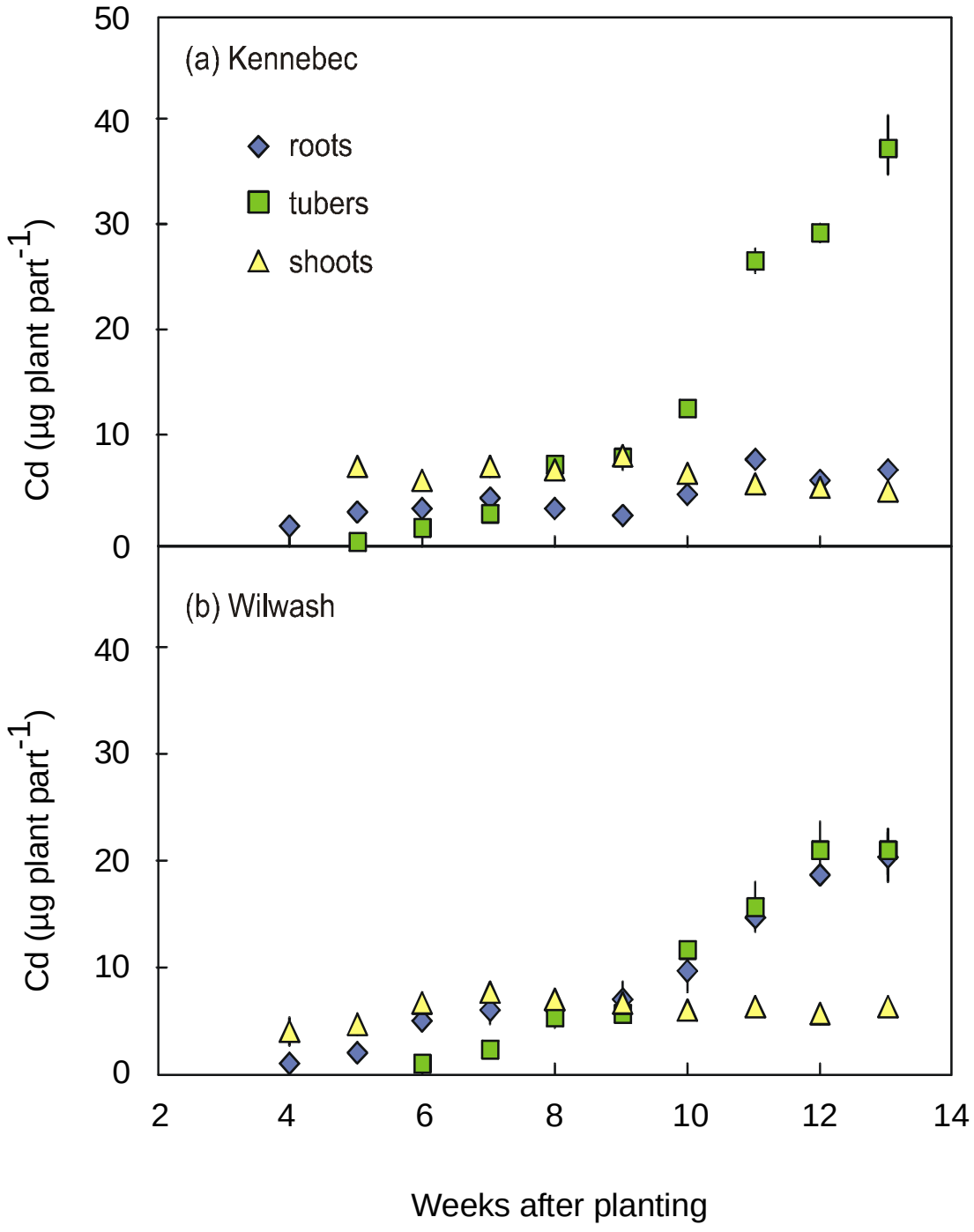


Figure 2.4 Distribution of Cd during growth of (a) Kennebec and (b) Wilwash. Each point is the mean ± SE of 4 plants.

2.4.2 Cadmium concentration in plant tissues

The concentration of Cd varied greatly depending on the particular tissue (Figures 2.5 & 2.6). The highest concentrations were found in the roots and the lowest concentration in the tubers. The large accumulation of Cd in the tubers (Figure 2.4) was not reflected in high concentrations because of the high tuber biomass (Figure 2.3). Importantly, in conformation with previous results (McLaughlin *et al*. 1994), the concentration of Cd in the tubers of cultivar Kennebec at final harvest was significantly higher than cultivar Wilwash (Figure 2.5). This difference could not be attributed to tuber yield, which did not differ significantly between cultivars. The differences in tuber Cd concentration were apparent after only 6 weeks and increased during tuber bulking (Figure 2.5).

The overall pattern of Cd accumulation is easiest to see by looking at changes in concentration in the tissues over time. For cultivar Kennebec, comparison of the growth data in Figure 2.3 with Cd concentrations in the various tissues (Figure 2.6) shows that in the case of the roots and shoots, once these tissues stopped growing, no further input of Cd into that tissue occurred. However large differences were apparent in Cd concentrations between the tissues. At final harvest, the concentration of Cd in the roots exceeded that of the tubers on a dry weight basis by more than a factor of ten and was approximately 5-fold higher than the average shoot concentration.

The main difference between the cultivars lay in the concentration of Cd in the roots. Whereas the concentration of Cd remained relatively constant in roots of Kennebec during tuber bulking, the concentration in roots of Wilwash continued to increase strongly (Figure 2.6b). The overall pattern during growth was for Kennebec to have having higher concentrations in the tubers and less in the shoots and roots than Wilwash.

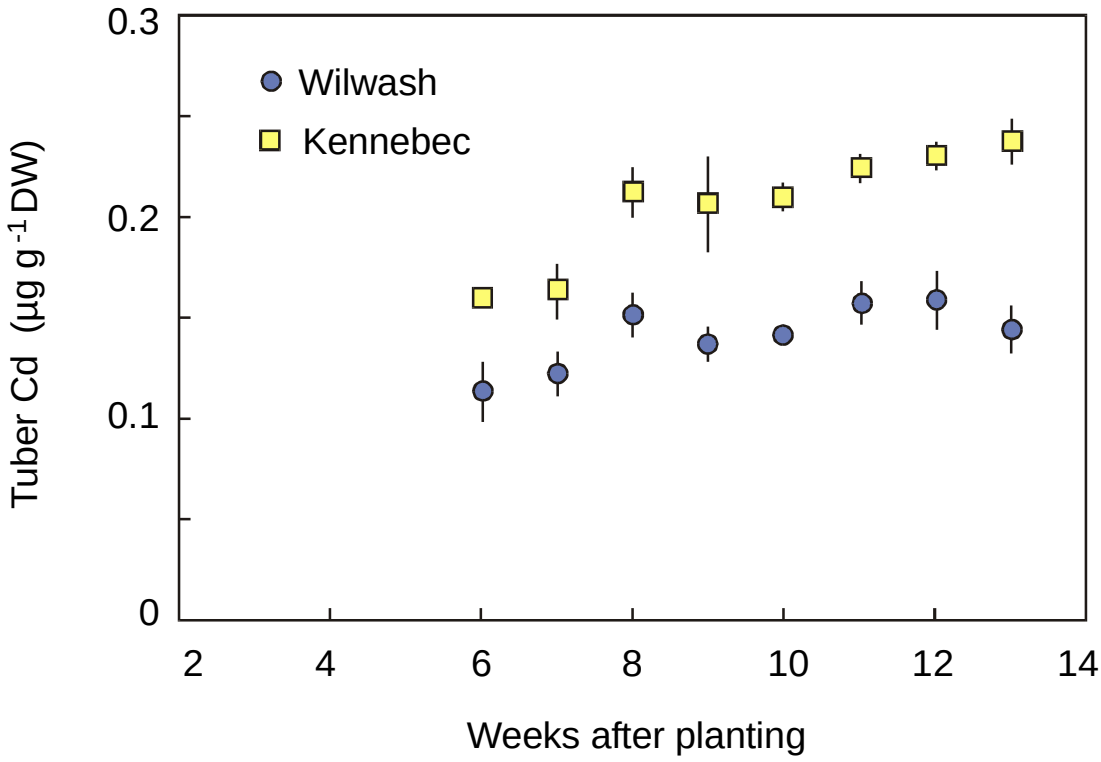


Figure 2.5 Concentration of Cd in tubers during tuber development. Each point is the mean ± SE of 4 plants.

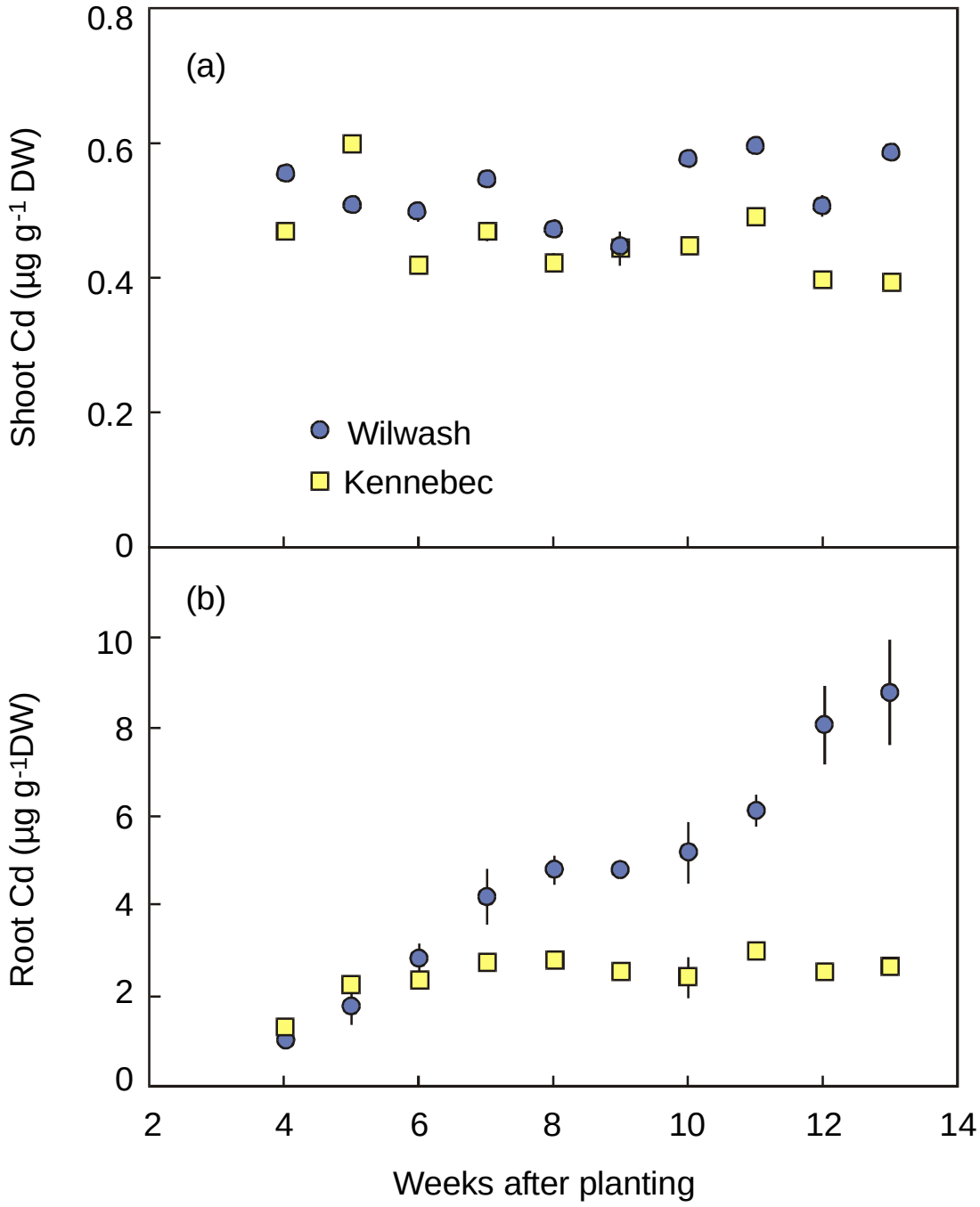


Figure 2.6 Concentrations of Cd in (a) shoots and (b) roots of Kennebec and Wilwash potatoes. Each point is the mean ± SE of 4 plants.

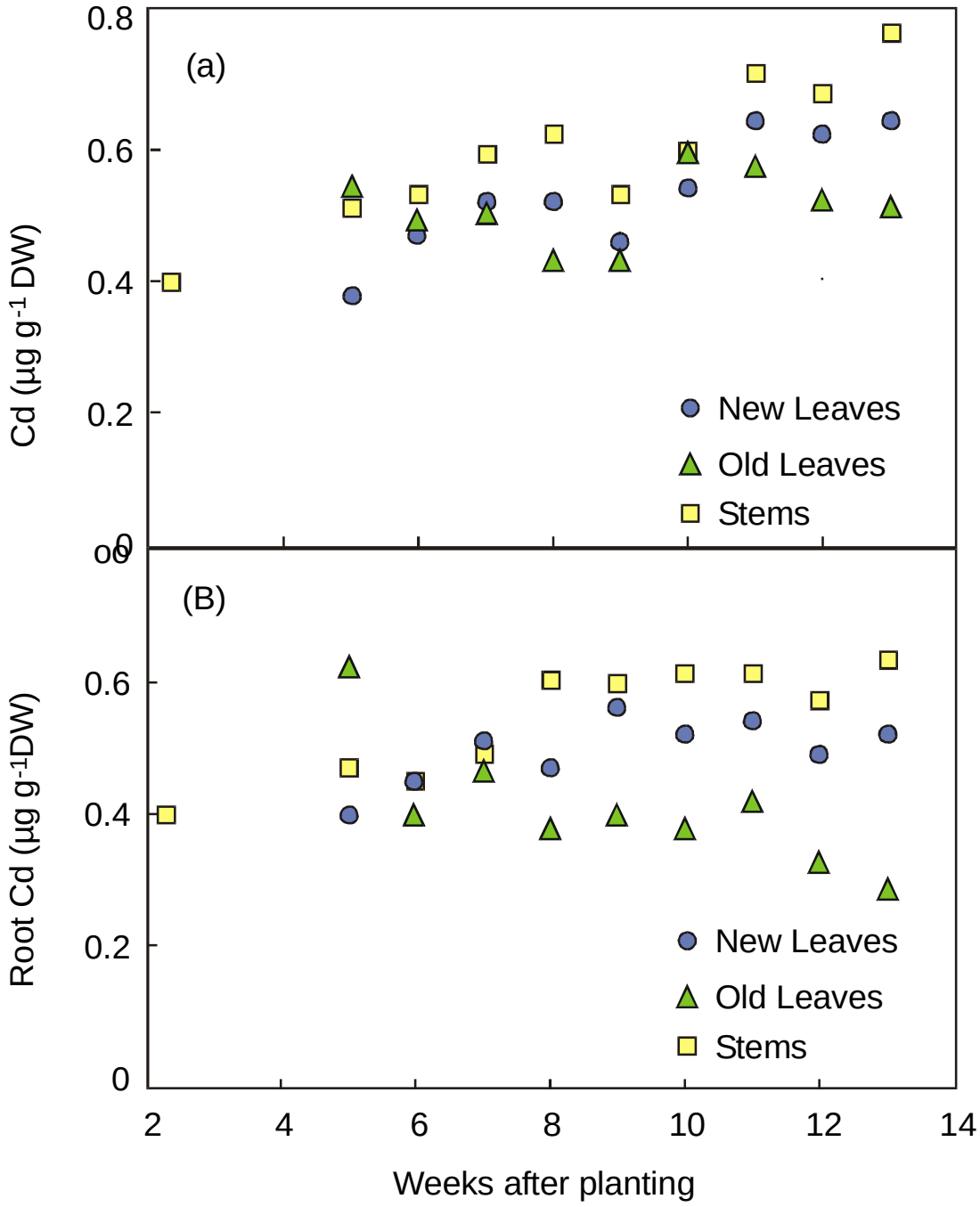


Figure 2.7 Concentrations of Cd in new leaves, old leaves and stems of (a) Wilwash and (b) Kennebec potatoes. .

2.4.3 Distribution of cadmium at final harvest

As the concentration of Cd in the tubers of potato at maturity is most important in terms of human consumption, the distribution of Cd between plant tissues at final harvest (13 weeks after planting) was observed more closely. Figures 2.8 and 2.9 show the distribution of Cd in non-tuber tissues and in all tissues. Differences in the distribution of Cd between tissues were evident between cultivars. At maturity, 76% of Cd was found in the tubers of Kennebec, 14% was found in the roots and 10% in the shoots (Figure 2.9). In comparison the distribution of Cd in Wilwash was 43% in the tubers, 42% in the roots and 14% in the shoots.

Although there was a large accumulation of Cd in the roots of cultivar Wilwash this did not give rise to an overall decrease in Cd content across the whole of the remainder of the plant. Instead, only the tuber Cd concentration was reduced while the shoot Cd concentration remained similar between the two cultivars.

2.4.4 Distribution of mineral nutrients

The differences in Cd distribution between varieties raise the question of whether Cd is preferentially sequestered into non-tuber tissues of cultivar Wilwash or whether the differences are due to factors also dictating the partitioning of other elements, notably nutrient elements. The profile of mineral elements in leaves suggests that Cd is treated differently.

Comparison of the concentrations of mineral elements in the two cultivars showed that in leaves the concentration in cultivar Kennebec were similar (Mn, K, B, Cu, S, Zn, P) or significantly higher (Fe, Ca, Mg) than in cultivar Wilwash (Table 2.5). Cadmium did not conform to this pattern and the concentration in cultivar Kennebec was nearly half that of cultivar Wilwash at final harvest.

Concentrations of all elements, including Cd, were higher in tubers of cultivar Kennebec (Table 2.5). The ratio of concentrations in leaves to concentration is tubers may be indicative of the ability of solutes to be mobilised from leaves into the phloem. Certainly, in the case of Ca where the ratios were 95 and 72 for cultivar Wilwash and Kennebec respectively, the results strongly support the view that phloem is the major pathway for loading of mineral elements into the tubers. The ratios for all other elements were much lower but roughly consistent with the expected mobility in the phloem. Magnesium, Mn and Fe displayed the greatest difference between leaf and tuber concentrations and Zn, S and P showed the lowest ratios (Table 2.6) Cadmium was intermediate between these extremes.

The higher concentrations of elements in tubers of Kennebec could not be attributed to differences in water content since the FW/DW ratios of the two varieties remained similar throughout the growth period (Figure 2.10).

Table 2.6 Comparison of the concentrations of Cd and mineral nutrients in mature leaves and tubers of potato cultivars Wilwash and Kennebec. Concentrations are means ± SE (n=4) expressed as μg g^{-1}DW.

Element	Leaves		Tubers		Ratio leaves:tubers	
	Wilwash	Kennebec	Wilwash	Kennebec	Wilwash	Kennebec
Ca	22750 ± 479	26750 ± 480	238 ± 24	370 ± 41	**95.4**	**72.3**
Mg	8125 ± 281	13025 ± 502	1245 ± 20	2425 ± 278	**6.5**	**5.4**
Mn	34.0 ± 0.6	28.5 ± 1.4	5.2 ± 0.1	10.4 ± 1.0	**6.5**	**2.7**
Fe	53.4 ± 1.6	70.6 ± 3.3	9.9 ± 1.5	20.4 ± 2.7	**5.4**	**3.5**
Cd	**0.51 ± 0.06**	**0.28 ± 0.03**	**0.15 ± 0.01**	**0.24 ± 0.01**	**3.5**	**1.2**
K	69750 ± 3092	70500 ± 3476	23250 ± 750	41750 ± 4347	**3.0**	**1.7**
B	27.3 ± 0.8	26.3 ± 1.8	10.1 ± 0.3	22.2 ± 3.4	**2.7**	**1.2**
Cu	4.6 ± 0.2	4.0 ± 0.3	3.0 ± 0.2	6.1 ± 0.4	**1.5**	**0.7**
S	2175 ± 48	2675 ± 85	1980 ± 108	3375 ± 202	**1.1**	**0.8**
Zn	26.9 ± 0.6	23.4 ± 0.7	25.2 ± 1.1	36.9 ± 1.4	**1.1**	**0.6**
P	1518 ± 40	1353 ± 23	2245 ± 108	3750 ± 357	**0.7**	**0.4**

These large variations in nutrient concentrations in tubers were not unique to the growth conditions employed in this study since field-grown tubers obtained from Dr Roger Kirkham of Agriculture Victoria showed very similar ratios between cultivars, with the exception of Ca (data presented in Appendix 1).

The concentrations of a range of nutrient elements were measured in shoots and tubers for each of the harvests. This data is summarised in Appendix 2.

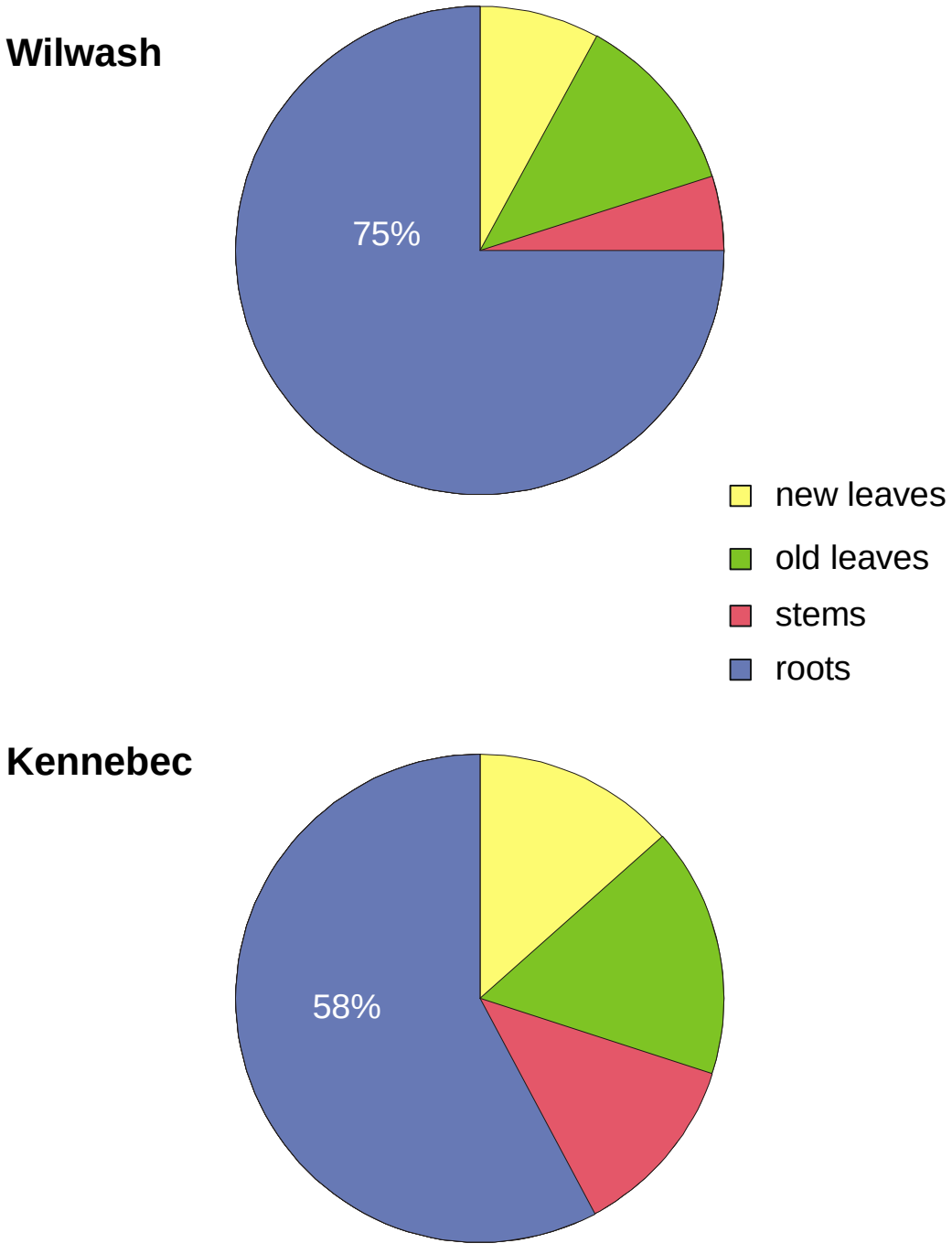


Figure 2.8 Distribution of Cd in non-tuber tissues of potato plants at harvest after 13 weeks growth.

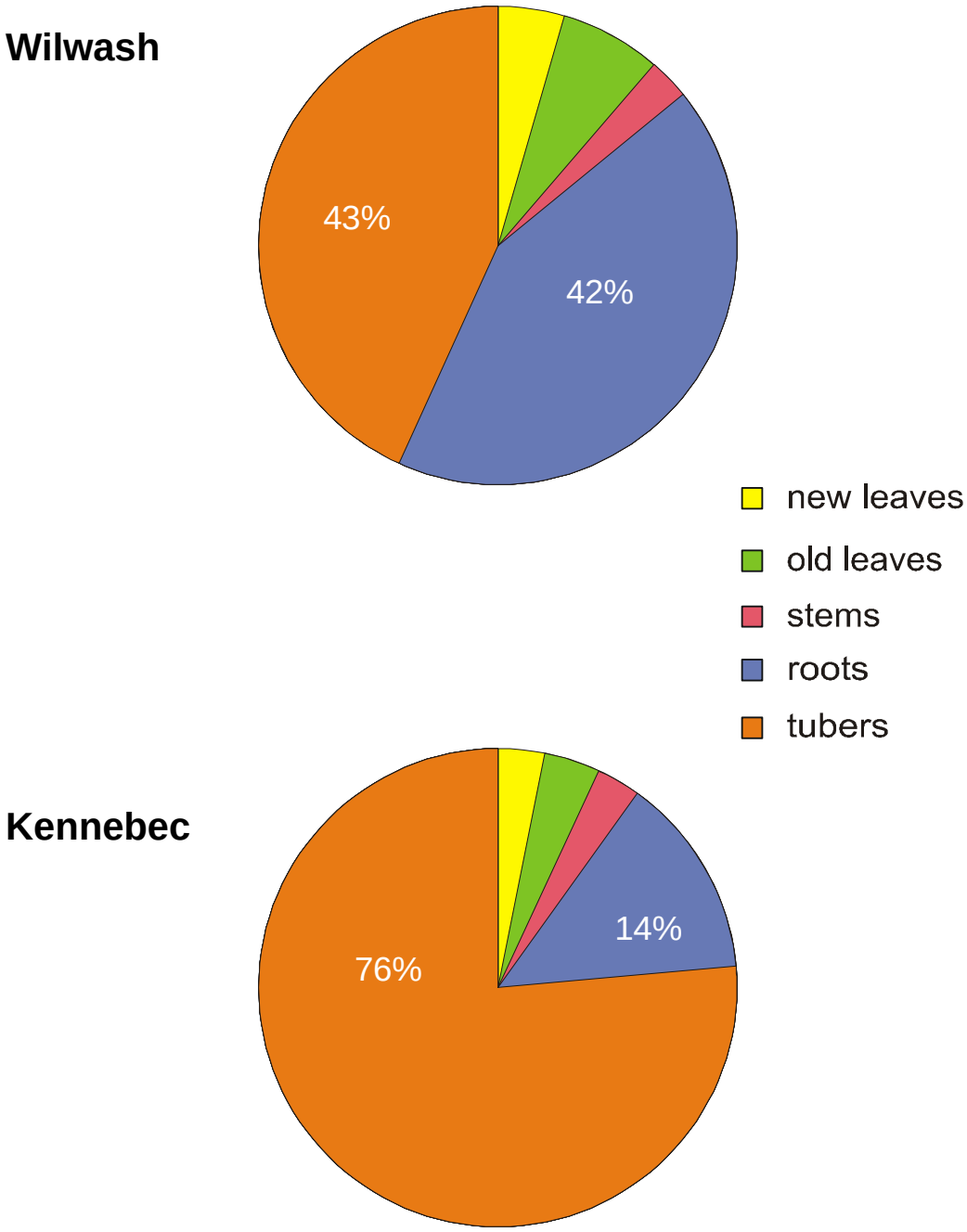


Figure 2.9 Distribution of Cd in all tissues of potato plants at harvest after 13 weeks growth.

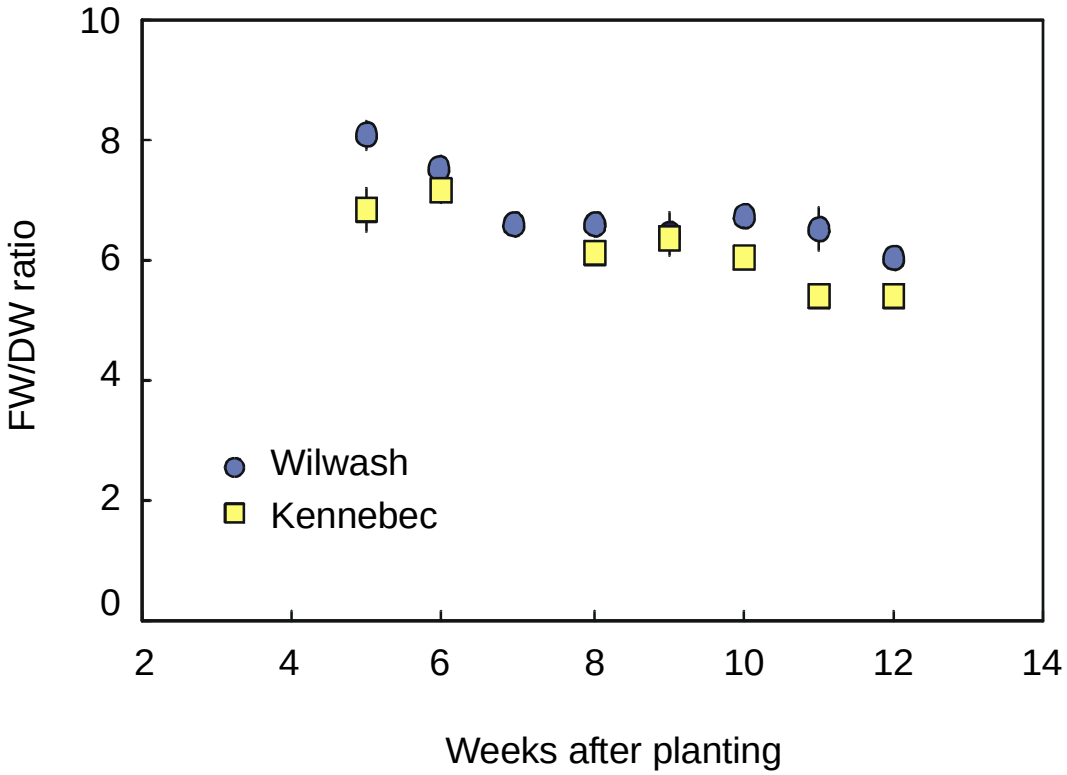


Figure 2.10 Fresh weight:dry weight ratios of tubers of Kennebec and Wilwash during growth. Each point is the mean ± SE of 4 measurements.

2.5 Discussion

This study sought to determine whether differences in tuber Cd concentration were attributable to naturally higher plant uptake by cultivar Kennebec or to differences in the distribution of Cd within the plant. Similarities in growth habit, along with the similar total Cd content and average plant Cd concentration, indicate that tuber differences could not be attributed to concentrating effects due to differences in growth or a higher uptake of Cd into the plant. In this case differences were clearly attributable to genotypic variations in the partitioning of Cd between the tubers and the rest of the plant, particularly the proportion of total Cd that was retained in the roots and leaves.

It was observed in this study that once fully expanded there was no significant net input of Cd into shoot tissue. This can be explained by either cessation of Cd import into the leaf tissue or an equilibrium between the transport of Cd into the leaf tissue (import) and re-translocation (export) to other plant tissues. As import of Cd to the leaf tissue is governed by xylem flow, it is unlikely that Cd stops being transported to the leaves as there is a continual need for xylem flow to replace transpirational losses of water. A closer look at shoot data (Fig 2.7) indicates that although there is a lack of overall net input of Cd into the shoots, on the whole, the concentration of Cd into the new leaves and stems continues to increase until final harvest. The equilibrium noted in net Cd uptake is brought about by decreases in the Cd content of the mature leaves. With elements that show a limited phloem mobility, such as Ca, a balance between import and export would be unlikely as only a small percentage is transported from the leaves to the tubers. However it is not unusual for nutrients/elements with a high phloem mobility to show equilibrium of import and export from fully expanded leaves (Marschner 1995). The fact that Cd appears to be continually retranslocated to the tubers from the mature leaves suggests relatively free movement within the phloem sap. It then seems possible that the most likely pathway of

Cd movement to the tuber is from the soil to basal roots to shoots in the xylem followed by retranslocation to the tubers in the phloem.

Of some surprise was the difference in concentrations of mineral nutrients in the tubers of the two varieties. In most cases the concentration of mineral nutrients in the tubers of Kennebec was 1.5 to 2 times greater than in the tubers of cultivar Wilwash. As with Cd, differences could not be attributed to alterations in growth conditions or the concentrating effects due to differences in plant dry weight. This may suggest that there are fundamental differences in the phloem loading mechanisms of the two cultivars. The ratio of concentration in the leaves to that in the tubers can be considered to indicate the level of phloem mobility of a particular element. A higher ratio indicates that a greater percentage of that particular element is remaining in the leaves and, therefore, not being mobilised to the tuber via the phloem. For all elements analysed, including Cd, the ratio of concentration in leaves to tubers was consistently higher in Wilwash than in Kennebec, supporting the assumption that differences in final tuber concentration between cultivars is related to the ability of elements to be loaded into the phloem or movement of the elements once loaded into the phloem.

However, the mechanisms governing the redistribution of mineral elements from the leaves to the tubers may not necessarily be related to those governing the movement of Cd. In cultivar Kennebec the concentrations of mineral elements in the leaves were similar or higher than cultivar Wilwash. Cadmium was the only element which had higher concentrations in the leaves of Wilwash. This suggests that Cd may be preferentially sequestered in the leaves despite a strong concentration gradient existing in the direction of the tubers. By sequestering Cd either into physical compartments such as vacuoles or into chemical complexes, access of Cd to transport in the xylem or phloem may be reduced. Previous studies on intracellular Cd transport have established that Cd is moved into the

vacuole by two main routes, either by complexation with phytochelatins (PCs) followed by active transport of the PC-Cd complex across the tonoplast (Vogeli-Lange and Wagner 1990, Ortiz *et al*. 1995, Salt and Rauser 1995), or by H^+/Cd^{2+} antiport (Salt and Wagner 1993). Plant tolerance to some heavy metals, including Cd, has been shown to be related to the ability to synthesise PCs (Rauser 1990, Xiang and Oliver 1998, Zhu *et al*. 1999, Cobbett 2000) and there are several levels at which variations in PC synthesis between cultivars might occur. Firstly, PC synthase is rapidly induced by exposure to Cd (Chen *et al*. 1997) and therefore natural variation in response of the induction process may lead to significant differences in PC synthesis. Secondly, the level of PC synthesis may also be controlled through modulation of the biosynthetic pathway leading to the synthesis of glutathionine, the immediate precursor of PCs (Yong *et al*. 1999, Zhu *et al*. 1999). Alternatively, higher accumulation of Cd in vacuoles might occur through a greater activity of the Mg-dependent ATP-ase that is believed to be responsible for the transport of the PC-Cd complex across the tonoplast (Salt and Rauser 1995). Variations between the cultivars in vacuolar acidity, for whatever reasons, would also serve to stimulate sequestration through the H^+/Cd^{2+} antiporter (Salt and Wagner 1993) independently of any contribution by phytochelatins.

The ability of Wilwash to preferentially compartmentalise Cd goes a long way to explain the large difference in root Cd concentration. Alternatively the concentration of Cd in a particular plant tissue may de determined by recycling of nutrients in the phloem and the competition between sinks. Both the roots and the tubers act as sinks for the movement of photosynthate from the leaves, and both are exposed to the other constituents of phloem such as amino acids and inorganic nutrients and non-nutrients such as Cd. Overall there can be little difference in the amount of photosynthate directed to the tubers and roots of the two cultivars since the differences in tuber yield and root growth are not significant.

Therefore, the likely mechanism for a greater accumulation of retranslocated Cd would be more efficient extraction of Cd from the phloem in the roots of Wilwash. For this there is no direct experimental evidence.

Differences in plant Cd distribution in this study have been considered with the assumption of tuber Cd being supplied by redistribution in the phloem from the basal roots. The possibilities exists for tuber Cd to be independent of the main pathway accepted for the transport of the majority of mineral nutrients. The use of alternative pathways, such as a direct link between the basal roots and the stolons, is discussed in the following chapter.

Chapter 3

Pathways of Tuber Cadmium Uptake

3.1 Introduction

The potato has a highly branched, fibrous root system (Cutter 1992) which is made up of four different types of roots (refer to Figure 3.1), as described by Kratzke and Palta (1985). The bulk of the root system is comprised of ‘basal’ roots which originate from the base of the stem. ‘Stem-stolon junction’ roots arise at the junction between stolons and the main stem, ‘stolon’ roots arise directly from nodes on the stolon and ‘tuber’ roots originate from the base of buds on the tuber. The characterisation of these different types of roots has led to speculation about the role of these roots in the mineral nutrition of the plant and tubers. As a result, many studies have investigated the function of the different parts of the root system. Kratzke and Palta (1985) used water soluble dyes to trace the pathway of water through Russet Burbank potatoes. It was found that basal roots supplied water primarily to the aerial parts of the plant and were of little importance in the water supply to the tuber. Even though there was some indication of a xylem connection between the basal roots and the stolon, the authors suggested that water supplied from the phloem would be sufficient in meeting needs arising from evaporation from the periderm. Previous studies have shown that water transport to the tuber occurs at night due to the reduction in transpirative demand of the leaves (Baker and Moorby 1969, Gandar and Tanner 1976). However, even after a 24 hour period, Kratzke and Palta (1985) found no evidence for the direct transport of water from the basal roots to the tuber. Stolon and tuber roots, however, were shown to provide water directly to the tuber, with tracer dye being found in the xylem ring of the

tuber two hours after application to these roots. In addition, the stolon roots also provided a path for water movement to the aerial parts of the plant.

While phloem provides most of the mineral nutrients and other compounds such as amino acids and carbohydrates essential for the growth and development of the tuber, nutrients such as Ca—which are considered highly immobile in the phloem—must rely on other uptake pathways if the nutritional needs of the tuber for these elements are to be met. Previous studies have provided strong evidence of Ca being supplied to the tuber via direct periderm uptake, or via stolon and tuber roots (Wiersum 1966, Krauss and Marschner 1971, Kratzke and Palta 1986). Whether or not Cd can utilise any of these additional pathways for uptake in to the potato tuber is unknown although previous studies in bacteria (Nies 1995) and in animal cells (Hinkle *et al*. 1992) reported competition between Ca and Cd for cellular uptake via ion channels. This competition may be attributable to the similarities in physical characteristics between Cd and Ca, such as charge (+2) and ionic radius (97 and 99 pm, respectively).

Regardless of the competition with Ca, the high mobility of Cd in the xylem sap suggests that the stolon and tuber roots may provide important uptake sites and make a significant contribution to the total Cd found in the tubers at harvest. The possibility also exists for Cd to enter the tuber via direct transport across the periderm.

3.2 Objectives

The aim of the experiments outlined in this chapter was to determine the origin of the Cd found in the tuber at harvest and to suggest possible pathways of uptake. Three main pathways for movement of Cd into the tubers seem possible:

Pathway 1 xylem transport from the basal roots to the leaves, followed by translocation in the phloem back to the tubers

Pathway 2 uptake from small roots on the stolon with transport directly to the tuber via the xylem

Pathway 3 direct uptake across the periderm of the tuber

A pot experiment was designed to distinguish between pathways 1 and 2 above. 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 ^{109}Cd was added to either 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 3 was determined by applying ^{109}Cd directly to the periderm of intact tubers and measuring any subsequent uptake into the tuber after 7 days.

3.3 Methods

3.3.1 Split-pot experiment

Seed tubers of cv. Kennebec were sprouted under a 24 hour light regime in washed Mt Compass soil (see Table 2.2) to the one to two leaf stage. Once sprouted, the seed pieces were transplanted to the bottom section of the pots. Plants were grown in an environmentally controlled growth cabinet and subject to a 12 hour day/night cycle with light irradiance of 275 µmol s^{-1} m^{-1}. Plants were subject to a day temperature of 25°C and a night temperature of 20°C.

The use of radio-labelled soil meant that this experiment had to be carried out in a growth cabinet registered for the use of radionuclides. The size of these cabinets limited the number of plants able to be grown to twelve. These were divided into three treatments, each with four replicates, as follows:

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

Pots were arranged randomly in the growth chamber.

3.3.2 Pot design

The pots allowed the separation of the roots from the tubers, with two main compartments (Figure 3.1). Both compartments consisted of a peatmoss/sand mixture (UC mix) 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 peatmoss 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 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. A 1 cm hole was found to be small enough to seal around the base of the stem but flexible so as not to restrict growth of the stem. The top compartment was filled with 2.5 kg of the peatmoss mix. Three weeks after emergence a further 2.5 kg of the mix was used for mounding.

The pots were successful in dividing the basal roots from the remainder of the root system, with no tubers or stolons being found in the bottom section of the pots. In addition, testing of the UC mix in the non-labelled compartments of each pot at the end of the experiment confirmed that no leakage of ^{109}Cd between sections had occurred.

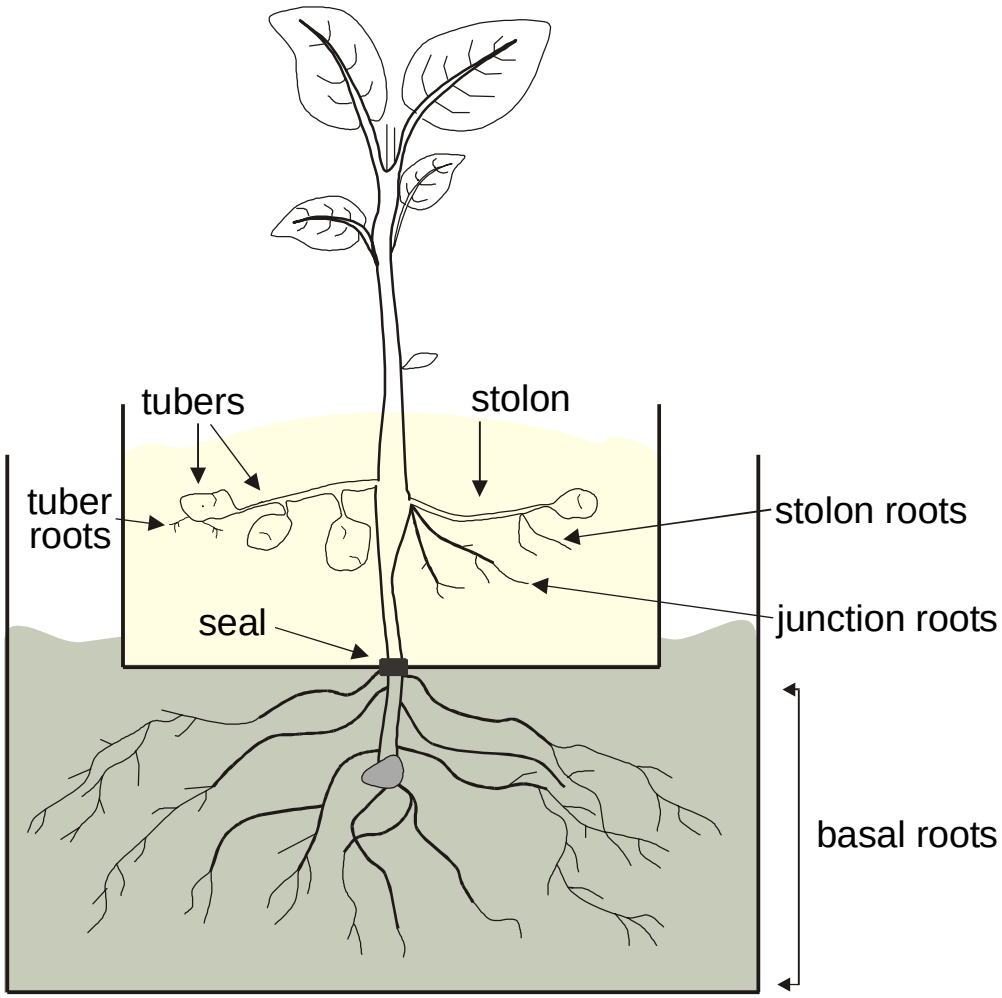


Figure 3.1 General anatomy of a mature potato plant and the arrangement of compartments for the split-pot experiment.

3.3.3 Soil parameters

The growth medium was a modified University of California Mix (UC mix), consisting of a 3:4 mixture of peatmoss and sand. The soil mix was made four weeks before the start of the experiment to allow equilibrium within the soil to be reached. Four hundred litres of course sand was sterilised at 100°C for 30 minutes and mixed with 300 litres of Eurotorf® peatmoss. Fertilisers were added as calcium carbonate and nitrophoska® (15-4-12) (Table 3.1). Calcium hydroxide, which is included in a standard UC mix, was not added to ensure that the pH of the growth medium was not too high. The pH of the UC mix at the beginning of the experiment, as measured with a 1:5 ratio in water, was 5.58.

Table 3.1 Components of University of California (UC) Mix, including analysis of nitrophoska.

Component		Amount
Coarse washed sand		400 litres
Eurotorf® peatmoss		300 litres
Calcium Carbonate		480 g
Nitrophoska		600 g
Nitrophoska® Analysis		
N	Total Nitrogen	15%
	5% NH_4	
	4% NO_3	
	1% NH_2	
	5% IBDU	
P	Total Phosphorus	3.90%
	3.9 % citrate soluble	
	(incl. 1.2 % water soluble)	
K	Potassium sulphate	12.40%
Mg	Magnesium carbonate	1.25%
Ca	Dicalcium phosphate	3.40%
S	Sulphates	5.30%
Fe	Iron oxide	0.30%
Cu	Copper oxides	0.0002%
Zn	Zinc oxide	0.0070%
B	Calcium borate	0.0100%
Mo	Molybdenum oxide	0.0003%

3.3.4 Addition of ^{109}Cd to the soil

Two weeks prior to planting 120 kg of the UC mix was labelled with carrier free ^{109}Cd to provide an activity of 4 kBq kg^{-1}. This was calculated on the basis of the expected concentration of Cd in the tuber at harvest combined with the specific activity required to produce an accurate analysis for each sample.

A total of 1.26 µL of ^{109}Cd, with a specific activity of 381 kBq µL^{-1}, was diluted into 6 litres of d.H_2O. The radioactive solution was applied to the UC mix by mixing 20 kg batches of soil with 1 litre each of solution in a cement mixer for 5 minutes. The solution was applied with a spray atomiser to ensure maximum distribution within the soil. The batches of labelled UC mix 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.

3.3.5 Plant harvest and analysis

Plants were harvested 10 weeks after emergence (70 DAP). The new leaves, old leaves and stems were harvested from each plant as described in Section 2.3.4. Tubers were carefully removed from the top section of the pots and the UC mix removed with a nylon brush, before being rinsed, peeled and dried. No tubers were found to be growing in the bottom section of any of the pots.

All plant material was dried, ground and digested using the methods described in Section 2.3.5. The activity of ^{109}Cd in each digested sample was measured using a gamma counter, and all activities decay corrected.

3.3.6 Direct uptake across the periderm

To determine whether Cd could be taken up directly by the tuber, another pot trial was undertaken. Three plants each of four cultivars, Kennebec, Wilwash, Desiree and King Edward, were grown in unlabelled UC mix under the same conditions as those described

above. Split-pots were used to ensure that the 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 litre of aerated nutrient solution (see Table 2.2) 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 litre solution containing 5 mM $CaCl_2$ and 1 µM $LaCl_3$ for 30 minutes. Tubers were then removed from the plants, rinsed in d.H_2O 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 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 as the tuber sections.

3.3.7 Autoradiography

The remaining tuber from each cultivar was used for autoradiography. A 0.5 cm longitudinal slice was taken from the centre of each tuber and freeze dried. Samples were placed in a Kodak Biomax Transcreen system for 48 hours at -70°C. The Transcreen

system consisted of a Kodak Biomax Transcreen LE intensifying screen and Biomax MS-1 film, mounted in a sealed, light-tight Biomax autoradiography cassette. Autoradiographs were developed manually using Kodak GBX Developer and Fixer. Both light and dark images of the tuber slices were taken.

3.4 Results

3.4.1 Split pot experiment

The different root systems were found to vary in their role of supplying Cd to different sections of the plant (Figure 3.2). In each instance the basal roots were found to be the main source of the ^{109}Cd found in the plant tissue at harvest. As expected, the majority of Cd in the above ground material was supplied by the basal root system. In addition, ^{109}Cd supplied to the basal roots was found to account for up to 85% of the Cd in the tuber.

Stolon roots growing in the tuber compartment, although not quantified, were found to be extensive and are clearly shown growing from the stolons in Figure 3.3. No tuber roots were apparent on any tubers. Uptake of ^{109}Cd from these roots was less than the basal roots, but still important, providing 15–20% of tuber Cd. Their importance 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, indicates a continuous xylem connection between these sections.

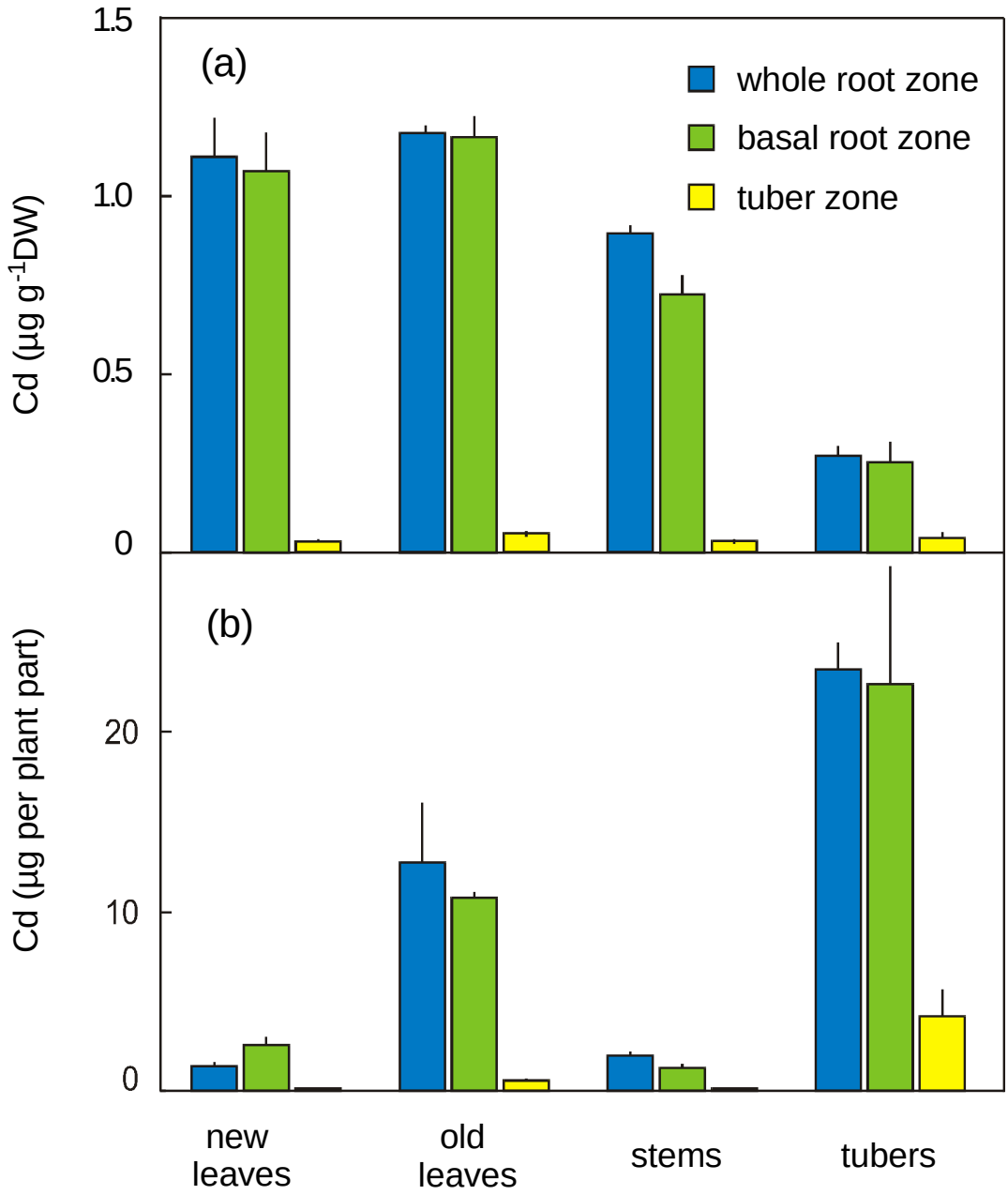


Figure 3.2 (a) Concentrations in potato tissues of ^{109}Cd applied to root and tuber compartments in the split-pot experiment after 10 weeks, and (b) total content of Cd in the different tissues. Values are the means ± SE of 4 plants cv. Kennebec.

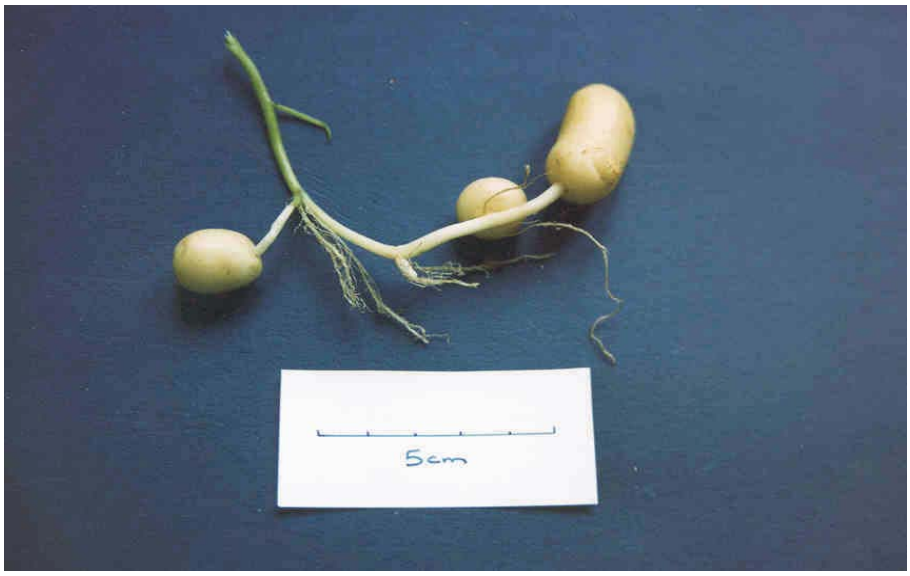


Figure 3.3 Developing tubers showing the occurrence of stolon roots

3.4.2 Direct uptake across the periderm

Monitoring of the ^{109}Cd activity in the nutrient solution showed no significant (<10%) depletion from all but one of the treatment solutions throughout the course of the experiment. The concentration of ^{109}Cd in one of the solutions containing a tuber of cv. Wilwash was found to have reduced by 10.5% at the time of harvest but this was not deemed a significant decrease to have affected the uptake of ^{109}Cd into the tuber.

The activity of ^{109}Cd (given in counts per minute) found in each section of the tuber cores is given in Table 3.2. After 7 days of growing in the nutrient solution ^{109}Cd was found in the periderm of most of the tubers. The activity of ^{109}Cd was highest in the periderm of cv. Wilwash and the lowest in cv. Kennebec. The presence of ^{109}Cd was found in the internal tissue of only one tuber. This was in the first 1 cm of the tuber core and is most likely a result of leakage due to deterioration of the periderm of that tuber. 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 radio-labelled solution. Table 3.2 also shows the concentration of ^{109}Cd calculated on the basis of the counts per minute in each section. The reliability of the concentrations derived from these measurements is dubious given the very low number of counts per minute and the proximity of these numbers to background levels.

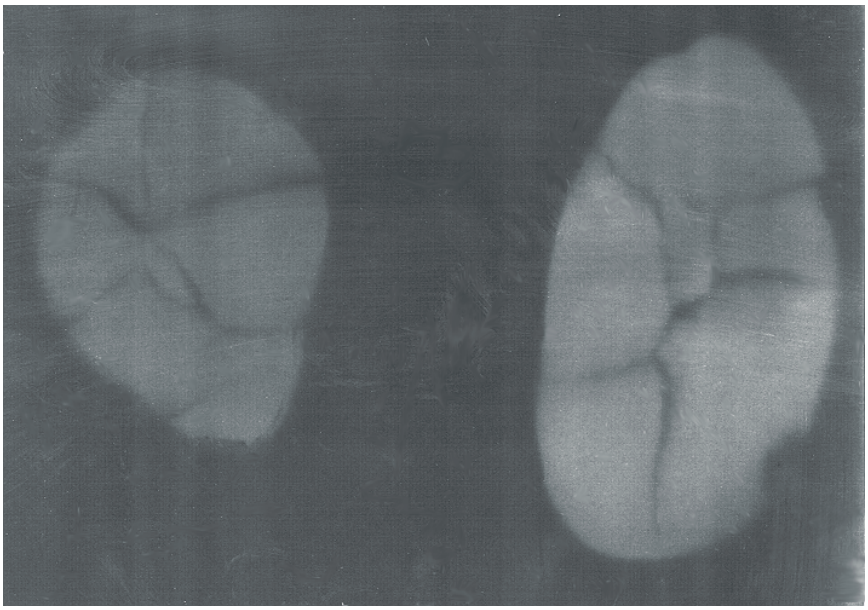
Table 3.2 Counts per minute of each sections of longitudinal cores of tubers grown in the presence of ^{109}Cd for 7 days. Numbers in parenthesis indicated the concentration (µg g^{-1} FW) of ^{109}Cd and numbers followed by an asterisk indicated that the cpm are not significantly (>50%) above background levels.

Section	Kennebec	King Edward	Desiree	Wilwash
Skin	17.6 (0.003)	24.3 (0.015)	11.3*	52.0 (0.063)
1	16.0*	21.6 (0.009)	10.7*	16.3*
2	9.6*	10.3*	10.3*	11.3*
3	11.6*	12.0*	10.5*	16.3*
4	10.9*	9.3*	15.2*	14.9*
5	10.6*	12.4*	10.7*	9.0*
6	9.0*	9.2*	12.4*	10.2*
7	10.0*	13.3*	13.5*	15.3*
Skin	15.3*	25.3 (0.022)	21.3 (0.009)	34.7 (0.029)

3.4.3 Autoradiography

Autoradiographs of longitudinal sections from taken from cultivars Wilwash and Kennebec are shown in Figure 3.4. Evidence of ^{109}Cd activity was only indicated in some sections of the periderm of cv. Wilwash and can be correlated to areas were the ^{109}Cd activity is in the proximity of 50 cpm. These images indicate that this autoradiography system is suitable for detecting levels of ^{109}Cd activity greater than 50 cpm.

(a)



(b)

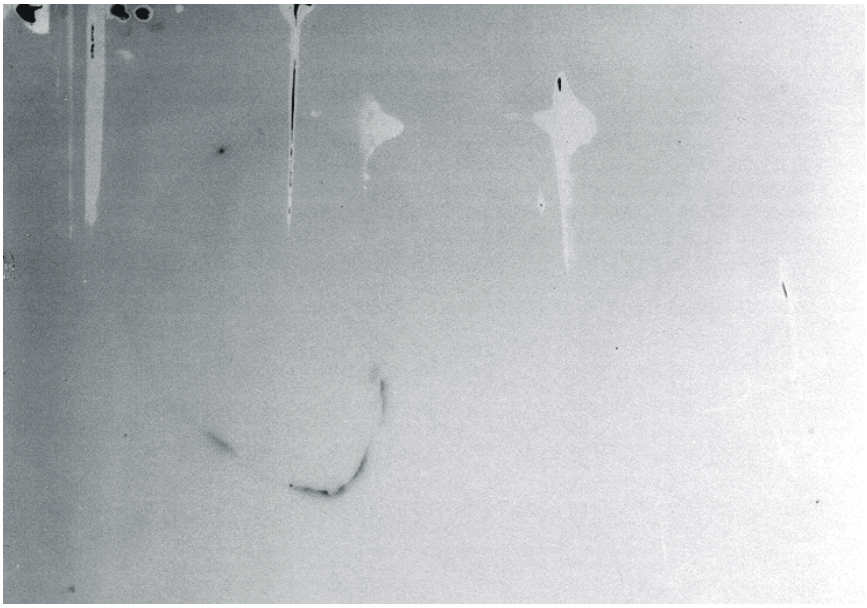


Figure 3.4 (a) photographic image and (b) autoradiograph of slices of tubers from cultivars Wilwash (left) and Kennebec following 7 days incubation in a solution containing ^{109}Cd.

3.5 Discussion

Previous studies have focused on the role of the root system in the movement of water and nutrients, such as Ca, P and K, to the tuber. The pathway of movement of other macronutrients and micronutrients has been suggested based upon their relative mobility in the phloem. Phloem mobile nutrients are likely to be transported to the tuber in the phloem via translocation from the leaves, whereas nutrients which are believed to travel almost exclusively in the xylem rely on alternative transport pathways, which may include direct uptake across the periderm or uptake from stolon or tuber roots.

Although the mobility of Cd in the phloem has not been directly determined, it is known to form complexes with molecules containing sulphydryl groups which are present in the phloem and are believed to provide a means for other divalent cations, such as Zn to travel in the phloem sap (Welch and Norvell 1999). It is likely, therefore, that Cd may be transported to the tuber in this way.

Results from the initial split-pot experiment did suggest that Cd has at least a moderate mobility in the phloem, with the ^{109}Cd supplied to the basal roots of the potato plants accounting for the majority of Cd found in the tuber at harvest. Following on from previous studies, this would suggest that the major pathway for Cd movement into the tuber is via translocation from the leaves to the tubers in the phloem.

An alternative possibility for the movement of Cd from the basal roots to the tuber, is by a direct xylem connection from the basal roots to the stolons via the underground portion of the stem, thereby bypassing the leaves and the phloem altogether. Although initial studies by Kratzke and Palta (1985) showed some evidence for the existence of such a connection, follow up studies found no indication that this pathway was used to transport Ca (Kratzke and Palta 1986). When additional Ca was added to the basal roots, the authors found no difference between the Ca concentration of the tubers when compared to the control plants.

The divided pot system used in this instance was similar to that used in the current study (see Section 3.3.2).

The fact that xylem flow is driven by the evaporative demand makes it unlikely that this 'bypass' pathway would be important in supplying Cd to the tubers. Although tubers are transpiring organs their evaporative demand in comparison to that of the leaves is negligible (Kratzke and Palta 1985) and is only likely to have some influence during dark periods when water loss from the leaves is minimised. In fact, the experiments by Kratzke and Palta (1985) where water soluble dyes were used to trace the movement of water from the basal roots to the tubers showed that only a slight trace of dye at the stem-stolon junction could be found after a 24 hour period when the tubers had been exposed to the air to increase their evaporative demand. In the majority of plants, the dye from the basal roots always bypassed the stolons. Even plants grown for over three months, and having multiple dark periods for potential movement along this route, gave no indication that this was a viable pathway for nutrient uptake into the tuber.

One further reason for suggesting that it is unlikely Cd could move from the basal roots directly to the stolons and tubers, is the concentration of Cd in the tubers at harvest, compared to that in the leaves. The concentration of different nutrients in growth sinks, such as the tuber, in relation to that in the source leaves, is often used as an estimation of phloem mobility (Baker and Moorby 1969, Marschner 1995). Highly phloem mobile nutrients, such as potassium and phosphate, are generally assumed to have similar concentrations in sink regions as in source regions, whereas phloem immobile nutrients have very low concentrations in the growth sinks. Baker and Moorby (1969) showed that this assumption was true for potatoes. The concentration of ^{45}Ca found in tubers was less than 1% of that in the leaves, whereas the ratio of ^{32}P in the tubers compared to the leaves was approximately 1:1. In addition, experiments using a strontium isotope (^{89}Sr) found that

less than 0.1% of the ^{89}Sr found in the leaves had moved to the tubers. Although Sr is not an essential nutrient of plants, it is believed to travel exclusively in the xylem (Wiersum 1966, Baker and Moorby 1969). The concentration of Cd in the tubers in this study was approximately 20% of that in the leaves, and although this is much less than for elements that are highly phloem mobile, the tuber to leaf ratio of Cd was comparable to that of other elements, such as Fe and B (Norbert Maier, unpublished data), that are known to have an intermediate phloem mobility (Marschner 1995).

Uptake from the basal roots could not account for all of the Cd found in the tuber at harvest. A significant portion (15-20%) was found to have originated from ^{109}Cd applied to the tuber zone. This suggested either uptake from the stolon and tuber roots, or direct uptake across the periderm of the tuber, or both.

The suggestion of Cd movement from the stolon roots directly to the tuber in the xylem is in keeping with the other studies in relation to Ca and water movement mentioned above. Water soluble dyes moved readily into the xylem ring of tubers when applied to the stolon or tuber roots (Kratzke and Palta 1985) and the application of additional ^{45}Ca to the tuber region resulted in a 3-fold increase of ^{45}Ca in the tuber (Kratzke and Palta 1986). The contribution of the stolon and tuber roots to the concentration of Cd in the tuber was not as great as for Ca, where it has been suggested that up to 60% of the Ca may enter the tuber from this pathway (Davies and Millard 1985). This is consistent with the suggestion that the tuber receives a large amount of Cd via import from the phloem.

The finding that ^{109}Cd applied to the tuber zone was recovered in the leaves reinforces the proposal by Kratzke and Palta (1985, 1986) that there exists a continuous xylem connection from the stolon roots to the aerial parts of the plant. This pathway would most likely provide additional water and nutrient movement during times of high evaporative demand from the leaves. With the existence of this pathway it could be proposed that Cd

could move from the stolon roots to the leaves in the xylem, followed by translocation from the leaves to the tubers in the phloem. This is unlikely, however, as the proportion of Cd moving from the stolons to the leaves was only very small and is not enough to account for the concentration in the tubers when compared to the concentration gradient from the leaves to the tubers when ^{109}Cd was applied to the whole root zone.

In addition to uptake by the stolon roots, the possibility that Cd could be transported directly across the periderm to the internal tissues of the tuber warrants some consideration. This uptake pathway was first proposed by Krauss and Marschner (1971) as an explanation for the high concentrations of Ca in the tuber relative to what was expected from a nutrient believed to be largely immobile in the phloem. They reported a rapid uptake of Ca when a solution containing ^{45}Ca was applied to the tuber surface.

Results from this study, however, indicate that uptake via movement across the periderm does not occur and that this proposed pathway is not a source of tuber Cd. Firstly, there was no significant depletion of Cd from the solutions in which the tubers were bathed (Figure 3.4). Secondly, although Cd was found in the periderm of most of the tubers, Cd did not move into the internal tissues from the periderm during the experimental period. It is likely that this pool of Cd was not available for transport to the internal tissues, possibly due to apoplastic binding in the cell walls. One tuber did show some ^{109}Cd activity within the first 5 mm of the internal tissues, but this was explained by the loss of integrity of the cortical cells due to deterioration of external layers of the tuber after being in solution for 7 days. However, no other tubers displayed any deterioration during this time.

Uptake of Ca along this pathway may well have been exaggerated in the past as uptake from roots on the stolon may not have been considered. Davies and Millard (1985) grew intact tubers in the presence of ^{45}Ca and measured the activity in different layers of the tuber. They concluded that although a large amount of Ca was found in the periderm, it

contributed very little to the Ca concentrations of the internal tissues. Although the ^{45}Ca was not supposed to be in contact with any roots, the ^{45}Ca found in the internal tissues, as displayed by autoradiograph, was associated mostly with the vascular ring and the area where the stolon connected to the tuber, indicating that this connection and any roots associated with it, may have been a more likely source of the internal Ca. The autoradiographs showed no ^{45}Ca associated with the cortical cells between the periderm and the vascular ring. In addition, the early work by Krauss and Marschner (1971), while effectively separating the tubers from the basal roots, did not effectively limit the application of ^{45}Ca to the tuber surface alone as it was applied as an aerosol and would have been available for uptake by any roots present on the stolon.

Data presented within this chapter provide strong evidence that Cd is not transferred directly to the tuber by a xylem connection from the basal roots. Instead, movement of Cd to the tubers must be via the shoots and ultimately involve transport within the phloem. Given the importance of long distance phloem transport in the supply of Cd to the tuber, further experiments designed to trace the movement of Cd in the xylem and phloem were carried out and are discussed in the following chapter.

Chapter 4

Long Distance Transport: the Role of Xylem and Phloem

4.1 Introduction

Long-term growth of potato plants in ^{109}Cd labelled soil revealed the overall pattern of Cd accumulation and distribution within the plant and implied that Cd has at least a moderate mobility in the xylem and phloem. However little information was provided on the short-term movement of newly absorbed Cd, including how Cd is exported from one tissue to another, and the direction of transport. For example, once Cd is loaded into the phloem in the leaves is it transported preferentially to the tubers, or does the root represent a viable sink with which the tuber competes?

Although Cd is a non-essential element for plants, it is taken up effectively by both the leaf and root systems (Senden and Wolterbeek 1990), hence ^{109}Cd can be used as an isotopic tracer to examine the role of xylem and phloem in long-distance transport of Cd to the tubers of potatoes. One method for determining the phloem mobility of an element is to apply an isotopic tracer to the leaf surface and monitor its subsequent movement throughout the plant. The movement of solutes in the xylem is unidirectional, driven by the 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.

Isotopic tracers have been used extensively to monitor long-distance transfer of most essential elements, but it is only recently that this technique has been used to examine the movement of Cd within the plant. Cakmak *et al*. (2000a) have reported substantial

genotypic variation in the uptake and translocation of leaf-applied ^{109}Cd in wheat. Cakmak *et al*.(2000b) also investigated the influence on zinc supply on the uptake and distribution of leaf-applied Cd.

4.2 Objectives

The aim of this experiment was to follow the pathway of Cd in both the xylem and the phloem of two potato cultivars. This was achieved by applying ^{109}Cd to either the roots or the leaves of mature plants and monitoring the pathway of Cd from the roots to the leaves, or from the leaves to the tubers. Movement of ^{109}Cd from the roots to the leaves was assumed to take place in the xylem and, conversely, any movement of ^{109}Cd from the leaves to other parts of the plant was assumed to be via translocation in the phloem.

4.3 Methods

4.3.1 Experimental design

The experiment studied three factors, cultivar type, site of application and the time after application that harvest occurred (Table 4.1). ^{109}Cd was applied to the 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 hours after application. When supplied to the roots, plants were harvested 30 and 48 hours after application. Each treatment was replicated three times.

Table 4.1 Time of plant harvest in terms of hours after application of ^{109}Cd to plant leaves and roots of cultivars Wilwash and Kennebec.

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

4.3.2 Plant growth

Seed tubers of cultivars Wilwash and Kennebec were planted in 15 L free draining pots containing 13 kg of UC mix (see Section 3.3.3) and grown under glasshouse conditions (Figure 4.1). As seed tubers often produce multiple sprouts, seedlings were thinned within the first week after emergence to ensure the growth of only one stem per plant. If at any time the main stem became divided the secondary shoot was removed from the plant. Four weeks after emergence the plants were mounded with a further 2 kg of UC mix.

Of the original 40 plants grown (20 of each cultivar), 30 were used in the experiment. Plants were chosen on the basis of similarities in size and growth habit. Plants that had only a single stem were used in preference to those that had a secondary stem removed.

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.

4.3.3 Addition of ^{109}Cd to the Plant

4.3.3.1 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 paint brush. This resulted in approximately 1 MBq being applied to each plant. The source leaf was usually the 4th or 5th leaf from the growing apex and did vary in size between plants (Figure 4.2). This variation meant that the volume of solution that could be successfully applied without saturating the leaf surface, also differed. The precise amount of isotope added was determined by weighing the vial containing the ^{109}Cd and the paint brush, before and directly after application, on a four figure balance. Table 4.2 shows the surface area of the source leaf for each plant and the amount of ^{109}Cd that was applied.

Figure 4.1 Glasshouse arrangement for long-term growth experiments.

Table 4.2 The surface area and amount of ^{109}Cd applied to the source leaf of each plant. The description of the source leaf indicates time of harvest after application (T1, T2 or T3), cultivar type (W=Wilwash, K=Kennebec) and the replicate number (1, 2 or 3)

Source Leaf	Surface Area	Added ^{109}Cd	
	cm^2	mL	MBq (total)
T1W1	61.21	0.469	0.938
T1W2	59.57	0.434	0.868
T1W3	85.33	0.513	1.026
T1K1	77.68	0.439	0.878
T1K2	72.42	0.787	1.574
T1K3	91.39	0.463	0.926
T2W1	71.24	0.439	0.878
T2W2	69.01	0.495	0.990
T2W3	91.61	0.603	1.206
T2K1	64.67	0.382	0.764
T2K2	85.12	0.374	0.748
T2K3	93.67	0.539	1.078
T3W1	73.22	0.445	0.890
T3W2	55.73	0.370	0.740
T3W3	56.63	0.520	1.040
T3K1	73.14	0.380	0.760
T3K2	54.02	0.419	0.838
T3K3	66.68	0.439	0.878

4.3.3.2 Soil application

^{109}Cd was applied to the root system of each plant via application to the base of the pots where the root activity was expected to be greatest. One litre 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 mixture. After 30 minutes 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.

4.3.4 Plant harvest and analysis

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 (Figure 4.2). 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 2.3.4.

All plant material was dried, ground and digested using the methods described in Section 2.3.5. One mL of each plant digest sample was added to 2 mL of d.H_20 in a 6 mL plastic tube and the activity of ^{109}Cd in the sample was measured using a gamma counter.

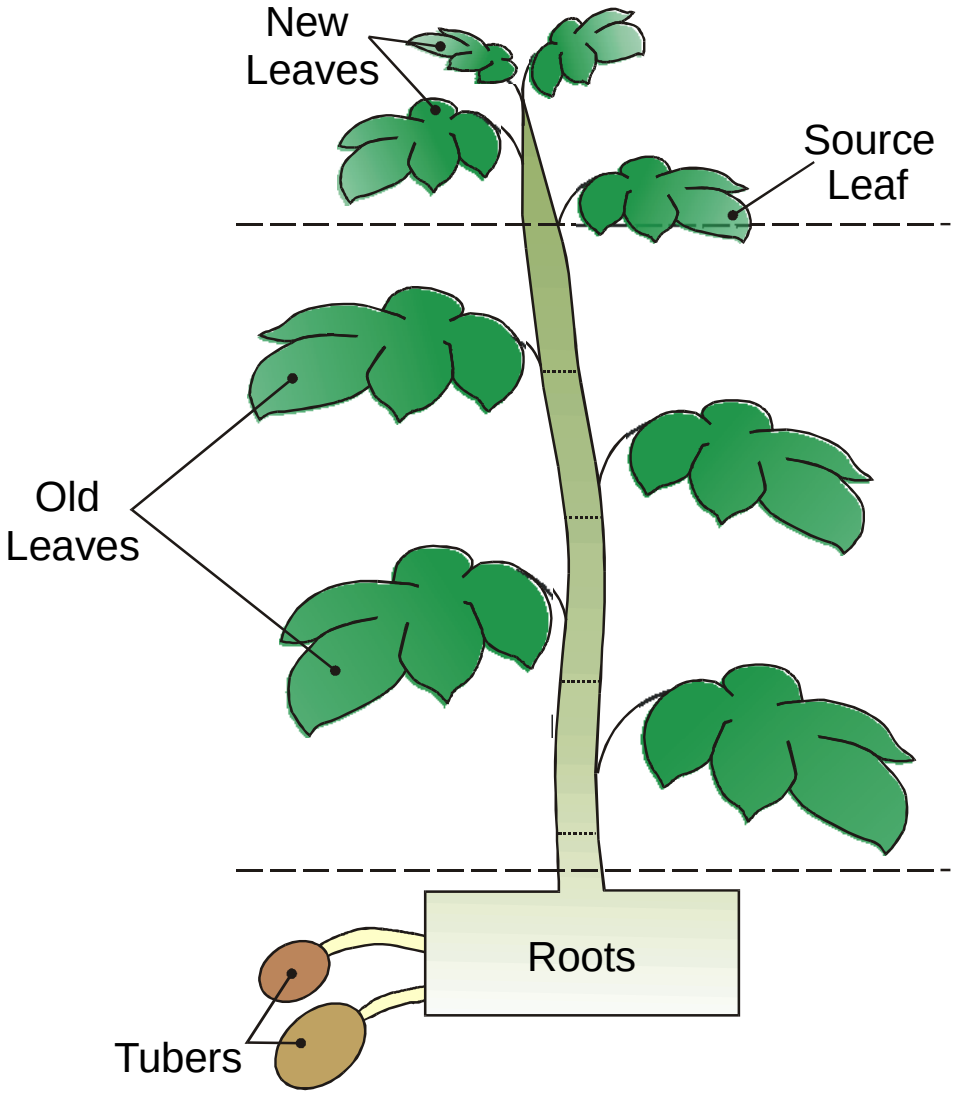


Figure 4.2 Diagram showing the site of application of ^{109}Cd to a single leaf, and the different tissues harvested to measure the movement of Cd from the source leaf.

4.4 Results

4.4.1 Soil Application

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 hours. After 48 hours more than 85% of the total plant-associated Cd was found in non-root tissues (Figure 4.3). 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. The concentrations of ^{109}Cd in the various tissues is shown in Figure 4.4. For clarity, the roots were omitted from the figure because their concentration was around 100-fold higher than in the other tissues, and it was unclear how much of this was adsorbed to the outside of the root. The amount attributed to the roots in Figure 4.3 must also be an overestimate due to the unknown apoplastic Cd. The highest Cd concentrations were found in the stems, much higher than in the leaves which in turn were higher than in the tubers. 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.

At 30 hours after application, no difference was noted in the total amount of Cd absorbed between cultivars, and the proportional distribution was approximately the same. However, after 48 hours significant differences between cultivars were apparent in the total amount of Cd, most of which was due to a much higher uptake into the stems of Kennebec (Figure 4.5).

Plants of both cultivars had approximately the same total mass and distribution of mass between plant parts, hence, differences in the Cd concentration were similar to those of Cd distribution.

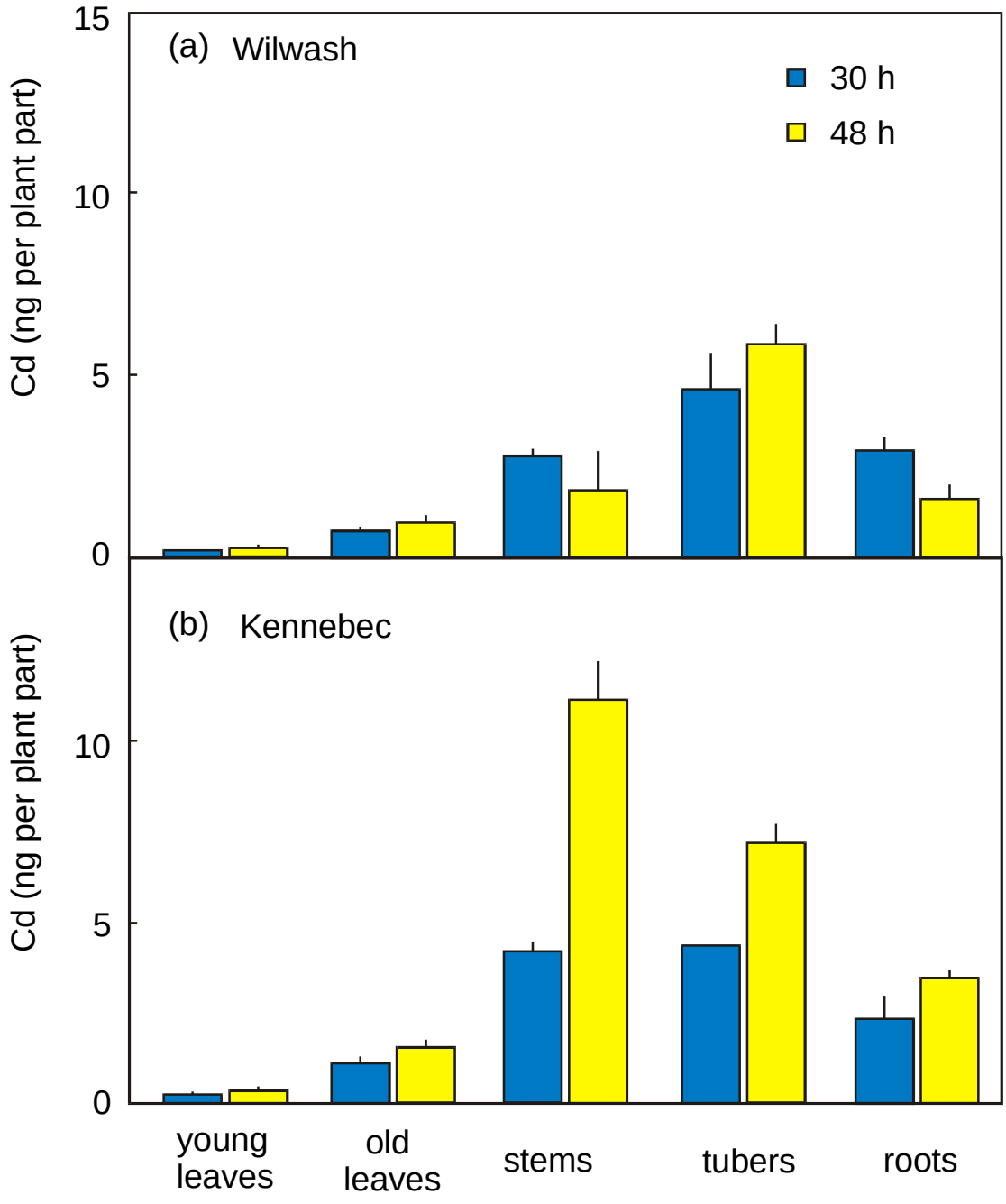


Figure 4.3 Distribution of ^{109}Cd in different tissues of Wilwash and Kennebec grown in soil labelled with ^{109}Cd. Each value is the mean ± SE of 3 plants.

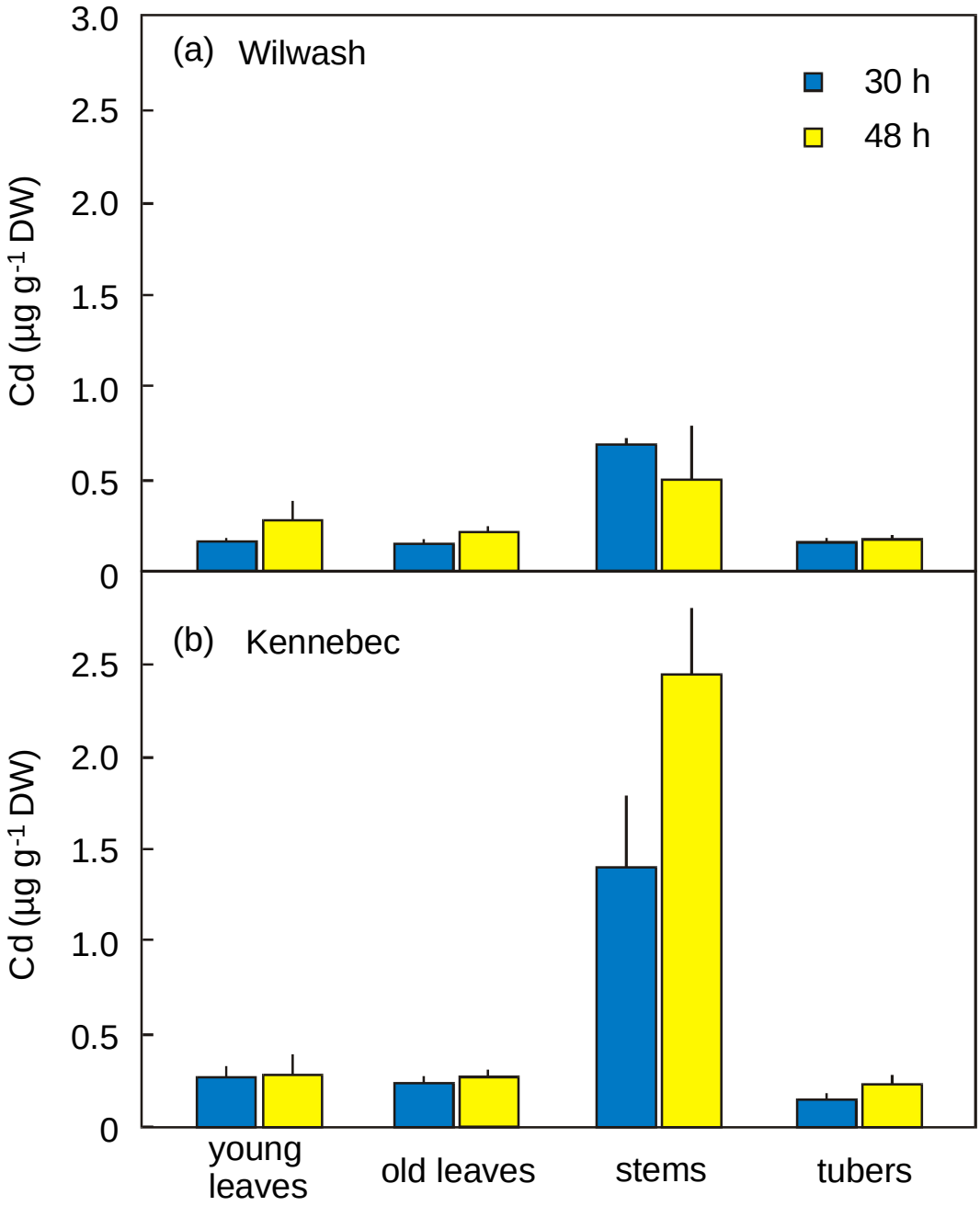


Figure 4.4 Concentration of ^{109}Cd in different tissues of Wilwash and Kennebec grown in soil labelled with ^{109}Cd. Each point is the mean ± SE of 3 plants.

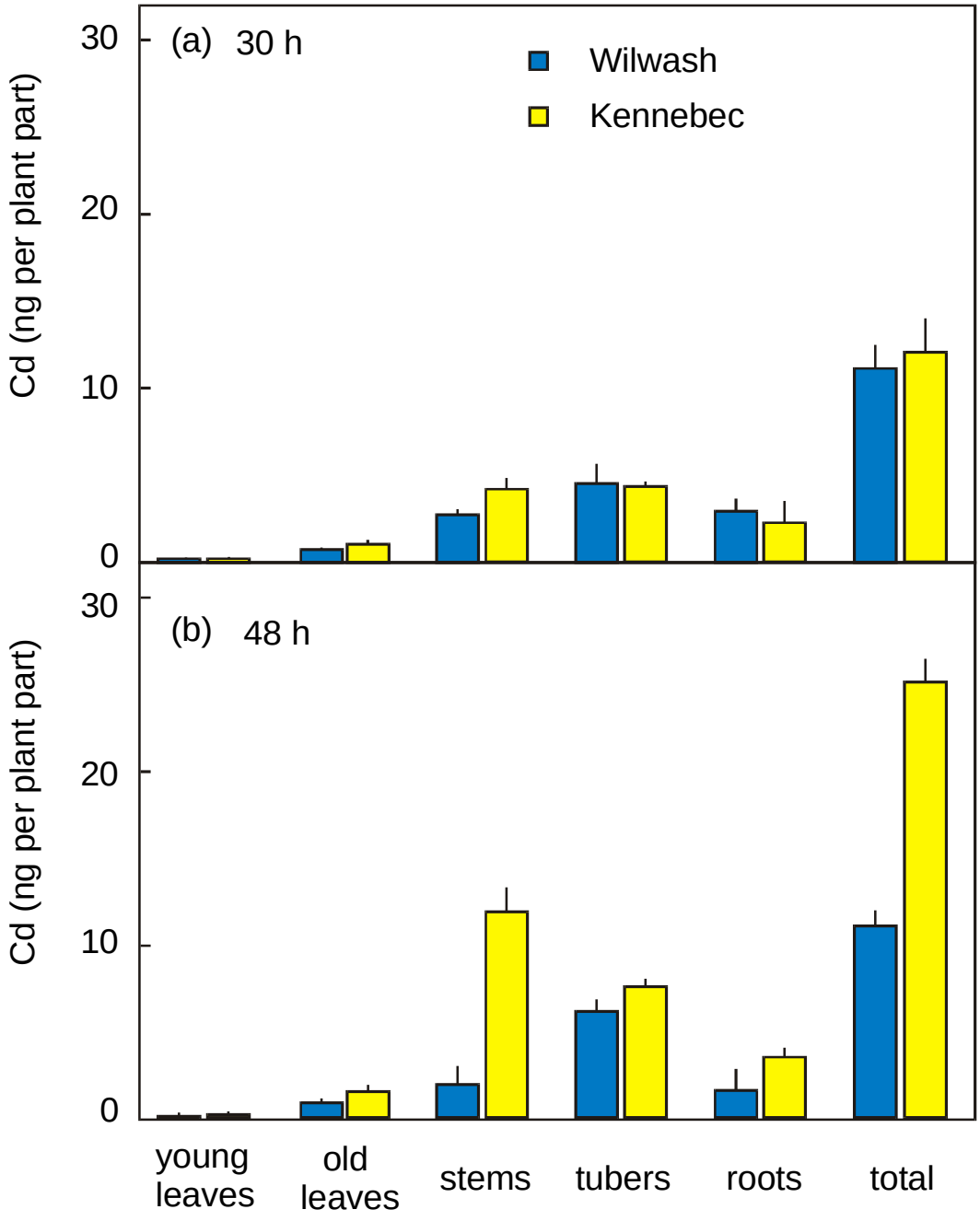


Figure 4.5 Comparison of the distribution of ^{109}Cd in different tissues of Wilwash and Kennebec grown in soil labelled with ^{109}Cd after (a) 30 h and (b) 48 h. Each value is the mean ± SE of 3 plants.

4.4.2 Leaf Application

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 hours ^{109}Cd was found in all sections of the plant, with the total amount absorbed increasing with each harvest time (Figure 4.6). By far the 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 been transported to the roots at each time interval but this represented only a small fraction of the total amount absorbed from the source leaf.

Both cultivars Wilwash and Kennebec showed similar foliar uptake. After 54 hours 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.

Despite the higher overall allocation of foliar-absorbed Cd to the tubers, the highest concentration was found in the stems of cultivar Kennebec (Figure 4.7), as occurred when Cd was absorbed from the soil (Figure 4.4).

Cultivars Wilwash and Kennebec showed the same overall trend in terms of concentration of Cd within the leaves and roots, with the notable exception of Kennebec having a higher concentration of added Cd in the stems and a lower concentration of added Cd in the tubers after 54 hours (the term ‘added’ is used to indicate Cd calculated on the basis of the specific activity of ^{109}Cd in the applied solution—it is additional to any unlabelled Cd already present).

Movement of added Cd within the phloem appeared to be rapid, with a significant increase in the concentration of added Cd in most plant parts noted between 24 and 54 hours. 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 4.8).

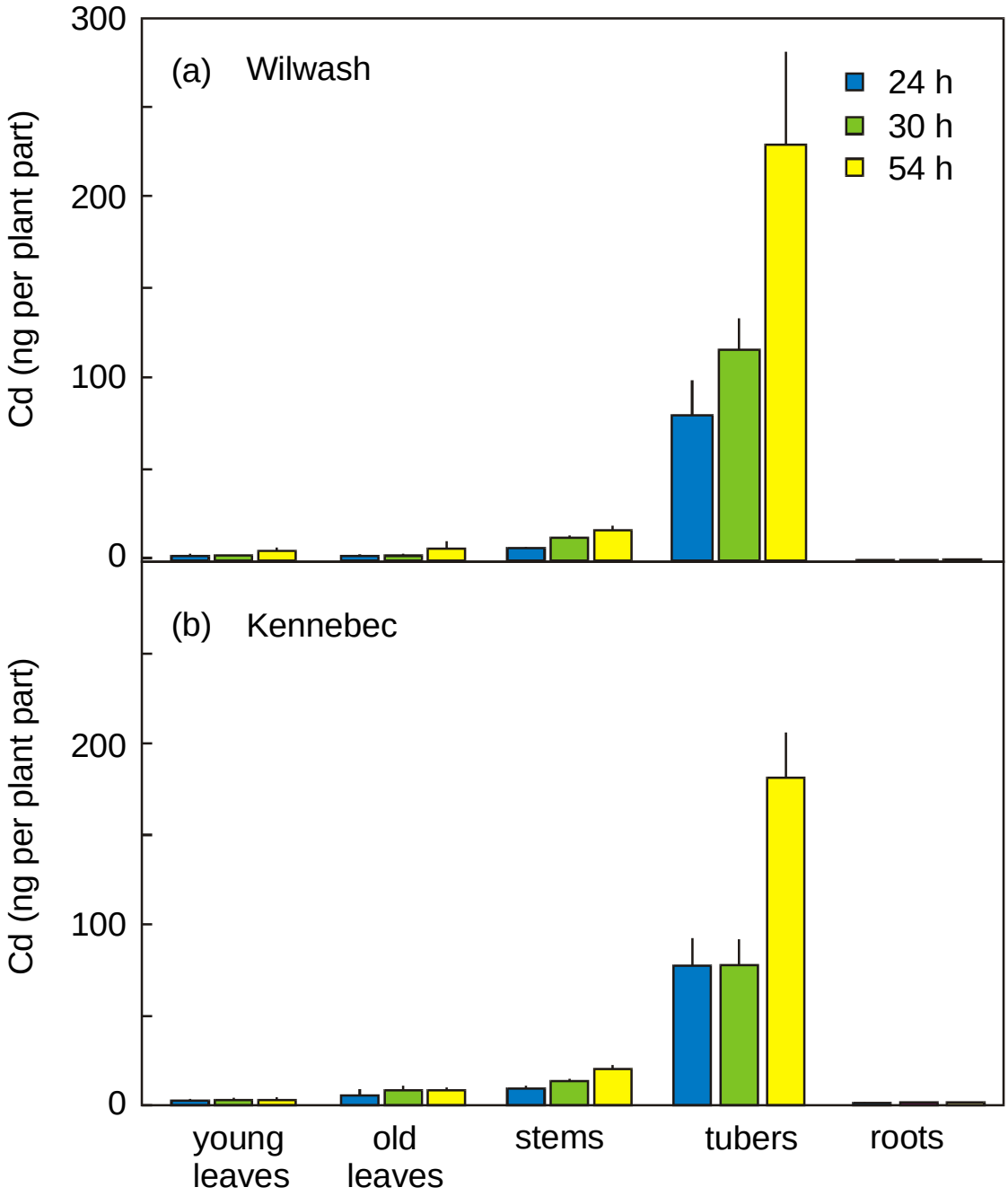


Figure 4.6 Cd content of various tissues of Wilwash and Kennebec following application of ^{109}Cd to a single leaf. Plants were harvested at 24, 30 or 54 h after leaf loading. Each point is the mean ± SE of 4 plants.

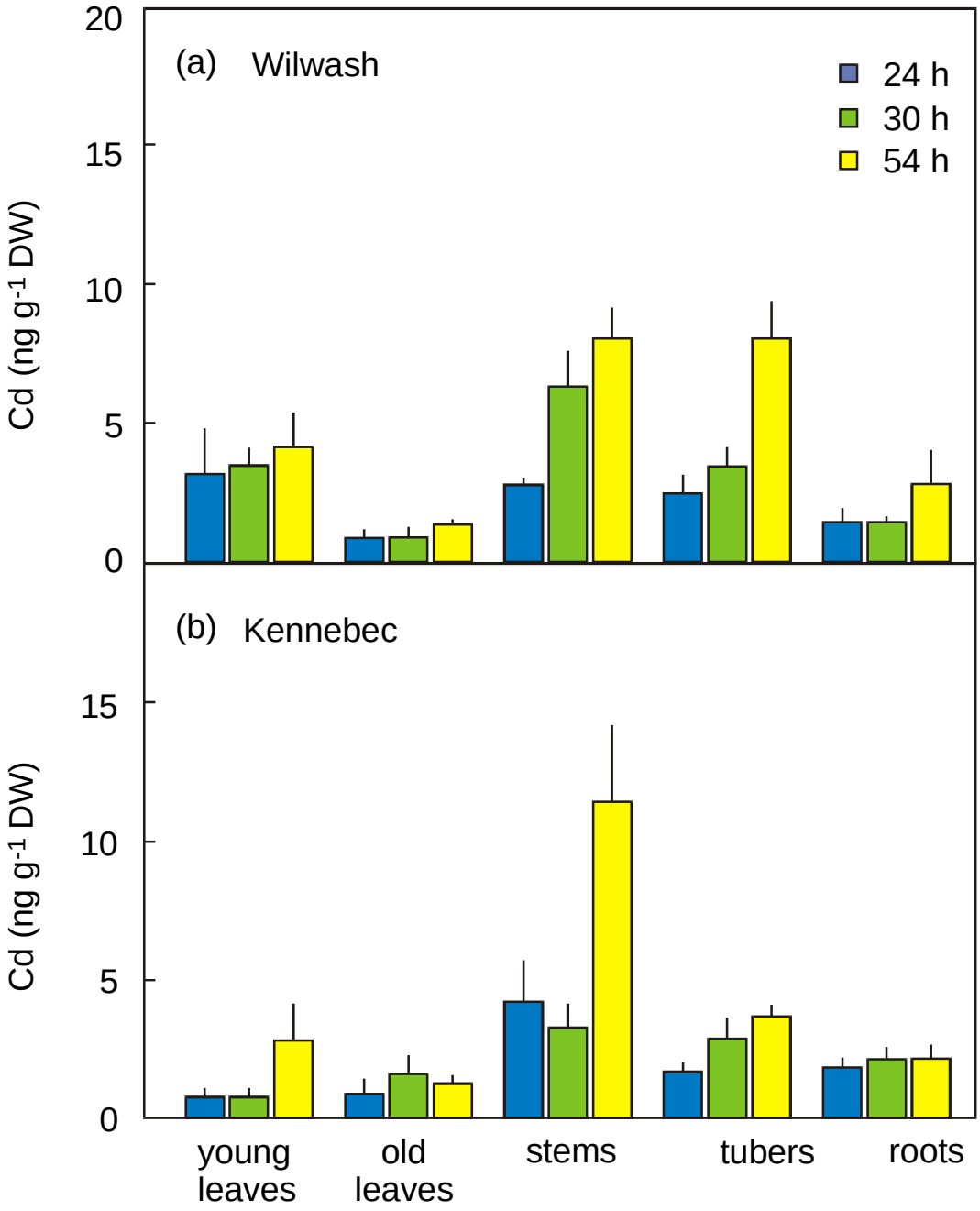


Figure 4.7 Concentrations of Cd in various tissues of Wilwash and Kennebec following application of ^{109}Cd to a single leaf. Plants were harvested at 24, 30 or 54 h after leaf loading. Each point is the mean ± SE of 4 plants.

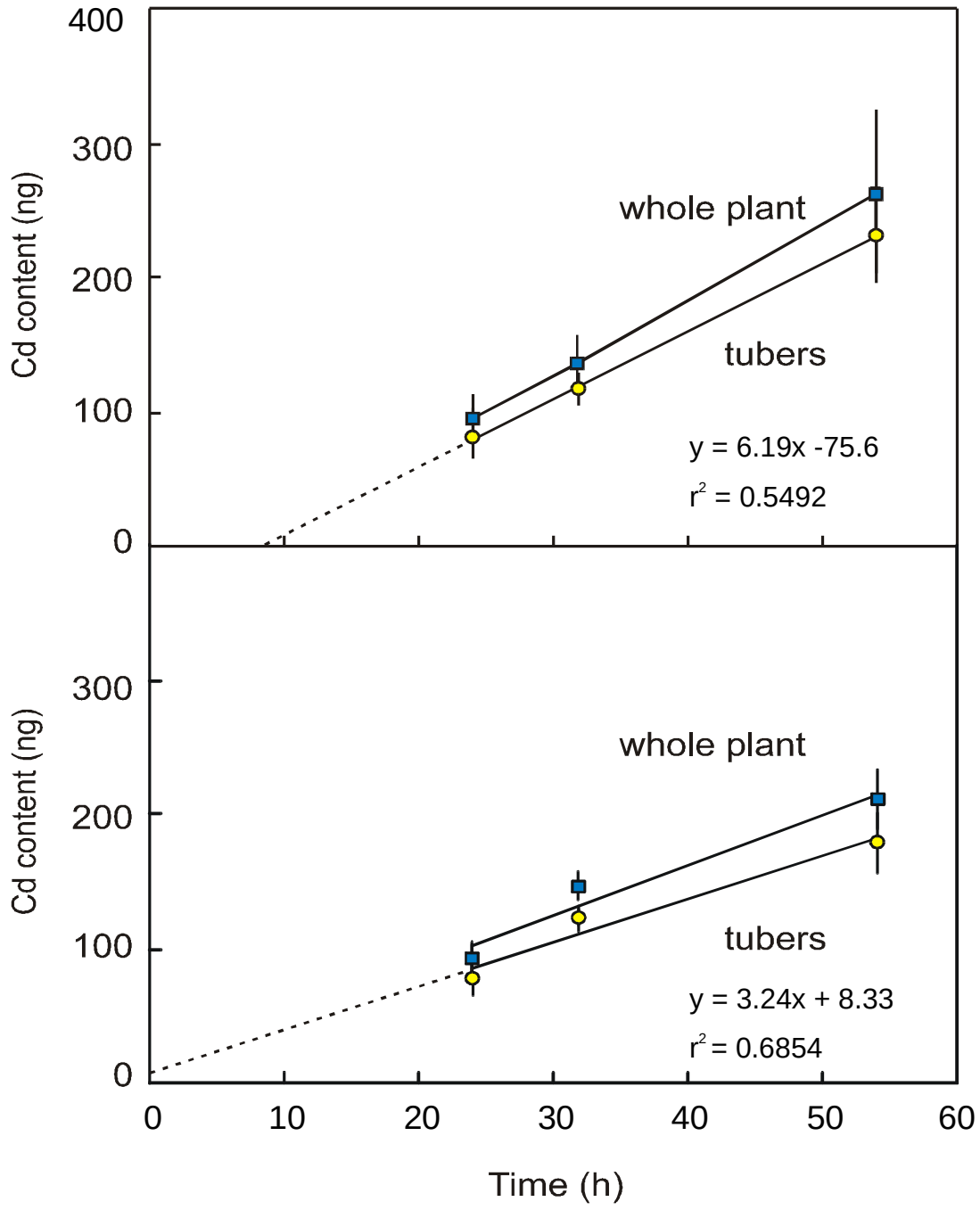


Figure 4.8 Cd content of tubers and whole plants of Wilwash and Kennebec following application of ^{109}Cd to a single leaf. Each point is the mean ± SE of 3 plants. The linear regression lines for the tuber Cd content were calculated using the individual data points not the mean values.

4.5 Discussion

The potato system is particularly useful in looking at long distance transport due to the dominant role of the phloem in supplying nutrient to the tubers. As seen in Chapter 2, after the onset of tuber bulking there is almost no growth of other tissues within the plant and no significant net input of Cd into non-tuber tissues (except the roots of cultivar Wilwash). Consequently the most active pathways are from the soil to the leaves in the xylem, due to the continual need for water to replace transpirational losses, and from the leaves to the tubers in the phloem. Results of the short-term experiment confirm 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. This supports findings of other authors who demonstrated phloem transport of leaf-applied Cd in wheat (Welch *et al*. 1999, Cakmak *et al*. 2000a) and soil-applied Cd in peanuts (McLaughlin *et al*. 2000).

During long-term growth experiments the concentration of Cd in the leaves at maturity was greater than in the stems. However, in the short-term experiment newly absorbed Cd was rapidly sequestered by the stems when applied to either the soil or to the source leaf. This does not preclude the possibility of the overall leaf Cd concentration being higher than the stems as the non-isotopic Cd pool was not measured during this experiment. Rather it focuses 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.

Senden and Wolterbeek (1990) regarded retention of Cd in the stem to be a plant dependent parameter determined by the volume of xylem flow and the anatomical xylem vessel characteristics, for example the number of exchange sites. It was assumed that the concentration of stem Cd could be largely attributed to binding in the cell walls of the xylem vessels and lateral leakage into cells of the surrounding structural tissues. However, the current data clearly show that the stems act as a sink even when Cd is transported in the

phloem (i.e. when it is applied to the leaf) and the high mobility suggests that Cd is not readily bound or precipitated.

The long-term uptake experiments indicated that the concentrations of Cd in the stems were similar or lower than those in the leaves (Figure 3.2). However the short-term experiments with added Cd applied to soil suggested that newly-absorbed Cd was preferentially sequestered from the xylem into stem tissue (Figure 4.4). Similarly, Cd applied to the leaves was accumulated to high concentrations in the stems (Figure 4.7), even though its movement must have occurred via the phloem. Thus the stem appears to be a site for removal of Cd from both xylem and phloem, and potentially a major transfer point between the two vascular systems. Data from Welch *et al*. (1999) also supports the possibility of solute exchange in the stem. They showed that in a split-root experiments with wheat, Cd absorbed by one root could be transported to the stem and then deposited in another root. This, presumably, has to involve some movement in the phloem.

This experiment does not shed any light on why Cd appears to be so mobile within the xylem and phloem, but it does suggest that some other mechanism, aside from concentration gradient, governs the movement of Cd once loaded in the xylem or the phloem, particularly if it exits and re-enters the vascular system at a number of points (i.e. the stem).

Investigations of phloem transport of other divalent cations point to the existence of specific facilitators for loading and translocation. Schmidke and Stephan (1995) reported that metal micronutrients Cu, Fe, Mn and Zn were loaded into the phloem of Ricinus in stoichiometric proportions to the endogenous chelator nicotianamine, but more recent work suggests that divalent metals are transported as complexes of metal binding proteins within the phloem (Krüger *et al*. 2001). Equivalent data is not available for Cd and it is therefore difficult to make any meaningful comment about its likely speciation in phloem. However the physical chemistry of Cd differs from these micronutrient cations in forming strong

complexes with sulphydryl groups (e.g. on proteins) and Cl, both of which are usually abundant in the phloem.

Previous studies (Cakmak *et al*. 2000a, Picchioni *et al*. 1995) have cited the importance of leaf surface characteristics in determining genotypic differences in the uptake of leaf-applied nutrients and divalent metals. Although it is difficult to accurately determine the amount of Cd that was taken up by the source leaf, the total amount of foliar applied Cd redistributed to other parts of the plant was not affected by cultivar type during this experiment. This suggests that transport processes, rather than structural or anatomical differences in the leaf, were responsible for any differences in the final distribution and concentration of foliar-applied Cd within each cultivar. It does not rule out the possibility of a higher absorption rate by one cultivar coupled with greater retention, perhaps through binding in the cell walls, in the source leaf. However, it seems unlikely that this process would be matched so well between cultivar that the same total amount of ^{109}Cd is redistributed to the remainder of the plant.

When discussing the cultivar differences of long-term uptake and distribution of Cd in Chapter 2, it was put forward that differences in the root concentration of Cd may be due to the recycling of nutrients in the phloem and the ability of the roots of cultivar Wilwash to compete more efficiently with the tubers for the movement of photosynthate from the leaves. The short-term loading experiments clearly showed that the roots (as well as other leaves) are a viable sink for Cd applied to the leaves, with significant concentrations of added Cd being noted in the roots after just 24 hours. However, remobilisation of added Cd from the source leaf to the roots did not differ significantly with cultivar. Perhaps more significant however, is the huge difference in the amount of leaf applied Cd that was allocated to the tubers compared to the roots. The amount of added Cd transported from the source leaf to the tubers was at least two orders of magnitude greater than that allocated to the roots and is likely a reflection of the massive input of phloem required during tuber

bulking. As a result it seems highly unlikely that re-translocation of Cd from leaves to roots could explain the large differences in root Cd content between the cultivars. More probable is that Cd absorbed from the soil is more strongly retained in the roots of Wilwash.

This work has demonstrated the high mobility of Cd in the xylem and phloem and the apparent importance of the stem as a conduit for Cd, and most likely other mineral elements, to the tubers. If the stem is indeed acting as a intermediary storage pool for mineral element transfer, it may be that structural or physiological differences exist between cultivars.

Chapter 5

Interactions Between Zinc and Cadmium

5.1 Introduction

Significant interactions have been found to occur between Zn and Cd in their accumulation by plants (Welch *et al*. 1999). In fact, several studies have examined the relationship between zinc application and Cd uptake and the resulting Cd/Zn ratios. Abdel-Sabour *et al*. (1988) showed that applied Cd significantly increased the ratios of Cd/Zn in Swiss chard and corn, and Takijima *et al*. (1973) reported a high correlation between the soil extractable Cd and Zn concentrations and the Cd/Zn ratio in rice. Increasing Zn application has been found to reduce Cd accumulation in flaxseed (Moraghan 1993, Grant and Bailey 1997), in lettuce and spinach (McKenna *et al*. 1993), and in durum wheat grain when grown on zinc deficient soils (Choudhary *et al*. 1994, Oliver *et al*. 1994). In contrast, no interaction was noted in studies by Cunningham *et al*. (1975) and White and Chaney (1980), and synergistic effects were found by Williams and David (1976).

Soil chemical processes could possibly explain inhibition of Cd with increased application of Zn. Hart *et al*.(2002) suggest that this is unlikely as Zn^{2+} and Cd^{2+} have been shown to compete for exchange sites on soil particle surfaces (Basta and Tabatabai 1992) and, hence, increasing the Zn should result in the displacement of Cd^{2+} from soil particles, leading to increased availability of Cd^{2+} in the soil solution and increased uptake. Instead, they suggest that Zn activity affects a biological process such as uptake or translocation.

Cataldo *et al*. (1983) proposed that Zn, along with some other micronutrient metals, might be a competitive inhibitor of Cd transport in higher plants and that both metals may employ a common transporter. Recent studies give further support to this hypothesis with a

family of Zn transporter genes (ZIPs) from *Arabidopsis* being found to be inhibited by Cd (Grotz *et al*. 1998). Results from solution culture experiments investigating transport interactions between Cd and Zn in bread and durum wheat seedlings (Hart *et al*. 2002) showed that Cd^{2+} uptake was inhibited by Zn^{2+}, and Zn^{2+} uptake was inhibited by Cd^{2+}. The authors concluded that the competitive interaction was due to the sharing by Cd and Zn of a common transport system at the root cell plasma membrane. In addition to competitively inhibiting Cd uptake at the plasma membrane, Zn has also displayed an inhibitory effect on phloem-mediated transport (Cakmak *et al.* 2000b), suggesting that interactions are not only important at the point of uptake (by the roots) but that they may be important in the resulting distribution of Cd within the plant.

Although Cd transport has been linked to other metal transport systems, inhibition of Cd uptake by Zn, particularly in Zn-deficient soils commonly found in Australia, is a useful agronomic option on a broad agricultural scale, as has been demonstrated by Oliver *et al*. (1994) for wheat and McLaughlin *et al*. (1995) for potatoes. The mechanisms responsible for this inhibition in potatoes have not yet been examined.

5.2 Objectives

The objective of this study was to further investigate the interactions between Zn and Cd by supplying potato plants grown in nutrient solutions with varying Zn/Cd concentration ratios. 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 with ^{65}Zn and ^{109}Cd in differing ratios so that the final treatments were a combination of high and low Cd, and high and low Zn.

5.3 Methods

5.3.1 Plant growth

Seed tubers of cultivars Wilwash and Kennebec were planted in approximately 4cm of perlite and misted with dH_2O until moist. Trays were placed in a growth cabinet and tubers were left to sprout at room temperature under a 24 h light regime for 3 weeks, at which time the seedlings were approximately 10 cm tall. Care was taken to ensure that they did not dry out at any time by regular misting with dH_2O. Seedlings of similar size and growth habit were chosen for the experiment and carefully removed from the trays 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. The composition of the nutrient solution was the same as that used for the uptake and partitioning experiment detailed in Chapter 2 (see Table 2.2). Containers were covered with aluminium foil to ensure no light entered the nutrient solution. At the end of the pre-growth period the final nutrient solution was analysed to check for depletion of macro- and micro-nutrients. No significant depletion was seen after 7 days, except for boron, which had decreased by a factor of 3.

5.3.2 Experimental design

At the beginning of the experimental period plants were placed in treatment pots containing 1 L of fresh nutrient solution (minus Cd and 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 5.1) with 3 replicates of each treatment. Plants were grown in the treatment solution for 7 days. They were checked daily and kept topped up to 1 L volume with the Cd- and Zn-free nutrient solution. The treatment solution consisting of

low Cd and low Zn contained concentrations of Cd (0.01 µM) and Zn (1 µM) consistent with the nutrient solution used in the previous pot experiment (see Chapter 2). In reference to this experiment the term 'low' is not meant to mean deficient.

Table 5.1 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

5.3.3 Plant harvest and analysis

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 minutes. 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 in Section 2.3.5. One mL of each plant digest sample was added to 2 mL of $d.H_20$ 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.

5.4 Results

5.4.1 Cadmium concentration

Kennebec showed a significantly higher concentration of Cd in the roots than Wilwash at both low and high external concentrations of Cd (Figure 5.1a). It was expected that an increase in Zn activity would result in competition with Cd for uptake and show a decreased Cd concentration in the roots. There was no evidence of competition between Cd and Zn in Wilwash at high and low Cd concentrations. However, some evidence of competition was noted for Kennebec when the external Cd concentration was high. In this case increasing the external concentration of Zn resulted in a significant decrease of Cd in the roots.

The amount of Cd transported from the roots to the shoots did not differ significantly between cultivars, with shoot concentrations between Wilwash and Kennebec being similar in all treatments (Figure 5.1b) despite the fact that the concentrations in the roots were significantly higher in Kennebec. There was no evidence of competition between Cd and Zn at low Cd concentrations, but there was an of Zn on the transport of Cd to the shoots in Wilwash at high external concentrations of Cd (Figure 5.1b).

5.4.2 Zinc concentration

Zinc concentrations in the roots of Kennebec showed a similar pattern as for Cd, with significantly higher concentrations noted at both low and high external Zn. A similar trend as for Cd was also noted when external Zn concentrations were high, with increased Cd resulting in a decrease in Zn concentration in Kennebec (Figure 5.2a). This suggests some level of reciprocal competition between Cd and Zn when both metals are supplied to the plant at high concentrations. Interestingly, high Cd inhibited Zn uptake at low Zn concentrations in roots of Wilwash but not in Kennebec. The opposite was true at high Zn

where Cd reduced Zn uptake in Kennebec, but the addition of Cd did not significantly affect Zn uptake (Figure 5.2a).

The effect of Cd on the transport of Zn to the shoots appeared to be small, however the overall trend was similar to that on uptake to the roots with some evidence of competition being noted in Wilwash at low external Zn concentration and in Kennebec at high external zinc concentration, but this might simply be a reflection of the differences in root Zn concentration (Figure 5.2b).

5.4.3 Whole plant uptake

When total plant uptake was examined on a dry root basis, the interactions between Cd and Zn on the uptake of these two metals appeared complex and variable. The overall trends between treatments were similar for both Zn and Cd uptake, but the apparent interactions varied between treatment and cultivar. 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 of Cd uptake. Similarly, when external Cd was high, significant inhibition of Cd by Zn was noted in Kennebec, but a significant increase in uptake of Cd was found in Wilwash (Figure 5.3a). The effects of Cd on Zn uptake virtually 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 external concentrations of Zn (Figure 5.3b).

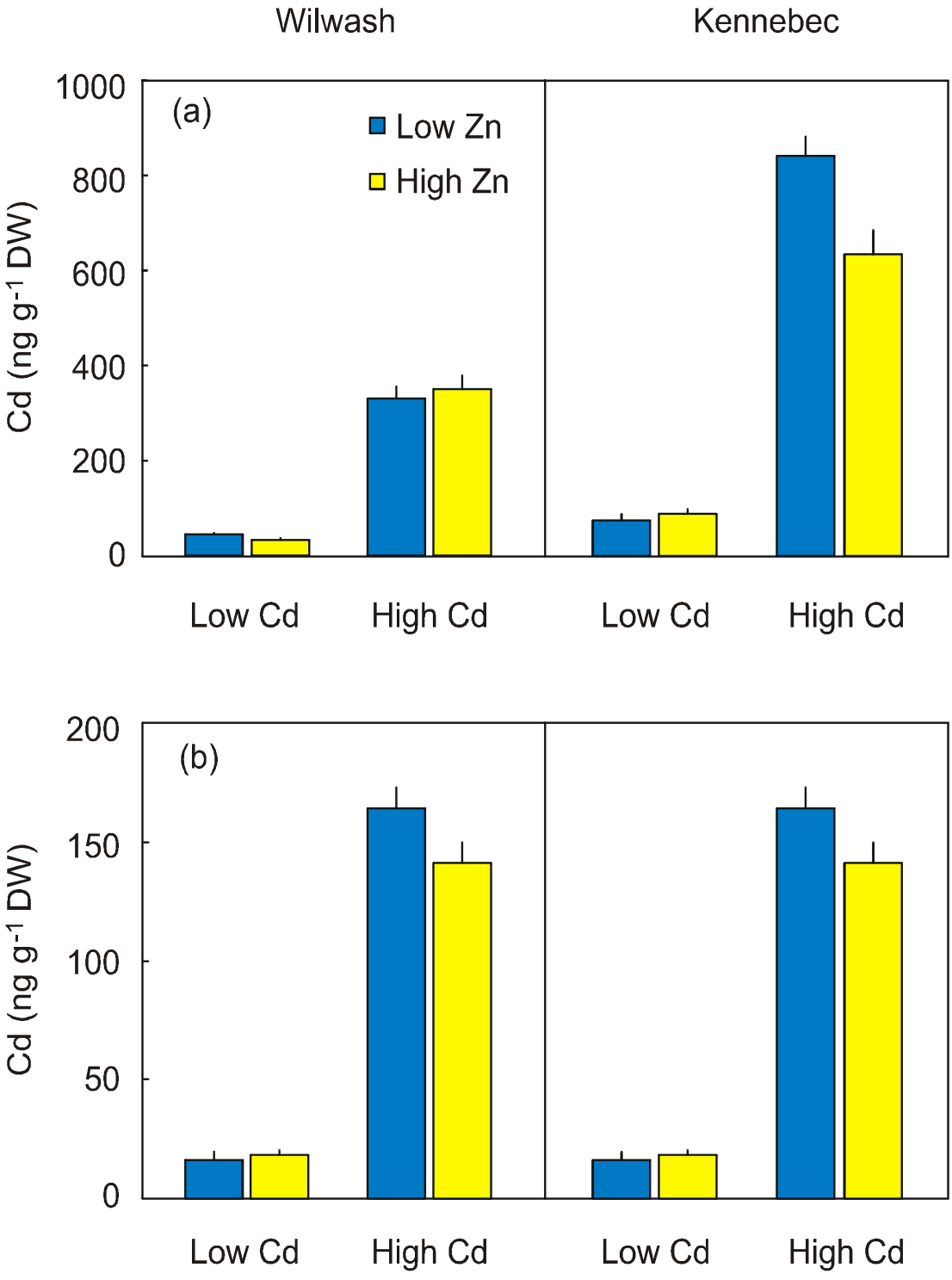


Figure 5.1 ^{109}Cd concentrations in (a) roots and (b) shoots of Wilwash and Kennebec as a function of the Zn concentration in the uptake medium. Plants were grown in nutrient solution for 7 days. Each point is the mean ± SE of 3 replicates.

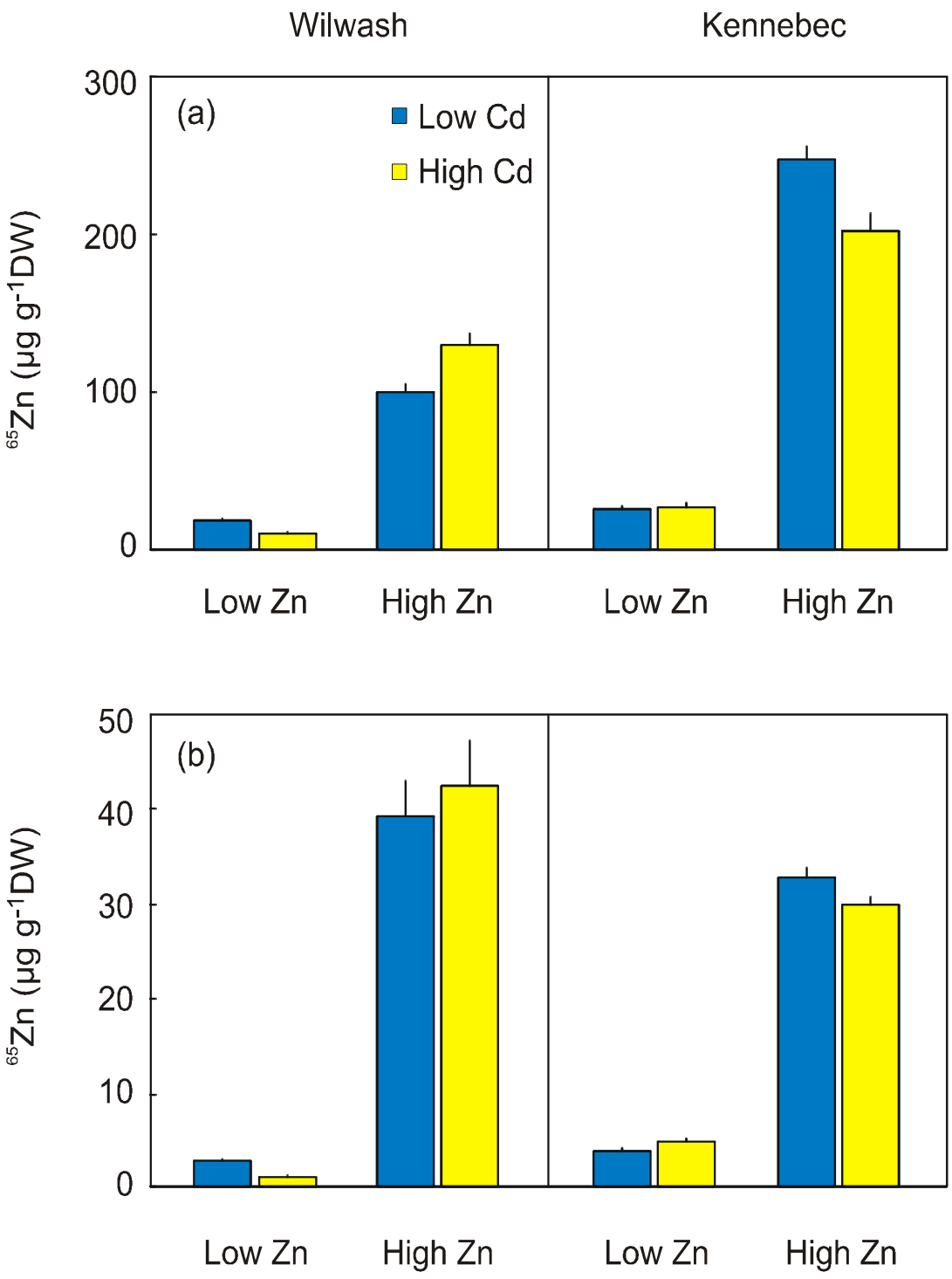


Figure 5.2 ^{65}Zn concentrations in (a) roots and (b) shoots of Wilwash and Kennebec as a function of the Cd concentration in the uptake medium. Plants were grown in nutrient solution for 7 days. Each point is the mean ± SE of 3 replicates.

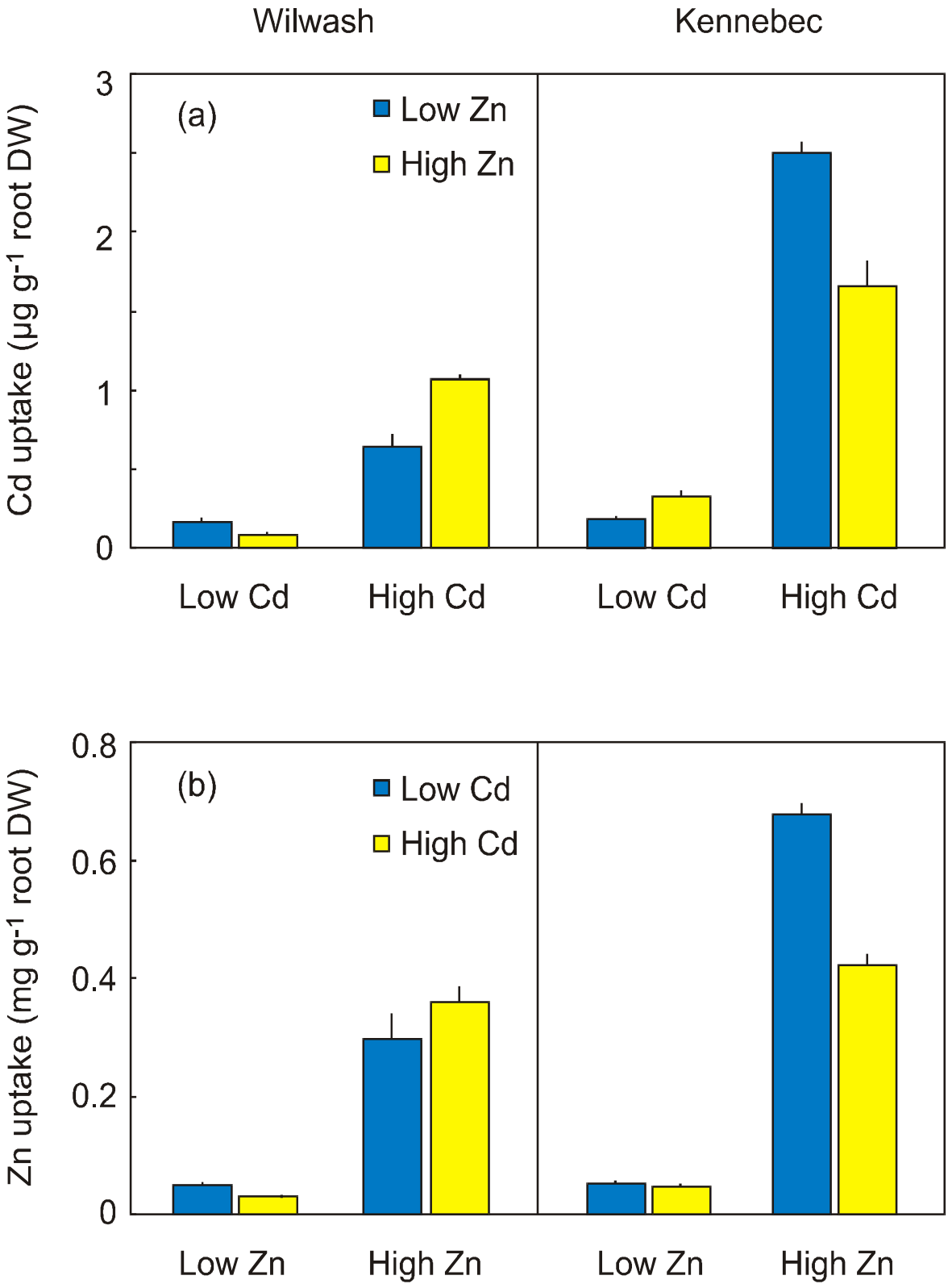


Figure 5.3 Influence of (a) Zn on uptake of ^{109}Cd and (b) Cd on uptake of ^{65}Zn. Uptake is expressed as the amount of Cd or Zn in the plant divided by the root DW. Plants were grown in nutrient solution for 7 days. Each point is the mean ± SE of 3 replicates.

5.5 Discussion

The results of these short-term uptake experiments provide some interesting insights into the consequences of addition of Zn to try to limit Cd uptake. It was clear that in Wilwash, addition of high levels of Zn would reduce Cd uptake but only under conditions where the Cd concentration was low. At high Cd concentrations, addition of Zn would actually increase the concentration of Cd. For Kennebec, high levels of Zn could limit Cd uptake from high external concentrations. Unfortunately at low Cd concentrations, Zn appeared to stimulate Cd uptake.

Mechanistically this data is hard to interpret, especially in the absence of any hard data on the pathways for membrane transport of Zn and Cd. It was quite clear from the reciprocal effects of Cd and Zn in the total uptake data (Figure 5.3) that there are significant interactions between the two elements. However, not all the results support a competitive interaction between Cd and Zn for uptake. For some treatments the effects were synergistic.

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 between Cd and Zn for uptake seen in some other plant species. For example, Hart *et al*. (2002) found that Cd uptake was inhibited by Zn, and Zn uptake was inhibited by Cd in both bread and durum wheats when grown in solution culture and supplied with ^{109}Cd and ^{65}Zn. These authors hypothesised that Cd and Zn share a common transport system on the root plasma membrane, and may therefore compete for binding sites. This seems reasonable for the competitive response of Zn on Cd uptake, however given the lower ratio of Cd to Zn, can this substitution hypothesis explain the Cd competition on Zn uptake? Interestingly, data

from Hart *et al*. (2002) showed that this transport system had a greater affinity for Cd than for the essential nutrient Zn, even though Cd was less abundant in the external solution.

In a recent review of nutrient uptake mechanisms in plants, Reid and Hayes (2003) proposed that trace metals may leak through non-selective cation channels and that this may be the dominant pathway for uptake at higher concentrations. At low concentrations though, uptake was considered to be due to more selective carriers that were induced in response to low plant status of that metal. It is in this higher affinity range that competition would be expected.

As Cd is a non-essential element it is unlikely that competition for uptake would occur at a transporter specifically targeting Cd. Two other possibilities seem likely—that Cd competes for uptake by a Zn transporter, or that both Cd and Zn compete for uptake by either a low affinity generic divalent cation transporter, or a high affinity transporter specific to another divalent metal cation. Recent molecular studies have indeed identified and characterised a family of Zn transporter genes (ZIPs) from the plant *Arabidopsis* (Grotz *et al.* 1998). The expression of these genes in a yeast mutant lacking both high and low affinity Zn uptake systems was found to decrease the resulting growth defect and led the authors to suggest that these genes might encode Zn transporters. The ZIP1 and ZIP3 genes were found to be expressed in the roots of Zn-deficient plants, while ZIP4 was found in both the roots and shoots of Zn-deficient plants. Further investigation of these transport genes found that Zn uptake by ZIP1 was not inhibited by Mn, Ni, Fe or Co, but was inhibited by Cd and Cu. ZIP3 was inhibited by Cd as well as Mn, Fe, Co and Cu.

A second family of transporter genes known to transport Cd is the Nramp family. Thomine *et al*. (2000) found that *Arabidopsis* Nramp genes encode metal transporters with a broad specificity, and contribute to the sensitivity of metals, including Cd, in plants.

In relation to the competitive inhibition demonstrated in the current study, is it likely that uptake of Cd and/or Zn by one of these described transporters could explain the observed results? In the case of Kennebec, competitive inhibition of Cd and Zn was noted only when the external concentration of Zn, was high. Under these conditions (10µM Zn) Grotz *et al*. (1998) found no evidence of the expression of the ZIP1 gene, although there was some small expression of the ZIP3 gene. In Wilwash there was some evidence of competition when the external concentration of Zn was low. Although these concentrations of Zn were not considered as deficient, they were an order of magnitude lower than the Zn-sufficient concentrations reported in Grotz *et al*. (1998) and raise the question as to whether expression of these transport genes could be occurring, and if so, does their occurrence differ between cultivars. Nramps are known to be expressed in both roots and shoots of *Arabidopsis* under non-limiting metal conditions (Thomine *et al*. 2000) and it seems likely that these genes encode metal transporters which transport both Zn and Cd along with other metal cations.

Although the physiological relevance of the function of transporters expressed in yeast and other non-plant systems is yet to be fully demonstrated in higher plants, the possible importance of these transporters in the role of both Cd and Zn uptake cannot be overlooked. It may be possible that differences in the expression, activities and specificities of such transporters ultimately relate to genotypic differences in Cd and Zn uptake and interaction.

From the current data it is clear that within the potato system the interaction between Cd and Zn is neither simple nor consistent. Stimulation, rather than competition of total Cd uptake was noted in Wilwash when the Zn supply was increased—in direct opposition with the response in Kennebec. Other studies have also reported stimulation of Zn uptake by Cd uptake (Williams and David 1976, Dudka *et al.* 1994, Welch *et al*. 1999), although with

little in the way of explanation. One possible explanation is that this apparent synergistic response may be due to the induction of phytochelatins (PCs). The synthesis of this group of metal binding polypeptides (poly(γEC)nG), is known to be stimulated by the presence of trace metals, (Grill *et al*. 1987) and rapidly induced by exposure to Cd (Chen *et al*. 1997). As discussed in Chapter 2, natural variations in the induction and synthesis of phytochelatins may occur between cultivars.

Overall, there is some evidence for reciprocal interactions between Cd and Zn in terms of uptake and distribution of Cd within the potato system. Although they are not clearly defined by the current experiments, it is apparent that these interactions are complex and vary from one cultivar to another. From an agronomic point of view, reducing Cd in the tubers does not appear to be as simple as increasing the application of Zn, as has been successful for some other crops. Whereas this may result in the desired decrease in Cd content of some cultivars (i.e. Kennebec), enhanced uptake of Cd may be seen in others (i.e. Wilwash).

Chapter 6

General Discussion

6.1 Introduction

Results from this study sought to provide some insight into the mechanisms affecting the uptake, translocation and partitioning of Cd within the potato plant, with particular focus on cultivar differences in tuber Cd concentration at maturity.

Initial studies supported the findings of field trials by McLaughlin *et al*. (1994), showing increased concentrations of Cd in the tubers of cultivar Kennebec compared to cultivar Wilwash at harvest. Whereas the cultivar responses seen in field trials may have been influenced by environmental variables, the glasshouse experiments were performed under controlled conditions allowing a more direct comparison between cultivars. Growth rate, habit and tuber yield were comparable such that any observed differences in Cd distribution could not be attributed to differences in morphology, vigour or mass of tubers, but must relate to variations in membrane transport and long distance translocation of Cd within the plants. From the long-term growth experiment it is apparent that for these cultivars of potato, the physiological processes of transport and allocation of Cd within the plant are the dominant factors—rather than uptake from the soil—in determining the amount of Cd present in the tuber.

6.2 Uptake of Cadmium

It was shown that Cd reaches the tuber by uptake from the roots, followed by transport in the xylem to the shoots, transfer from the xylem to the phloem, and then transport in the phloem to the tuber. As total uptake did not account for the differences between Wilwash

and Kennebec, it is necessary to look for possible mechanistic differences that may determine the fate of Cd after it has been absorbed by the roots.

Initial entry of Cd into the root is through the apoplast along a continuous pathway comprising the cell wall continuum and intercellular spaces. This pathway is terminated at the endodermis by the Casparian strip. For Cd to continue it must then enter the symplastic pathway via transport across the plasma membrane of the endodermal cells. As no difference was noted in total Cd uptake it seems unlikely that this part of the pathway is limiting.

Once present in the cytoplasm of the root cells there are two main alternatives for movement of Cd. One is to be transported to the tracheids and vessels of the xylem, and hence into the xylem stream, and the other is to be sequestered into the vacuoles where it is likely to form complexes with organic ligands. Given the large accumulation of Cd in the roots of Wilwash it would seem that a fundamental difference in xylem loading or vacuolar sequestration is occurring.

Two main mechanisms for transport of Cd into the vacuole have been proposed; the active transport of Cd into via a Cd^{2+}/H^{+} antiporter (as reported by Salt and Wagner 1993 for oat root vesicles), and transport across the tonoplast as a phytochelatin complex (Vögeli-Lange and Wagner 1990). Differences in the activity of a Cd^{2+}/H^{+} antiporter or natural variation in the induction of phytochelatins in response to Cd may explain the accumulation of Cd in the roots of Wilwash.

If the synthesis of phytochelatins in one cultivar was induced at lower concentrations of Cd than another cultivar, it would serve to increase the retention of Cd in phytochelatin-rich tissues (e.g. roots), thereby limiting its redistribution to other tissues (e.g. leaves, tubers). However, given the very low concentrations of Cd in the soil solution, it is unclear why an increase in the synthesis of phytochelatins would occur for cultivar Wilwash. Although, if

Cd loading into the xylem stream was limiting, could it be possible that this would lead to a 'build up', or increase in free Cd activity in the cytoplasm, resulting in induction of phytochelatins? Further studies focussing on the concentration of phytochelatins and other ligands generally believed to complex Cd in the vacuole of potato cultivars would be required to test this hypothesis further.

6.3 Transport within the Plant

In many plants, the only pathway for transport of nutrients and mineral elements to sinks, such as fruits and other storage, is via the phloem. However, the potato root system is different in that it allows a direct xylem connection to the tubers via roots located on the stolons and tubers themselves. The existence of these roots is most likely due to the need to supply large amounts of phloem immobile nutrients, such as Ca, to the tuber. Indeed, these different uptake pathways have been shown to be important in the supply of Ca, as well as water, to the tubers. Although data from the initial pot trial suggested some mobility of Cd in the phloem, it was not directly tested in that experiment, and the possibility remained that tuber Cd could be facilitated through uptake by these roots. The split-pot experiment described in Chapter 3 aimed to clarify this issue and to define clearly the pathways for movement of Cd into the tuber.

Cadmium transported from the basal roots accounted for the vast majority of Cd in the rest of the plant, including the tubers. Uptake from the stolon and tuber roots represented only a minor fraction of Cd found in the tubers. There was no evidence of the proposed 'bypass' pathway (from the basal roots directly to the stolons, via a xylem connection), or uptake directly across the periderm, contributing significantly to Cd uptake into the tuber. The major uptake pathway therefore appears to be from the roots to shoots, in the xylem, followed by translocation to the tubers in the phloem.

Application of ^{109}Cd to both the leaves and the roots, demonstrated that Cd has a high mobility in both the xylem and the phloem. The rapid uptake and redistribution of Cd from both soil and foliar sources underlines the ease with which it is able to move around plants, using both vascular systems. Interestingly, Cd appeared to be sequestered in the stem tissue when applied to both the roots and shoots, and it was suggested that the stem may be a site for removal from, and/or transport between, both the xylem and phloem, or may act as a storage pool for mineral element transfer. Instead of just being present in the phloem during movement of photosynthate from the leaves to the tubers, Cd appears to be actively loaded and unloaded into various tissues even though it is not essential nutrient, or present in concentrations toxic to the plant.

The high mobility of Cd in the phloem suggests that it is not being transported as a divalent cation, but is complexed with other compounds (such as phytochelatins and phytometallophores) that have a high affinity for binding metals, so as to prevent precipitation (e.g. as oxides, hydroxides and phosphates). As has been discussed above, differences in the formation or induction of these complexes may vary naturally between cultivar and provide some insight as to why Cd is retained in the roots and shoots of Wilwash.

A further interesting point to come out of this study is the differences in partitioning of other nutrient elements, which must ultimately impact on tuber quality parameters (e.g. specific gravity, post-harvest characteristics). The concentrations of most mineral nutrients present in the tubers at maturity were significantly greater in cultivar Kennebec. For all elements analysed, including Cd, the ratio of the concentration in leaves to tubers was consistently higher in Wilwash than in Kennebec. In cultivar Kennebec the concentrations of most mineral elements in the leaves were similar or higher than cultivar Wilwash. However for Cd, leaf concentrations were significantly higher in Wilwash. So, despite a

strong concentration gradient movement of Cd from the leaves to other tissues, Cd appeared to be preferentially sequestered in the leaves of Wilwash perhaps by compartmentation in vacuoles or through the formation of chemical complexes with phytochelatins as discussed in Chapter 2.

6.4 Agronomic Implications

There appear to be two factors that determine the concentration of Cd in tubers – the total uptake into the plant and the partitioning of absorbed Cd between the various tissues. Uptake into the plant will obviously be a function of the external concentration of Cd combined with a range of soil variables (e.g. pH, salinity, other cations) that have not been examined in detail here. Reduction in tuber Cd might be achieved either by selecting cultivars with low uptake, or by selecting cultivars which preferentially allocate or retain Cd in non-tuber tissues. In the two cultivars examined in this study, differential allocation rather than differences in uptake was the dominant factor. Whether this is generally true of potatoes has yet to be established, but for those cultivars in which total Cd uptake is similar, it may be possible to use the relationship between root and tuber Cd concentration as an indicator of a cultivar's ability to retain Cd within its non-tuber tissues. For Wilwash this ratio was 61 compared to 11 for Kennebec, which is a close reflection of the ratios of the total content in roots and tubers of 0.98 in Wilwash and 0.18 in Kennebec. Moreover, the differences in allocation begin to develop from the initiation in tuber bulking and decrease slightly in Kennebec but increase in Wilwash over time. From a practical perspective, measuring the ratio of root:tuber Cd could be used as a cultivar screening tool. It remains for this to be tested for a wider range of cultivars, as the comparison here of only two cultivars leaves this a somewhat speculative hypothesis.

Soil-added Zn has been linked to decreases in tuber Cd in several studies and has been suggested as a broad scale agricultural tool for limiting the uptake of Cd by various crops.

Data from this work highlighted the possible shortcomings of this practice when applied to potatoes. Of greatest note was that the ability of Zn to reduce Cd uptake was dependent upon cultivar and the concentration of Cd in the external media. For cultivar Wilwash the 'expected' competitive response was noted when the external concentration of Cd was low, but when the concentration of Cd in the external media was high, increasing Zn served to increase Cd uptake. In practice this may mean that for some cultivars, ameliorating Cd contaminated soil with Zn may actually stimulate Cd uptake, a response which is highly undesirable.

The potential of added Zn to reduce Cd uptake from contaminated soils seems more favourable for cultivar Kennebec, as the data showed the addition of Zn acted to decrease Cd uptake. However this was only true when Cd in the external solution was high. When the external Cd concentration was low, additional Zn resulted in increased Cd uptake. Again this highlights the danger of adopting Zn as a general preventative measure against Cd uptake. In this instance, excess Zn added to a soil with only low concentrations of Cd, may result in an increase in Cd uptake. In a cultivar such as Kennebec, the effect may be exacerbated when you consider that it was shown that the 'additional' Cd is unlikely to be sequestered in the roots, but be distributed to the tuber.

Appendix 1

Following comparison of mineral elements in the tubers of Kennebec and Wilwash, it was noted that the concentration of almost all elements, including Cd, was higher in Kennebec. These large variations in nutrient concentrations were surprising. In order to ensure that this affect was not due to the growth conditions employed in this study, field-grown tubers of the two cultivars were obtained from Dr Roger Kirkham of Agriculture Victoria, and the elemental concentrations determined. With the exception of Ca, the ratios of mineral element concentrations of field-grown tubers of Wilwash and Kennebec were comparable with the data presented in Chapter 2.

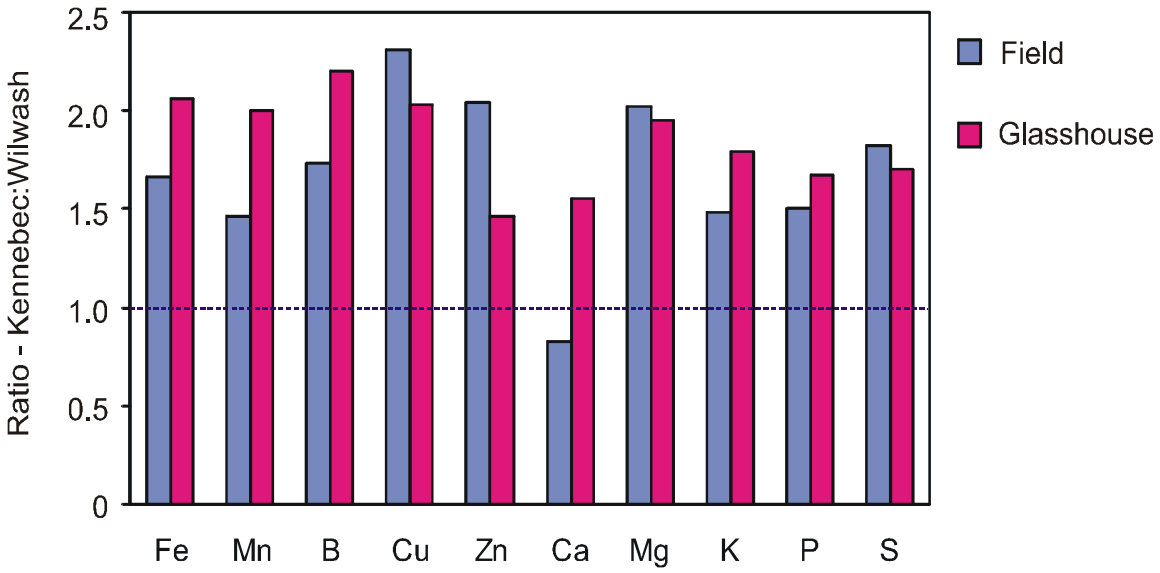


Figure A1 Comparison of the ratios of elemental concentrations in field-grown and glasshouse-grown tubers of Wilwash and Kennebec. The field-grown tubers were supplied by Dr Roger Kirkham of Agiculture Victoria. The dashed line indicates a ratio of 1:1.

Appendix 2

As part of the initial pot trial presented in Chapter 2, the concentrations of a range of nutrient elements were measured in shoots and tubers for each of the harvests. Although a detailed discussion of these results is beyond the scope of this thesis, some general observations have been made:

- Concentrations of most mineral elements varied markedly throughout the course of the growing period.
- Leaf concentrations of all nutrients, except magnesium (Mg), were similar between the two cultivars.
- A higher concentration of Na was noted in the leaves of Kennebec, compared to Wilwash.
- Concentrations of all nutrients in Kennebec tended to increase above the nutrient levels in Wilwash at a time consistent with the onset of tuber bulking.
- With the exception of Ca, all nutrient levels in Wilwash remained relatively constant, while those of Kennebec tended to increase with age.

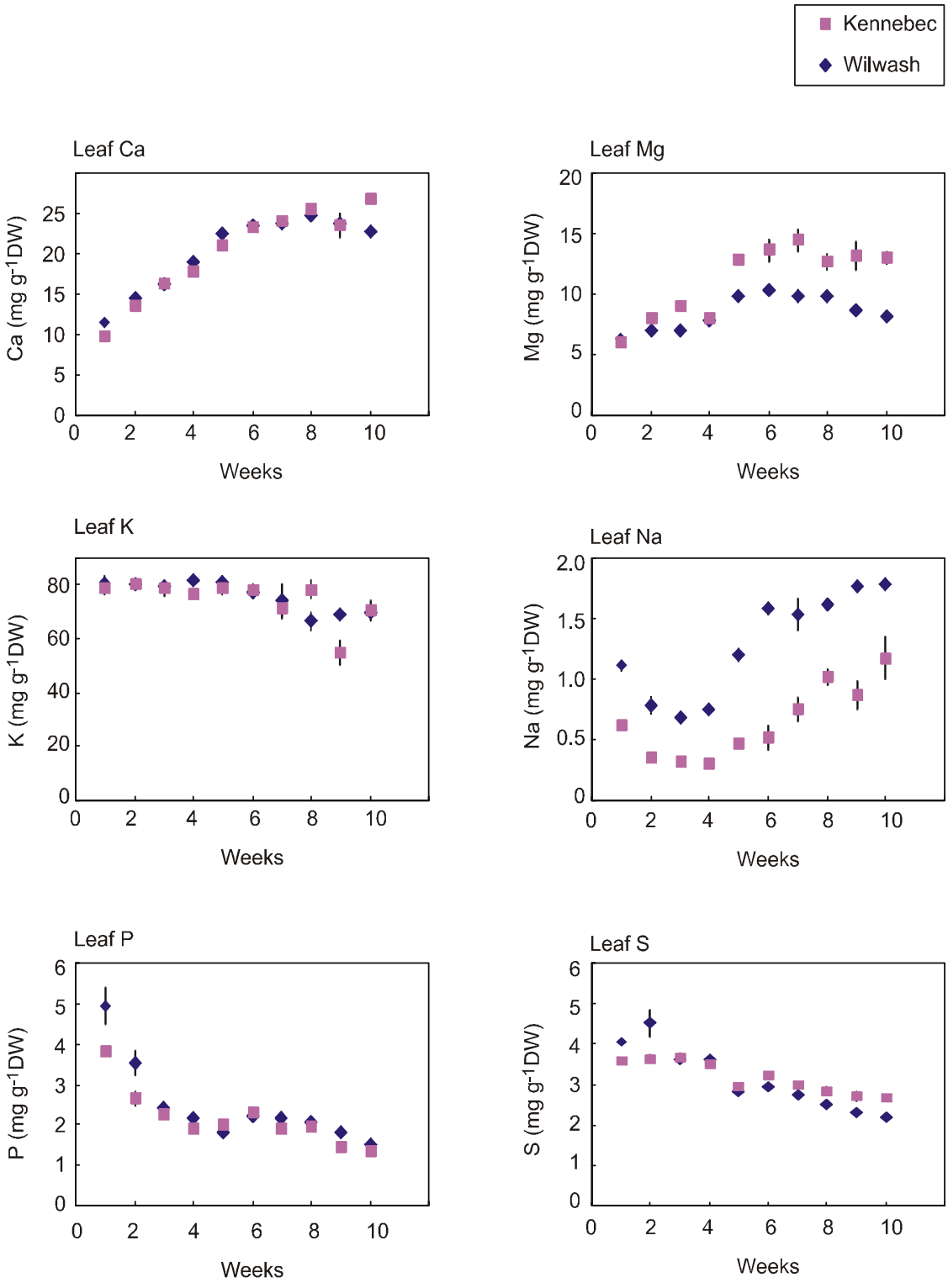


Figure A2.1 Concentrations of macronutrients and Na in leaves of potato cultivars Kennebec and Wilwash. Plants were grown in sandy soil with a defined liquid nutrient solution as described in Section 2.3.2. Each point is the mean ± SE of 4 plants.

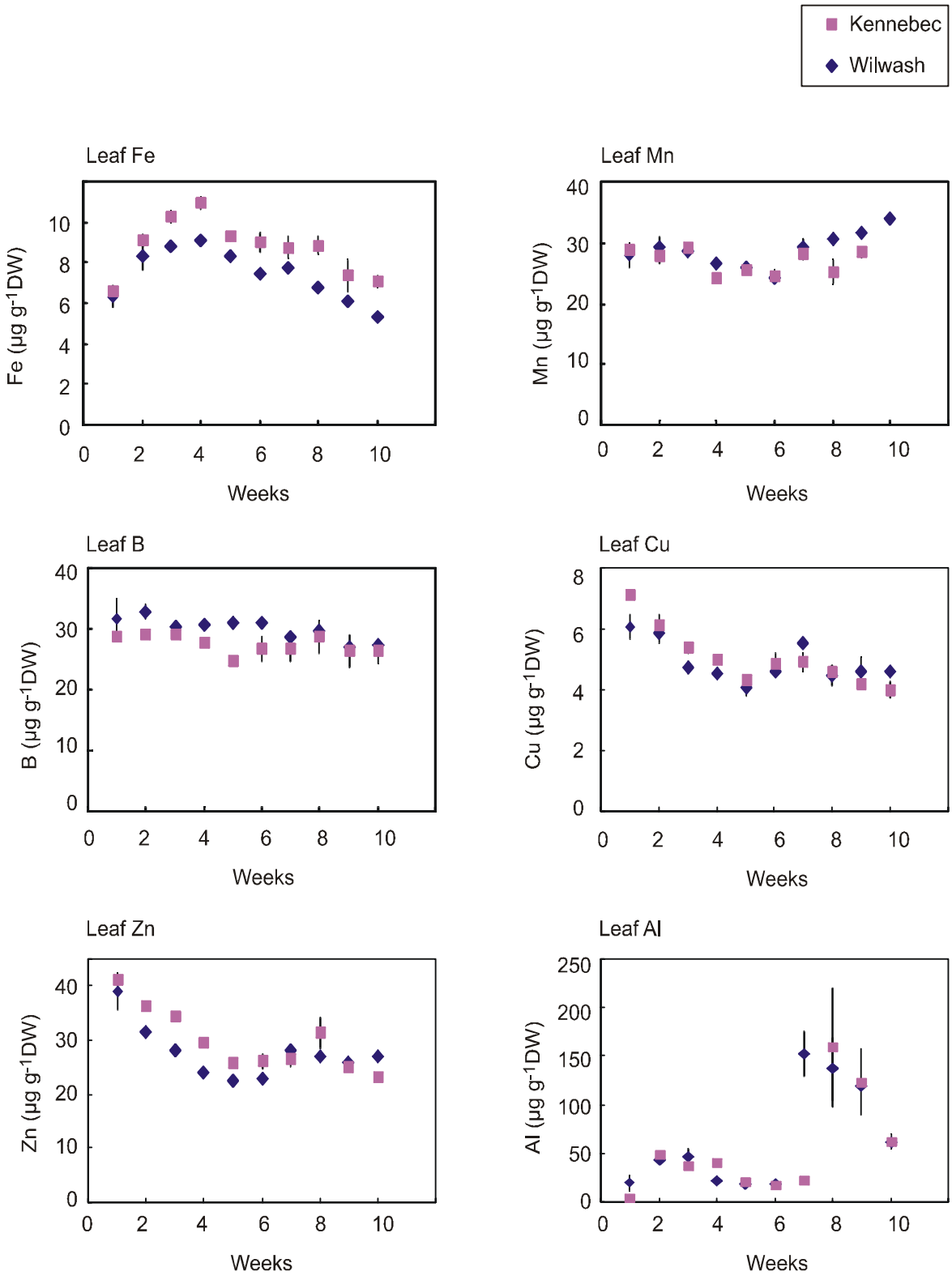


Figure A2.2 Concentrations of micronutrients and Al in leaves of potato cultivars Kennebec and Wilwash. Plants were grown in sandy soil with a defined liquid nutrient solution as described in Section 2.3.2. Each point is the mean ± SE of 4 plants.

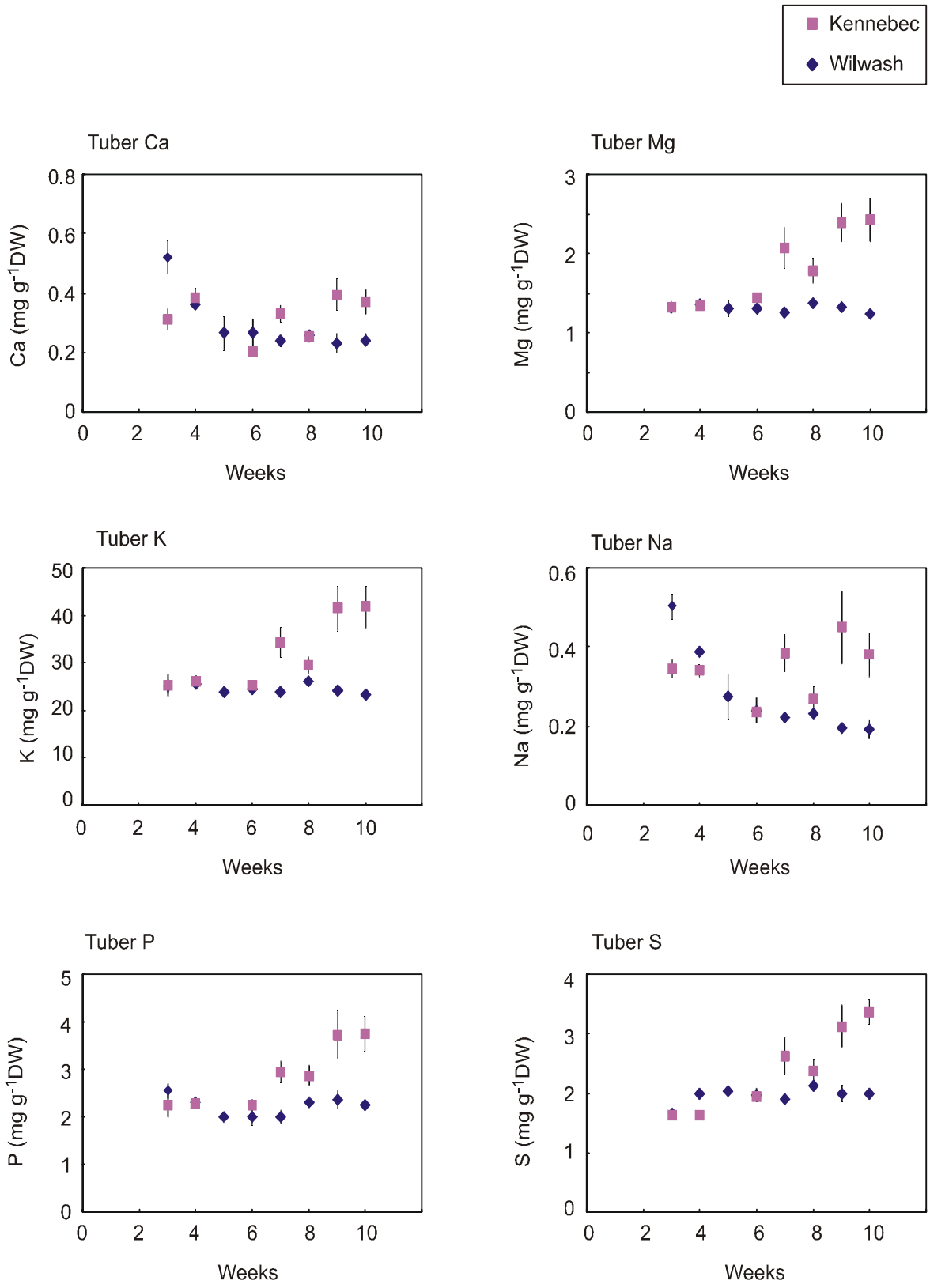


Figure A2.3 Concentrations of macronutrients and Na in tubers of potato cultivars Kennebec and Wilwash. Plants were grown in sandy soil with a defined liquid nutrient solution as described in Section 2.3.2. Each point is the mean ± SE of 4 plants.

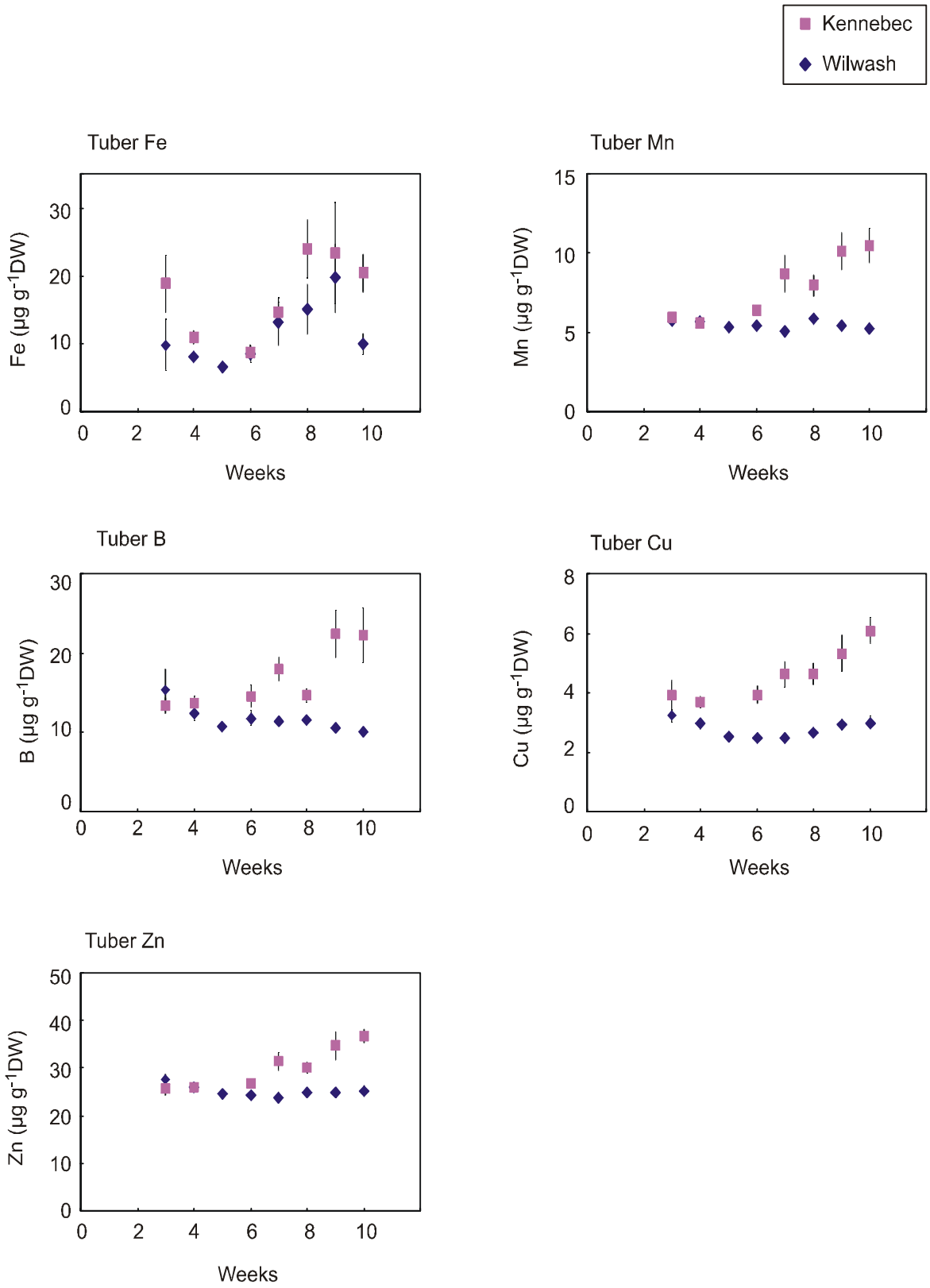


Figure A2.4 Concentrations of micronutrients in tubers of potato cultivars Kennebec and Wilwash. Plants were grown in sandy soil with a defined liquid nutrient solution as described in Section 2.3.2. Each point is the mean ± SE of 4 plants.

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