Plant, Cell and Environment (2003) **26**, 1657–1672

Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe

N. ROOSENS[1,2], N. VERBRUGGEN[2], P. MEERTS[3], P. XIMÉNEZ-EMBÚN[4] & J. A. C. SMITH[1]

[1]*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK,* [2]*Laboratoire de Physiologie et de Génétique Moléculaire des Plantes, Université Libre de Bruxelles, Campus Plaine – CP242, Bld. du Triomphe, B-1050 Brussels, Belgium,* [3]*Laboratoire de Génétique et Ecologie Végétales, Université Libre de Bruxelles, Chaussée de Wavre 1850, B-1160 Brussels, Belgium and* [4]*Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Avda. Complutense s/n, E-28040 Madrid, Spain*

ABSTRACT

***Thlaspi caerulescens* J. & C. Presl is a distinctive metallophyte of central and western Europe that almost invariably hyperaccumulates Zn to > 1.0% of shoot dry biomass in its natural habitats, and can hyperaccumulate Ni to > 0.1% when growing on serpentine soils. Populations from the Ganges region of southern France also have a remarkable ability to accumulate Cd in their shoots to concentrations well in excess of 0.01% without apparent toxicity symptoms. Because hyperaccumulation of Cd appears to be highly variable in this species, the relationship between Cd tolerance and metal accumulation was investigated for seven contrasting populations of *T. caerulescens* grown under controlled conditions in solution culture. The populations varied considerably in average plant biomass (3.1-fold), shoot : root ratio (2.2-fold), Cd hyperaccumulation (3.5-fold), shoot : root Cd-concentration ratio (3.1-fold), and shoot Cd : Zn ratio (2.6-fold), but the degree of hyperaccumulation of Cd and Zn were strongly correlated. Two populations from the Ganges region were distinct in exhibiting high degrees of both Cd tolerance and hyperaccumulation (one requiring 3 μM Cd for optimal growth), whereas across the other five populations there was an inverse relationship between Cd tolerance and hyperaccumulation, as has been noted previously for Zn. Metal hyperaccumulation was negatively correlated with shoot : root ratio, which could account quantitatively for the differences between populations in shoot Zn (but not Cd) concentrations. On exposure to 30 μM Cd, the two Ganges populations showed marked reductions in shoot Zn and Fe concentrations, although Cd accumulation was not inhibited by elevated Zn; in the other five populations, 30 μM Cd had little or no effect on Zn or Fe accumulation but markedly reduced shoot Ca concentration. These results support a proposal that Cd is taken up predominantly via a high-affinity uptake system for Fe in the Ganges populations, but via a lower-affinity pathway for Ca in other populations. Total shoot Cd accumulated per plant was much more closely related to population Cd tolerance than Cd hyperaccumulation, indicating that metal tolerance may be the more important selection criterion in developing lines with greatest phytoremediation potential.**

Correspondence: J.A.C. Smith. Fax: +44 1865275074; e-mail: andrew.smith@plants.ox.ac.uk

Key-words: *Thlaspi caerulescens*; cadmium; cation selectivity; metal hyperaccumulation; metal tolerance; shoot : root ratio; zinc.

INTRODUCTION

Thlaspi caerulescens J. & C. Presl (Brassicaceae) is a characteristic species of metalliferous soils in central and western Europe capable of surviving on unusual substrates including mine wastes and smelter sites (Reeves *et al*. 2001). In addition to a high degree of metal tolerance, the species is renowned for its ability to accumulate Zn, Ni and Cd to exceptional concentrations in its above-ground biomass. This capacity for metal 'hyperaccumulation' is comparatively rare in the plant kingdom, being known from approximately 400 species of vascular plants in total (Baker & Brooks 1989; Reeves & Baker 2000). The adaptive significance of this phenomenon is not yet fully understood. One plausible hypothesis is that high tissue metal concentrations provide a defence against herbivores and pathogens (Boyd 1998; Pollard *et al*. 2002). In addition to questions concerning their ecology and evolutionary origins, metal-hyperaccumulator plants are proving valuable for investigating fundamental aspects of metal-ion homeostasis in cells (Baker *et al*. 2000; Persans & Salt 2000; Clemens, Palmgren & Krämer 2002; Pollard *et al*. 2002) and the possibilities afforded by phytoremediation as a means of decontaminating metal-polluted soils (Chaney *et al*. 1997; Brooks *et al*. 1998; Salt, Smith & Raskin 1998; McGrath, Zhao & Lombi 2002).

Compared with the majority of hyperaccumulator species, *T. caerulescens* is unusual in being able to accumulate several different metals to very high concentrations in its

shoot biomass. Hyperaccumulation of Zn, to concentrations exceeding 1% of shoot dry biomass, appears to be a constitutive feature of this species in its natural habitats, even when growing on soils not especially enriched in Zn, implying particularly effective mechanisms for acquisition and accumulation of this metal (Meerts & Van Isacker 1997; Escarré *et al*. 2000; Reeves *et al*. 2001). When growing on serpentine soils, *T. caerulescens* can also hyperaccumulate Ni to concentrations well in excess of 0.1% (Reeves *et al*. 2001). Even more remarkably, certain populations of *T. caerulescens* have been shown to hyperaccumulate Cd, a highly toxic element for which there is no known essential function in vascular plants (Marschner 1995), to concentrations exceeding 0.01% of shoot dry biomass (Lloyd-Thomas 1995), with values up to 0.3% being recorded in field specimens from the Ganges region in France (Robinson *et al*. 1998; Reeves *et al*. 2001). Reports of high shoot Pb concentrations in *T. caerulescens* have not proved reproducible under laboratory conditions and are most likely the result of surface contamination, whereas hyperaccumulation of Mn and Co, although readily observable in solution culture, have not been recorded for this species in natural populations (Baker, Reeves & Hajar 1994; Reeves *et al*. 2001). These characteristics raise important questions about the underlying mechanisms of metal-ion uptake, translocation and sequestration in *T. caerulescens*, including the extent to which specific systems exist for individual metals or are shared between different metals.

Although metal hyperaccumulation appears generally to be a species-wide property (Baker *et al*. 2000; Macnair 2002; Pollard *et al*. 2002), there is considerable interest in determining the extent of natural variation in this trait, since this could lead to a better understanding of the genetic basis of hyperaccumulation. Studies under controlled conditions have demonstrated genetic differences between certain populations of *T. caerulescens* in their ability to hyperaccumulate Zn and Ni (McGrath *et al*. 1993; Lloyd-Thomas 1995; Pollard & Baker 1996; Meerts & Van Isacker 1997; Escarré *et al*. 2000; Assunção *et al*. 2001; Pollard *et al*. 2002). However, the relationships between hyperaccumulation, plant metal tolerance and soil metal content for the different populations – and hence clues as to the possible adaptive significance of metal hyperaccumulation – have been rather difficult to discern. For Zn tolerance in *T. caerulescens*, there is some evidence that tolerance is positively correlated with soil Zn content in the natural habitat, but that it is inversely related to the capacity for Zn hyperaccumulation (Ingrouille & Smirnoff 1986; Lloyd-Thomas 1995; Meerts & Van Isacker 1997; Escarré *et al*. 2000; Assunção *et al*. 2001). Inter-population differences in metal hyperaccumulation are not always easy to recognize, as tests are required at a range of metal concentrations to distinguish true hyperaccumulation in tolerant plants from the non-specific effects of membrane damage and 'breakthrough' of elements to the shoot in more sensitive plants (Baker 1981; Pollard *et al*. 2002). For Cd, there have been less extensive studies of interpopulation variation in *T. caerulescens*, but plants from the Ganges region in France are particularly notable for their ability to accumulate over 1% Cd on a shoot dry-biomass basis without apparent toxicity symptoms (Escarré *et al*. 2000; Lombi *et al*. 2000).

Because hyperaccumulation of Cd is such an unusual biological phenomenon and the ecological significance of this trait remains enigmatic, we investigated the relationship between Cd hyperaccumulation and metal tolerance in seven diverse populations of *T. caerulescens* from western Europe. The only other species currently known to hyperaccumulate Cd is *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz (= *Cardaminopsis halleri* (L.) Hayek), which can accumulate Cd to over 0.01% in its shoot dry biomass (Dahmani-Muller *et al*. 2000; Küpper *et al*. 2000; Reeves *et al*. 2001; Bert *et al*. 2002). *Arabidopsis halleri* shares certain similarities with *T. caerulescens*, in that hyperaccumulation of Zn is consistently observed in all natural populations of this species whereas Cd hyperaccumulation is more variable, and plants from non-metalliferous sites may show higher degrees of metal hyperaccumulation under controlled conditions than those from metalliferous sites (Bert *et al*. 2000, 2002, 2003). In the present study, we have examined populations of *T. caerulescens* from both mine sites and a serpentine site to compare the extent of natural variation in Zn and Cd hyperaccumulation in plants from highly contrasting substrates. The aim was to clarify the relationship between Cd hyperaccumulation and tolerance in *T. caerulescens* and to determine whether there is any association between the capacity for metal hyperaccumulation and other plant growth characteristics for the different populations.

MATERIALS AND METHODS

Plant material

Seeds of *Thlaspi caerulescens* J. & C. Presl (Brassicaceae) were obtained from populations in Bradford Dale, Clough Wood and Hafna (all British Isles), Prayon (Belgium), Puente Basadre (Spain), St Félix-de-Pallières and Les Malines (both France). Chemical characteristics of soils sampled from the natural habitat of these plants are shown in Table 1. Bradford Dale, Clough Wood and Hafna (Ingrouille & Smirnoff 1986; Baker *et al*. 1994; Lloyd-Thomas 1995), and St Félix-de-Pallières and Les Malines (Reeves *et al*. 2001) are all Pb/Zn mine spoil sites; Prayon is a Zn/Cd smelter site (Denaeyer-De Smet & Duvigneaud 1974); and Puente Basadre is a serpentine site (Reeves & Brooks 1983; Lloyd-Thomas 1995). All the soils are characterized by high concentrations of total (acid-soluble) Zn, Pb and Cd, except for the serpentine site at Puente Basadre, which contains low amounts of these elements but is highly enriched in Ni. Prayon has the highest mean soil concentrations of Zn and Cd, whereas Bradford Dale and Hafna show the highest mean values for Pb (Table 1).

Plant cultivation

Seeds were germinated on sterilized sand moistened with deionized water. After germination, the sand was moistened with 0.2-strength nutrient solution (see below). At 5

Table 1. Total metal concentrations (mg kg^{-1} dry mass) and pH of soil samples collected from *Thlaspi caerulescens* sites

Site	Soil pH	Soil metal concentration (mg kg^{-1})				
		Zn	Pb	Cd	Ni	Cu
Bradford Dale[a]	6.6 ± 0.2	9890 ± 779	20600 ± 3620	107 ± 11	97 ± 6	134 ± 17
Clough Wood[a]	6.4 ± 0.1	25900 ± 1050	1777 ± 2020	363 ± 18	123 ± 5	172 ± 8
Hafna[a]	5.8 ± 0.3	35200 ± 2590	17000 ± 1670	96 ± 6	44 ± 3	76 ± 5
Prayon[a]	6.9 ± 0.2[c]	75700 ± 13500	9620 ± 1460	667 ± 85	211 ± 57	4000 ± 648
Puente Basadre[a]	–	72 ± 1	35 ± 5	4 ± 0	2300 ± 115	20 ± 1
St Félix-de-Pallières[b]	–	64360–6654	46050–1931	265–35	–	–
Les Malines[b]	–	61710–35230	28390–10610	316–223	–	–

[a]Data from Lloyd-Thomas (1995). Soil pH was determined in 1 : 1 (w/v, H_2O) samples. Metals were analysed after digestion of samples in a 1 : 20 (w/v) mixture with 4 : 1 (v/v) HCl : HNO_3. Values are means ± SE for either 20 samples (Bradford Dale, Clough Wood, Hafna, Prayon) or five samples (Puente Basadre).
[b]Data from Reeves *et al*. (2001). Metals were analysed after digestion of samples in a 1 : 80 (w/v) mixture with 1 : 1 (v/v) HCl : HF. Figures are ranges encompassing the maximum and minimum recorded values.
[c]Additional measurements of soil pH from Prayon gave values in the range 4.7–6.2 (P. Meerts, unpublished results).

to 7 d after germination, seedlings were transferred to 1.2-L vessels (10 plants per vessel) containing a nutrient solution consisting of modified 0.1-strength Hoagland solution no. 1 macronutrients (Hoagland & Arnon 1950) comprising 0.5 mM KNO_3, 0.1 mM KH_2PO_4, 0.4 mM $Ca(NO_3)_2$ and 0.2 mM $MgSO_4$, together with modified 0.2-strength Long Ashton micronutrients (Hewitt & Smith 1975) comprising 20 μM FeEDDHA [ferric ethylenediamine-di-(2-hydroxyphenylacetate): Duchefa Biochemie BV, Haarlem, The Netherlands], 2.0 μM $MnSO_4$, 10 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 10 μM H_3BO_3 and 0.1 μM Na_2MoO_4. Plants were grown in solution culture in a glasshouse with supplementary illumination provided by sodium-vapour lamps to give a photoperiod of 16 h per day. Night temperature was maintained at 15 °C and day temperature at a minimum of 25 °C. The nutrient solution was aerated continuously and replaced every 7 d.

Metal treatments and sampling procedures

After 21 d in solution culture, the standard nutrient solution was supplemented with either $CdSO_4$ or $ZnSO_4$ to give final concentrations of 3 or 30 μM $CdSO_4$, and 10 or 100 μM $ZnSO_4$. The nutrient solution used in these treatments was aerated continuously and renewed every 3 d. Each treatment was replicated in two vessels, each containing 10 plants, and all experiments were conducted independently either two or three times.

Plants were harvested either 14 or 31 d after initiation of the metal treatment. Roots and shoots were washed with 10 mM Na_2EDTA solution (2 × 5 min) to remove surface-bound metals, and were then rinsed twice with deionized water. Plant material was oven-dried at 70 °C to constant mass, weighed, and ground to a fine powder.

For collection of xylem sap, plants were grown in standard nutrient solution for 60 d before the addition of 30 μM $CdSO_4$ and/or 100 μM $ZnSO_4$ to the solution. After 72 h of metal treatment, the stem was cut through at the base of the rosette with a GEM® single-edge stainless steel uncoated industrial blade (American Safety Razor Company, Verona, VA, USA). The remaining length of the stem was carefully sealed to a short length of Tygon® flexible plastic tubing (Norton Performance Plastics Corporation, Akron, OH, USA) using Parafilm® M (American National Can, Chicago, IL, USA), with the roots still immersed in aerated nutrient solution. Xylem sap was collected as root-pressure exudate in the tubing for 12 h. Each treatment was replicated in three vessels, each containing two plants, and this experiment was conducted twice.

Elemental analysis

Weighed subsamples (50 mg on average) of the oven-dried, ground plant tissues were digested in 3 mL of concentrated (69%, v/v) HNO_3 (AnalaR; Merck Ltd, Poole, Dorset, UK) using a microwave digestion system (MDS 2000; CEM Microwave Technology Ltd, Buckingham, UK). After appropriate dilution, Zn, Cd, Fe, Ca and K were measured in an air–acetylene flame by atomic absorption spectrophotometry (AAS) using a double-beam optical system with deuterium arc background correction (AAnalyst 100; Perkin-Elmer Ltd, Beaconsfield, Buckinghamshire, UK). For elemental determination in xylem sap, an aliquot of sap was diluted with 0.4 M HNO_3 and analysed by flame AAS.

Statistical analysis

One-way ANOVA followed by the T′-method (a modified Tukey test for multiple *post-hoc* comparisons between treatments with unequal sample sizes: Sokal & Rohlf 1995) was used to analyse the effect of Cd concentration on plant growth and the elemental composition of shoots and roots. Two-way ANOVA, with population and Cd treatment as main effects, was also performed to test for significant differences in the effect of Cd treatment between populations. Correlations between Cd and Zn concentrations in the

shoot, and between shoot metal concentrations and shoot : root biomass ratio, were tested by means of the Pearson correlation coefficient, as well as by general linear modelling (GLM) according to Grafen & Hails (2002). All data were log-transformed prior to analysis to stabilize the variance. Differences were considered not significant at values of $P > 0.05$. There was no evidence for significant differences between any treatment means in the two or three independent experiments performed, so values from individual experiments were combined to increase the power of the analysis. Computations were performed using SYSTAT version 5.0 (Systat Software Inc., Richmond, CA, USA) or MINITAB Release 12.1 (Minitab Inc., State College, PA, USA).

RESULTS

Growth responses of *T. caerulescens* populations to cadmium

To standardize experimental treatments, plants were precultivated as seedlings for 1 week on dilute nutrient solution, transferred to standard nutrient solution for 3 weeks, and then exposed to Cd and elevated Zn for either 14 or 31 d, so that plants were either 6 weeks old or approximately 8 weeks old, respectively, at the time of harvest. Two-way ANOVA showed that the seven *T. caerulescens* (*T.c.*) populations exhibited significant differences both in root and shoot biomass production in the absence of Cd ($P < 0.001$) and in their responses to Cd treatment ($P < 0.001$).

In the absence of Cd, plants from the Prayon population had the highest biomass after both 6 and 8 weeks of growth, followed by the two populations from southern France, Les Malines and St Félix-de-Pallières (Fig. 1). After 8 weeks, the total root plus shoot dry biomass of *T.c.* Prayon was 3.1-fold greater than that of the Hafna population, which had the slowest growth rate. The populations also showed significant differences in shoot : root dry biomass ratio ($P < 0.001$), the highest ratio (5.9) being observed in *T.c.* Prayon and the lowest in *T.c.* Bradford Dale and *T.c.* Clough Wood (which averaged 2.7; Fig. 1). There was no evidence, based on visual symptoms, that exposure to 100 μM $ZnSO_4$ for up to 31 d was toxic to any of the seven populations.

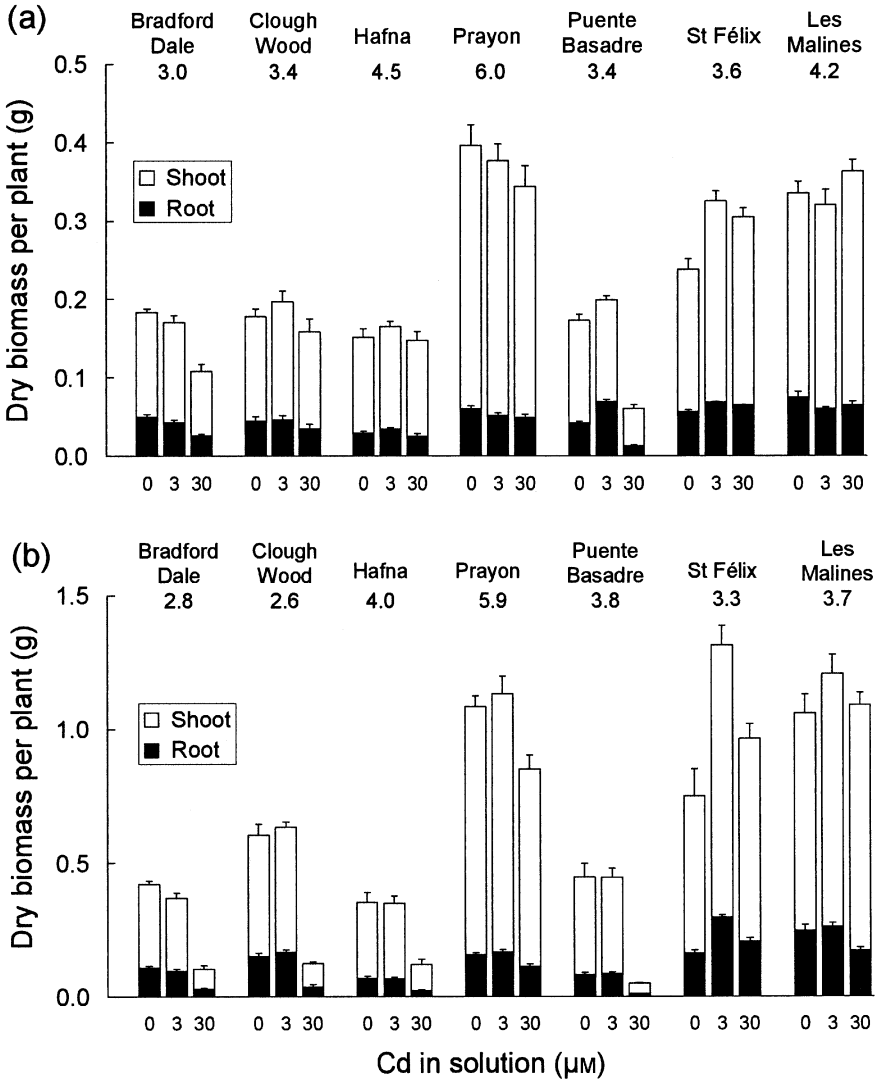

Figure 1. Shoot (white bars) and root (black bars) dry biomass for seven populations of *Thlaspi caerulescens* exposed for (a) 14 d or (b) 31 d to either 3 or 30 μM $CdSO_4$, compared with controls not exposed to Cd. Plants were precultivated on standard nutrient solution as described in Materials and methods for 4 weeks before exposure to Cd. At the beginning of the experimental treatment, all solutions (with or without Cd) were supplemented with additional $ZnSO_4$ to give a total Zn concentration of 100 μM. Values are means +1 SE ($n = 9, 9, 9, 14, 9, 8$ and 8 plants on average for the seven populations Bradford Dale, Clough Wood, Hafna, Prayon, Puente Basadre, St Félix-de-Pallières and Les Malines, respectively). The numbers beneath the names of the seven populations are shoot : root dry biomass ratios for each population averaged for all three Cd treatments (0, 3 and 30 μM).

None of the populations showed a significant growth reduction when exposed to 3 μM $CdSO_4$ in the nutrient solution, after either 14 or 31 d of treatment, and none of these plants showed toxicity symptoms such as leaf necrosis. In fact, growth of *T.c.* St Félix-de-Pallières was stimulated by 3 μM Cd, which caused a 37% increase in total plant biomass compared with the control after 14 d, and a 75% increase after 31 d (both $P < 0.001$: Fig. 1).

When exposed to 30 μM $CdSO_4$, all populations showed reduced growth compared with the zero-Cd controls after 31 d of treatment, except for *T.c.* St Félix-de-Pallières and *T.c.* Les Malines (Fig. 1b). The serpentine population, Puente Basadre, was the most sensitive to 30 μM Cd: after 1 week of treatment, all plants exhibited some necrosis (data not shown); after 2 weeks, necrosis on the young leaves was pronounced and the old leaves were desiccated, at which time plant biomass was only 35% of the control (Fig. 1a); and during the third and the fourth weeks of treatment, most of these plants died. The three British populations were the next most sensitive to 30 μM Cd, presenting the same symptoms of leaf necrosis about 5 d later (*T.c.* Bradford Dale) or 10 d later (*T.c.* Clough Wood and *T.c.* Hafna) than *T.c.* Puente Basadre (data not shown). After 14 d of exposure to 30 μM Cd, *T.c.* Bradford Dale was the only other population to show significant growth reduction ($P < 0.001$), although, after 31 d of exposure, necrosis and markedly reduced growth were apparent for all three British populations (Fig. 1b); *T.c.* Bradford Dale was the only one of these in which some plants had died after 31 d. *T.c.* Prayon was more tolerant than any of these four populations, showing only a 22% reduction in biomass production after 31 d of exposure to 30 μM Cd. For the two populations from the south of France, 30 μM Cd had either no significant effect (*T.c.* Les Malines) or caused a small increase ($P < 0.01$) in growth (*T.c.* St Félix-de-Pallières) compared with the zero-Cd controls after both 14 and 31 d of treatment, although after 31 d a number of *T.c.* St Félix-de-Pallières plants showed slight leaf chlorosis.

Based on these growth data and visual toxicity symptoms, the ranking of the seven populations of *T. caerulescens* with respect to Cd tolerance is thus as follows: Puente Basadre < Bradford Dale < Clough Wood ≈ Hafna < Prayon < Les Malines < St Félix-de-Pallières.

Zinc and cadmium accumulation in *T. caerulescens* populations

After 14 d of cultivation on 100 μM $ZnSO_4$ in the absence of Cd, plants from all populations had Zn concentrations in their shoot dry biomass either close to or exceeding the value of 10 g kg^{-1} (i.e. 1.0%, w/w) conventionally used to define Zn hyperaccumulation in plants growing in their natural habitats (Fig. 2a). Nevertheless, ANOVA showed that the seven populations differed significantly in shoot Zn accumulation ($P < 0.001$), with mean values ranging from 15.2 g kg^{-1} in *T.c.* Bradford Dale to 7.5 g kg^{-1} in *T.c.* Prayon. Exposure to either 3 or 30 μM $CdSO_4$ for 14 d had no significant effect on Zn hyperaccumulation except in *T.c.* St Félix-de-Pallières and *T.c.* Les Malines (Fig. 2a), which both showed a 30% reduction in shoot Zn concentration at 30 μM Cd (both $P < 0.01$).

After 14 d of treatment with 3 μM $CdSO_4$ in the presence of 100 μM $ZnSO_4$, all populations showed shoot Cd concentrations exceeding the value conventionally used to define Cd hyperaccumulation (0.1 g kg^{-1} or 0.01%, w/w), the highest shoot Cd concentration being observed in *T.c.* St Félix-de-Pallières (0.97 g kg^{-1}) and the lowest in *T.c.* Prayon (0.28 g kg^{-1}: Fig. 2b). Exposure to 30 μM $CdSO_4$ for 14 d produced six- to 10-fold higher shoot Cd concentrations, ranging from maximum values of 7.4 and 7.0 g kg^{-1} in *T.c.* Puente Basadre and *T.c.* St Félix-de-Pallières, respectively, to a minimum of 2.0 g kg^{-1} in *T.c.* Prayon (Fig. 2b).

In view of the severe impairment of growth in some populations after 31 d of exposure to 30 μM Cd (Fig. 1b), a meaningful comparison of plant metal contents after the longer treatment period is problematic. Apart from the visual symptoms of acute Cd toxicity in *T.c.* Puente Basadre, the populations from both Bradford Dale and Clough Wood showed clear evidence of 'breakthrough' of metals to the shoot after exposure to 30 μM Cd for 31 d, with shoot Zn concentration in these populations increasing to 144 and 193%, respectively, of the control values (data not shown). Thus, to explore further the relationship between metal hyperaccumulation and metal tolerance in the seven populations, our analysis focused on plants exposed to Cd for only 14 d, before the more non-specific effects of severe metal toxicity became apparent.

For all populations, the concentrations of both Zn and Cd after 14 d of metal treatment were considerably higher in shoot than root dry biomass (Fig. 2), although the shoot : root concentration ratios for these metals varied significantly ($P < 0.001$) between populations (Table 2). For Zn, this ratio did not show any consistent trend with Cd treatment but differed nearly seven-fold between populations, ranging from an average of 10.5 for the three treatments in *T.c.* Bradford Dale to an average of 1.7 in *T.c.* Puente Basadre. The highest shoot : root concentration ratio for Cd of 6.5 was also found in *T.c.* Bradford Dale, which was approximately three-fold higher than the lowest values observed in the four populations *T.c.* Prayon, Puente Basadre, St Félix-de-Pallières and Les Malines. For Cd, the two Ganges populations were distinct from the other five in showing either no effect of external Cd concentration on shoot : root metal-concentration ratio (*T.c.* Les Malines), or even an increase ($P < 0.05$) in this ratio at 30 μM Cd (*T.c.* St Félix-de-Pallières: Table 2).

For the seven populations of *T. caerulescens* considered together, there was a highly significant correlation between shoot concentrations of Zn and Cd (Fig. 3, plotted on a log–log scale to linearize the relationship), both for plants exposed to 3 μM Cd and to 30 μM Cd (both $P < 0.001$). However, there were significant differences between populations in their absolute values for Cd : Zn concentration ratios in shoot dry biomass (Table 3). At 3 μM Cd, the two Ganges populations, *T.c.* St Félix-de-Pallières and *T.c.* Les Malines, showed significantly ($P < 0.001$) higher shoot

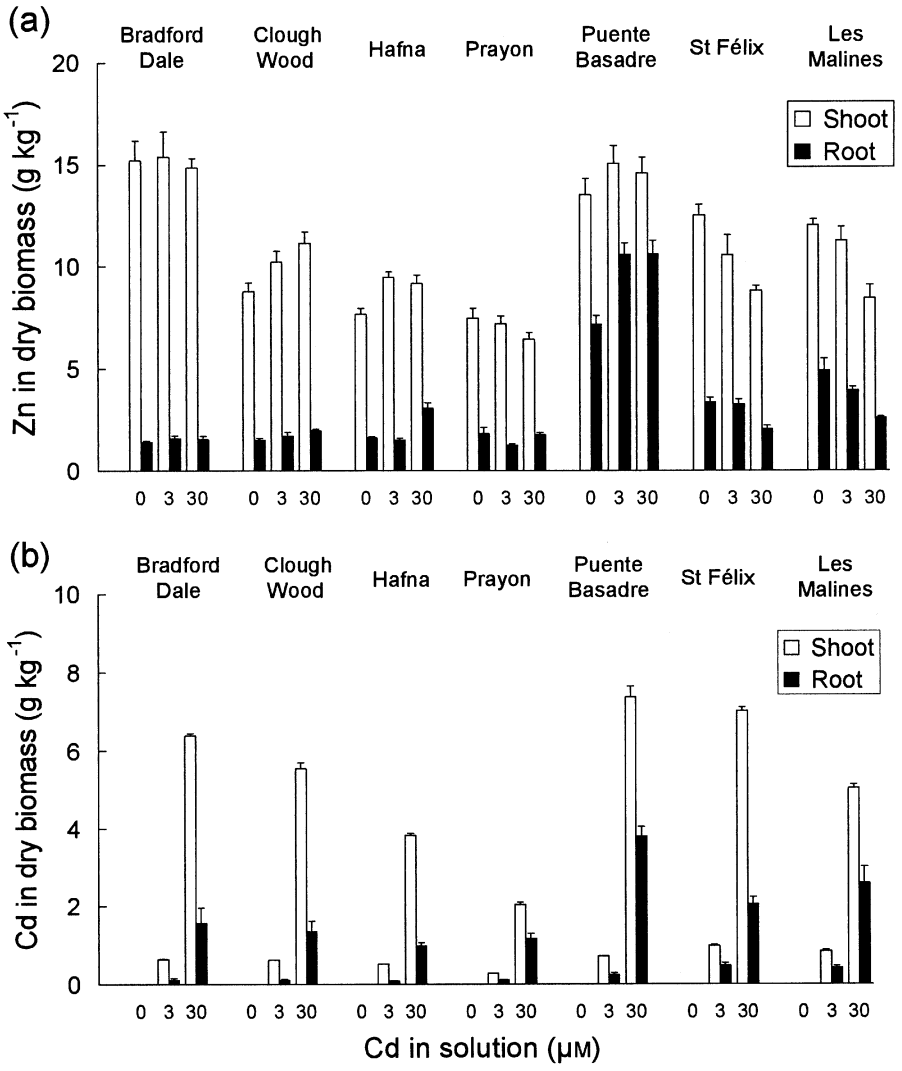

Figure 2. Concentrations of (a) Zn and (b) Cd in shoot (white bars) and root (black bars) dry biomass of 6-week-old plants for seven populations of *Thlaspi caerulescens* cultivated for 14 d in nutrient solution with 100 μM $ZnSO_4$, or in the same solution supplemented with 3 or 30 μM $CdSO_4$. Values are means +1 SE (n = 10, 9, 8, 11, 10, 8 and 8 plants for the seven populations Bradford Dale to Les Malines, respectively, reading from left to right).

Table 2. Ratio of Zn or Cd concentration in shoot to root dry biomass of 6-week-old plants for seven populations of *Thlaspi caerulescens* cultivated for 14 d in standard nutrient solution containing 100 μM $ZnSO_4$, or in the same solution supplemented with either 3 or 30 μM $CdSO_4$

Cd in solution (μM)	Zn shoot : Zn root concentration ratio						
	Bradford Dale	Clough Wood	Hafna	Prayon	Puente Basadre	St Félix	Les Malines
0	^{aA} 10.6 ± 0.7 (8)	^{bB} 6.0 ± 0.5 (10)	^{bcA} 4.7 ± 0.2 (8)	^{bcAB} 4.6 ± 0.6 (8)	^{dA} 1.9 ± 0.1 (10)	^{cAB} 3.8 ± 0.2 (8)	^{dA} 2.6 ± 0.3 (8)
3	^{aA} 10.8 ± 1.5 (10)	^{bB} 6.5 ± 0.5 (8)	^{bcB} 6.3 ± 0.4 (8)	^{bcA} 5.2 ± 0.7 (10)	^{dA} 1.9 ± 0.1 (8)	^{cA} 3.3 ± 0.2 (9)	^{dA} 2.9 ± 0.1 (9)
30	^{aA} 10.2 ± 0.9 (8)	^{bB} 5.5 ± 0.2 (8)	^{cC} 3.1 ± 0.2 (8)	^{dB} 3.8 ± 0.3 (10)	^{eB} 1.4 ± 0.1 (9)	^{bdB} 4.5 ± 0.5 (8)	^{dA} 3.4 ± 0.2 (8)

Cd in solution (μM)	Cd shoot : Cd root concentration ratio						
	Bradford Dale	Clough Wood	Hafna	Prayon	Puente Basadre	St Félix	Les Malines
3	^{aA} 6.5 ± 0.6 (10)	^{aA} 5.6 ± 0.1 (8)	^{aA} 5.7 ± 0.3 (8)	^{bA} 2.5 ± 0.1 (10)	^{bA} 2.5 ± 0.1 (8)	^{bA} 2.2 ± 0.3 (9)	^{bA} 2.1 ± 0.2 (9)
30	^{aB} 4.5 ± 0.1 (8)	^{aB} 4.4 ± 0.5 (8)	^{aB} 4.0 ± 0.2 (8)	^{bB} 1.8 ± 0.1 (10)	^{bA} 2.1 ± 0.2 (9)	^{aB} 3.5 ± 0.1 (8)	^{bA} 2.0 ± 0.2 (8)

Values are means ± SE (n = number of samples in parentheses); different superscripts denote values significantly different at P = 0.05 (lower case letters for comparisons within rows, upper case letters for comparisons within columns).

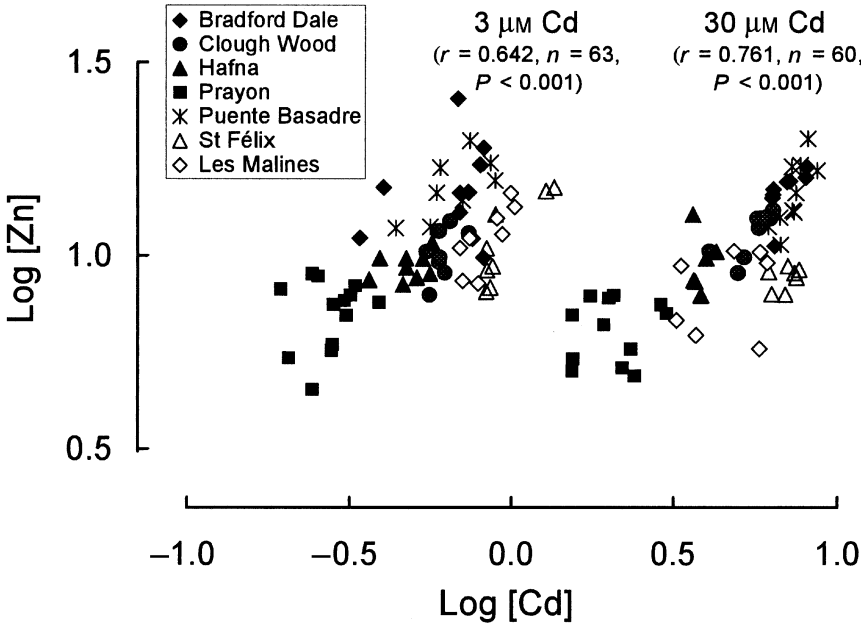

Figure 3. Correlation between Zn and Cd concentrations in shoot dry biomass of 6-week-old plants for seven populations of *Thlaspi caerulescens* cultivated for 14 d in nutrient solution containing 100 μM $ZnSO_4$ and either 3 or 30 μM $CdSO_4$. Values are plotted as $\log_{10}$ of the shoot metal concentrations in g kg^{-1}. Results of the correlation analysis performed by the method of least squares are indicated for the two data-sets on the Figure. Analysis using GLM showed that the relationship between shoot Zn and shoot Cd concentrations was significant at $P < 0.01$ when the influence of plant population was statistically eliminated.

Cd : Zn concentration ratios than the other five populations. At 30 μM Cd, values for shoot Cd : Zn concentration ratio varied by a factor of 2.6, showing a maximum of 0.80 in *T.c.* St Félix-de-Pallières, which was significantly ($P < 0.001$) higher than the value for all other populations; the shoot Cd : Zn ratio for *T.c.* Les Malines was only significantly higher than those for *T.c.* Prayon ($P < 0.001$) and *T.c.* Hafna ($P < 0.01$). By means of statistical elimination in GLM (Grafen & Hails 2002), it could be shown that the correlation between shoot Zn and Cd concentrations was still significant at $P < 0.01$ when the influence of plant population was eliminated.

Relationship between metal accumulation and shoot : root ratio

Shoot : root dry biomass ratio varied by a factor of two between the different populations of *T. caerulescens* (Fig. 1), so we investigated whether this showed any association with shoot metal hyperaccumulation. A highly significant negative correlation was found between shoot metal concentration and shoot : root ratio (Fig. 4), both for Zn (in all three treatments combined) and Cd (in the two Cd treatments). For example, *T.c.* Prayon consistently showed the highest shoot : root ratio (Fig. 1) and lowest shoot metal concentrations (Fig. 2) of the seven populations (cf. Fig. 4). Sensitivity analysis demonstrated that a significant correlation remained when the values from any single population were excluded (data not shown), and the correlations were still significant for Zn and Cd (at $P < 0.01$ and $P < 0.05$, respectively) when the influence of plant population was statistically eliminated in GLM. The correlation was not a general one, as shoot concentrations of Fe, Ca and K (cf. Fig. 5) exhibited no significant association with shoot : root ratio.

Effect of cadmium on iron, calcium and potassium accumulation

As 30 μM Cd reduced Zn accumulation in shoots of *T.c.* St Félix-de-Pallières and *T.c.* Les Malines but not in the other five accessions (Fig. 2a), we investigated whether Cd

Table 3. Ratio of Cd to Zn concentration in shoot dry biomass (both in units of g kg^{-1}) of 6-week-old plants for seven populations of *Thlaspi caerulescens* cultivated for 14 d in standard nutrient solution containing 100 μM $ZnSO_4$ and supplemented with either 3 or 30 μM $CdSO_4$

Cd in solution (μM)	Shoot Cd: Zn concentration ratio						
	Bradford Dale	Clough Wood	Hafna	Prayon	Puente Basadre	St Félix	Les Malines
3	^{abA} 0.05 ± 0.01 (10)	^{bA} 0.06 ± 0.00 (8)	^{bA} 0.05 ± 0.01 (8)	^{aA} 0.04 ± 0.00 (13)	^{aA} 0.04 ± 0.01 (9)	^{cA} 0.08 ± 0.01 (9)	^{cA} 0.08 ± 0.00 (9)
30	^{acB} 0.48 ± 0.02 (8)	^{acB} 0.49 ± 0.02 (8)	^{abB} 0.41 ± 0.02 (8)	^{bB} 0.31 ± 0.04 (13)	^{acB} 0.47 ± 0.05 (11)	^{dB} 0.80 ± 0.03 (8)	^{cB} 0.59 ± 0.08 (8)

Values are means ± SE (n = number of samples in parentheses); different superscripts denote values significantly different at $P = 0.05$ (lower case letters for comparisons within rows, upper case letters for comparisons within columns).

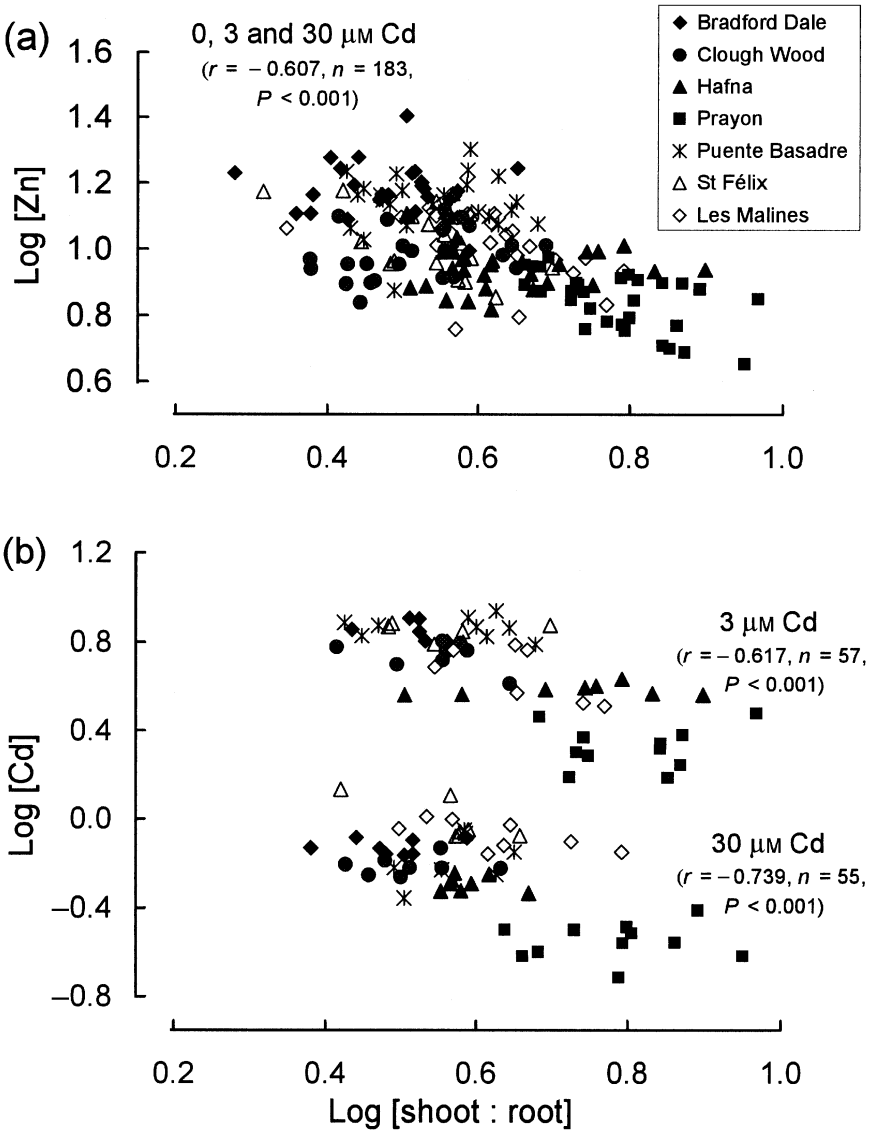

Figure 4. Correlation between shoot concentrations of (a) Zn and (b) Cd and shoot : root dry biomass ratio of 6-week-old plants for seven populations of *Thlaspi caerulescens* cultivated for 14 d in nutrient solution with 100 μM $ZnSO_4$, or in the same solution supplemented with 3 or 30 μM $CdSO_4$. Values are plotted as $\log_{10}$ of the shoot metal concentrations in g kg^{-1} and $\log_{10}$ of the shoot : root dry biomass ratio. Results of the correlation analysis performed by the method of least squares are indicated on the Figure for the three data-sets. Analysis using GLM showed that the relationship between shoot Zn concentration and shoot : root ratio was significant at $P < 0.01$, and that between shoot Cd concentration and shoot : root ratio was significant at $P < 0.05$, when the influence of plant population was statistically eliminated.

differentially affected the accumulation of other elements in these populations. In the absence of Cd, there were no significant differences between the seven populations in their shoot concentrations of Fe, Ca and K, which averaged 0.10, 30 and 53 g kg^{-1}, respectively (Fig. 5). For the statistical analysis of the effect of Cd on Fe accumulation (Fig. 5a), two outliers were first eliminated from the data-set obtained for *T.c.* Puente Basadre on the basis of Dixon's test for outliers (Sokal & Rohlf 1995). Analysis by the T′-method then showed that 3 and 30 μM Cd reduced shoot Fe accumulation by *T.c.* St Félix-de-Pallières to 68 and 59%, and by *T.c.* Les Malines to 78 and 64%, of their control values, respectively (all differences significant at $P < 0.001$). The only other statistically significant effects were of 30 μM Cd on *T.c.* Prayon and *T.c.* Bradford Dale, in which shoot Fe accumulation was reduced to 81 and 88% of their control values, respectively (both $P < 0.05$). (If the two outliers were not eliminated from the Puente Basadre data-set, statistical analysis using the less conservative minimum significant difference test indicated that 30 μM Cd significantly reduced shoot Fe accumulation only in *T.c.* St Félix-de-Pallières and *T.c.* Les Malines, and apparently increased shoot Fe accumulation in *T.c.* Puente Basadre, probably as a result of non-specific breakthrough of metals to the shoot associated with the high sensitivity of this population to Cd.)

Conversely, 30 μM Cd reduced shoot Ca accumulation significantly in all populations except *T.c.* St Félix-de-Pallières and *T.c.* Les Malines (Fig. 5b). In the four mine populations, 30 μM Cd caused relatively similar reductions in Ca accumulation (to between 62 and 72% of the control values: all $P < 0.001$), whereas in the serpentine population *T.c.* Puente Basadre the reduction in Ca accumulation was less pronounced (to 83% of the control value: $P < 0.05$). There was no significant effect of Cd on shoot K accumulation in any population (Fig. 5c).

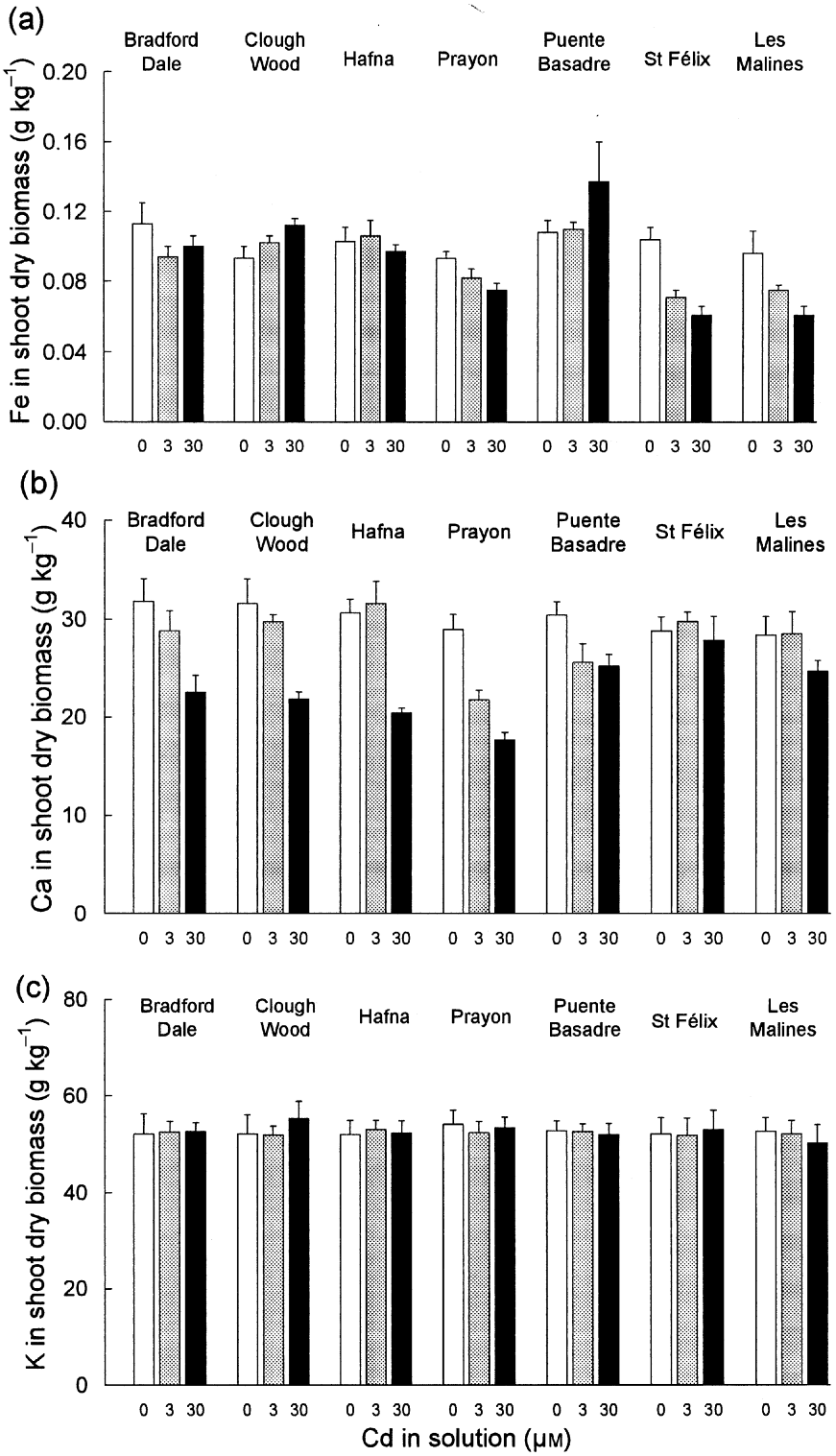

Figure 5. Concentrations of (a) Fe, (b) Ca and (c) K in shoot dry biomass of 6-week-old plants for seven populations of *Thlaspi caerulescens* cultivated for 14 d in nutrient solution with 100 μM $ZnSO_4$, or in the same solution supplemented with 3 or 30 μM $CdSO_4$. Values are means +1 SE (n = 12, 8, 8, 14, 10, 9 and 9 plants for the seven populations Bradford Dale to Les Malines, respectively, reading from left to right).

Effect of zinc on cadmium hyperaccumulation

Cd hyperaccumulation was further investigated in the three most contrasting populations of *T. caerulescens*, namely Prayon (a moderately Cd-tolerant population with the lowest shoot Cd concentrations), Puente Basadre (the least tolerant population), and St Félix-de-Pallières (the most tolerant population). When exposed to 30 μM Cd at two different Zn concentrations, shoot Cd accumulation was 45% lower ($P < 0.01$) at 100 μM Zn compared with 10 μM Zn in *T.c.* Prayon, but was unaffected by Zn concentration in *T.c.* Puente Basadre and *T.c.* St Félix-de-Pallières (Fig. 6). Values for shoot Cd : Zn ratio were lowest for *T.c.* Prayon, intermediate for *T.c.* Puente Basadre, and highest

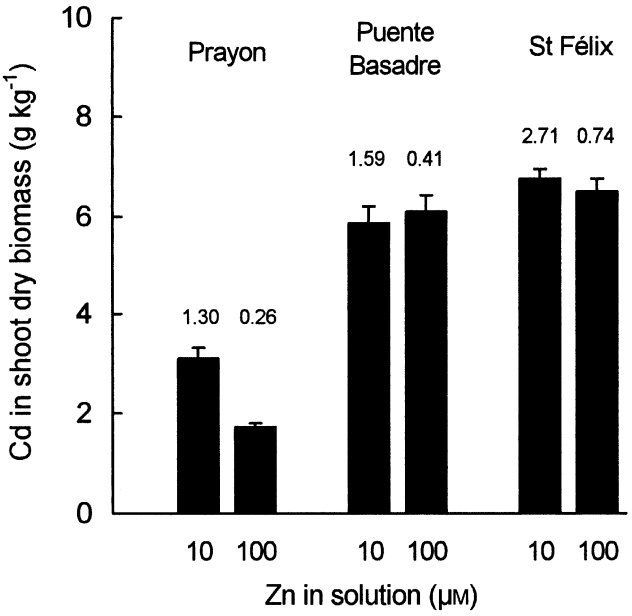

Figure 6. Cd concentration in shoots of 6-week-old plants for three populations of *Thlaspi caerulescens* cultivated for 14 d in nutrient solution containing 30 μM $CdSO_4$ and either 10 or 100 μM $ZnSO_4$. Values are means +1 SE (n = 9, 14, 9, 11, 9 and 8 plants for the six values, respectively, reading from left to right). The numbers above the bars indicate the average shoot Cd : Zn ratio for each treatment.

for *T.c.* St Félix-de-Pallières (Fig. 6), both at 100 μM Zn (as in the experiment in Table 3) and at 10 μM Zn.

To study the effect of Cd on root-to-shoot transfer of metals more directly, samples of xylem sap were taken from root-pressure exudate of plants exposed to Cd for 72 h. Zn concentrations in the xylem sap did not differ markedly between the three populations, but xylem Cd concentrations in *T.c.* St Félix-de-Pallières were more than double ($P < 0.001$) those in *T.c.* Puente Basadre and *T.c.* Prayon (Table 4). In agreement with the results of longer-term shoot metal accumulation (Fig. 6), xylem Cd concentration was 39% lower at 100 μM Zn than at 10 μM Zn in *T.c.* Prayon ($P < 0.001$) but was not affected by Zn exposure in the other two populations. Xylem Zn concentrations were 8.2-fold higher on average at 100 μM Zn compared with 10 μM Zn, and at 10 μM Zn were unaffected by the addition of 30 μM Cd in this short-term experiment.

Total shoot accumulation of zinc and cadmium

Zinc and Cd phytoextraction were evaluated for the seven populations of *T. caerulescens* following 31 d of exposure to 100 μM Zn plus 30 μM Cd by multiplying total above-ground biomass by the concentration of Zn or Cd in the shoot biomass (Fig. 7). The two French populations, *T.c.* St Félix-de-Pallières and *T.c.* Les Malines, accumulated the largest amounts of both Zn (Fig. 7a) and Cd (Fig. 7b) per plant, a function of their high biomass production on 30 μM Cd combined with a relatively high level of Cd hyperaccumulation (cf. Figs 1b & 2b). These two populations extracted on average 1.7-fold and 2.4-fold more Zn and Cd, respectively, than *T.c.* Prayon, the next most efficient population in terms of total metal accumulated per plant, and 14-fold and 15-fold more Zn and Cd, respectively, than *T.c.* Puente Basadre, the least efficient population (all differences significant at $P < 0.001$).

DISCUSSION

Although once considered a strict metallophyte, *T. caerulescens* is now known to occur on a number of substrates besides Zn/Pb/Cd mine and smelter wastes, including Ni-rich serpentine outcrops and a variety of non-metalliferous soils, especially in montane and arctic–alpine habitats (Ingrouille & Smirnoff 1986; Lloyd-Thomas 1995; Meerts & Van Isacker 1997; Escarré *et al.* 2000; Reeves *et al.* 2001). The species is highly variable and includes forms sometimes recognized as separate subspecies, or even as distinct species, but the more unified view of the taxon is currently supported by isozyme data (Koch, Mummenhoff & Hurka 1998). A distinctive feature of *T. caerulescens* across its range is hyperaccumulation of Zn almost irrespective of the total Zn concentration of the local substrate (Reeves *et al.* 2001). The ability of this species to hyperaccumulate Cd to >0.01% of shoot dry biomass was first noted in laboratory experiments (Vázquez *et al.* 1992; Baker *et al.* 1994; Brown *et al.* 1995; Lloyd-Thomas 1995; Knight *et al.* 1997;

Table 4. Concentrations of Cd and Zn in xylem sap of 60-day-old plants for three populations of *Thlaspi caerulescens* cultivated in standard nutrient solution with either 10 or 100 μM $ZnSO_4$ and exposed to either 0 or 30 μM $CdSO_4$ for 72 h

	Metal concentration in xylem sap (μM)					
	Prayon		Puente Basadre		St Félix	
Treatment	Cd	Zn	Cd	Zn	Cd	Zn
0 μM Cd, 10 μM Zn	–	^{aA} 84 ± 3 (8)	–	^{abA} 72 ± 5 (8)	–	^{bA} 63 ± 5 (7)
30 μM Cd, 10 μM Zn	^{aA} 32 ± 1 (7)	^{aA} 93 ± 5 (7)	^{aA} 36 ± 3 (8)	^{aA} 73 ± 9 (6)	^{bA} 75 ± 8 (9)	^{aA} 75 ± 11 (9)
30 μM Cd, 100 μM Zn	^{aB} 19 ± 3 (7)	^{aB} 700 ± 75 (9)	^{aA} 34 ± 4 (7)	^{aB} 610 ± 48 (9)	^{bA} 85 ± 7 (7)	^{aA} 662 ± 75 (10)

Values are means ± SE (n = number of samples in parentheses); different superscripts denote values significantly different at $P = 0.05$ (lower case letters for comparisons within rows for the respective elements, upper case letters for comparisons within columns).

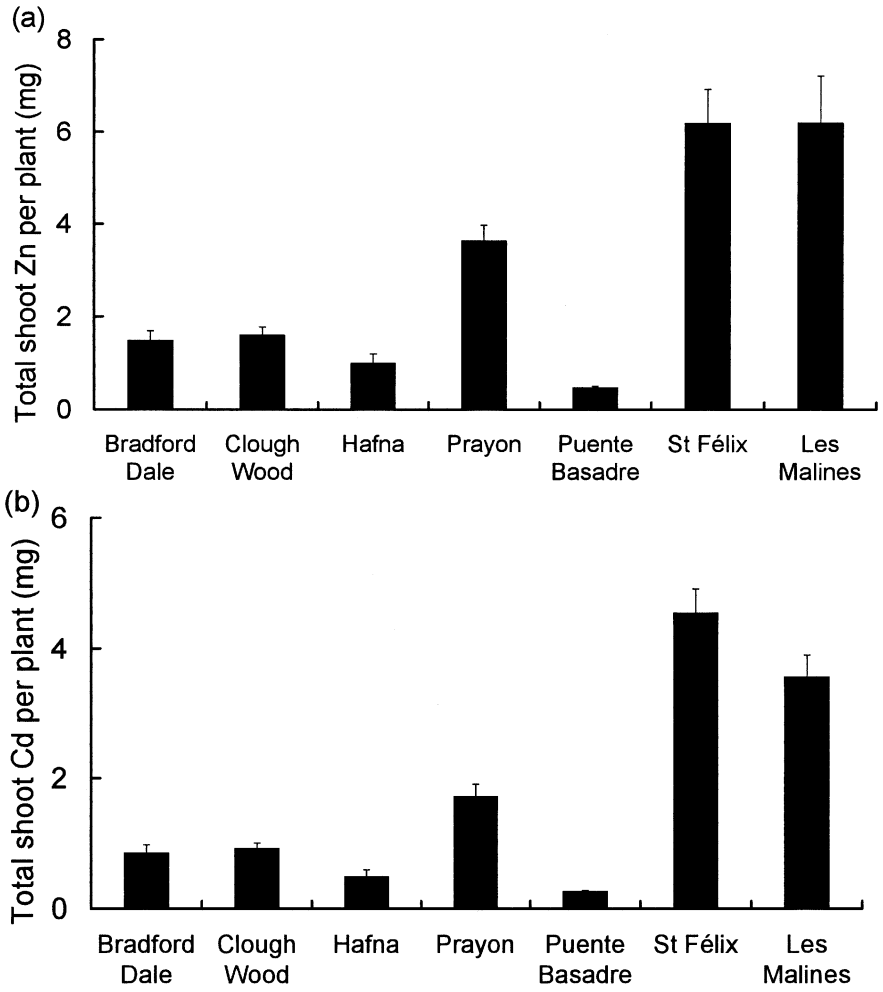

Figure 7. Total content of (a) Zn and (b) Cd in the shoots of 60-day-old plants for seven populations of *Thlaspi caerulescens* cultivated for 31 d in nutrient solution containing 100 μM $ZnSO_4$ and 30 μM $CdSO_4$. Values are means +1 SE of the metal content per plant ($n = 8$ plants for all values except Prayon, for which there were 10 and 9 plants for Zn and Cd determinations, respectively).

McGrath, Shen & Zhao 1997), and has been observed in the field in plants from the Zn/Cd smelter site at Prayon in Belgium (Lloyd-Thomas 1995) and from former Pb–Zn mining areas in the Ganges region of southern France (Robinson *et al.* 1998; Reeves *et al.* 2001). There is evidence for significant genotypic differences between populations in their ability to hyperaccumulate Cd (Escarré *et al.* 2000; Lombi *et al.* 2000), but the extent of natural variation in metal-hyperaccumulation potential in *T. caerulescens*, and its relationship to metal tolerance, has been explored much less fully for Cd than for Zn.

The present study confirms that there are considerable between-population differences in both Cd tolerance (Fig. 1) and Cd hyperaccumulation (Fig. 2) in *T. caerulescens*. To be able to recognize non-specific 'breakthrough' of metals to the shoot – which is thought to be consequence of severe metal toxicity causing membrane damage and uncontrolled release of metals into the xylem (Baker 1981; Pollard *et al.* 2002) – we assayed tissue metal accumulation in plants exposed to two concentrations of Cd (3 or 30 μM) for two different treatment periods (14 or 31 d). The responses in plant biomass production, as well as visual toxicity symptoms, indicated that the serpentine population from Spain (Puente Basadre) was the most sensitive to Cd, followed by the three mine populations from the British Isles (Bradford Dale, Clough Wood and Hafna) and then the Belgian population from Prayon. In contrast, growth of the two Ganges populations was either unaffected (Les Malines) or was stimulated (St Félix-de-Pallières) by Cd (Fig. 1), confirming the exceptional Cd-tolerance of populations from this region of France (Robinson *et al.* 1998; Escarré *et al.* 2000; Lombi *et al.* 2000).

Insofar as the serpentine population was the most sensitive to Cd, these results provide some evidence for a relationship between Cd content of the parent soil and plant Cd tolerance (Table 1), but more precise correlations are difficult to draw because of uncertainty over the most appropriate measure of bioavailable soil metals. In some of the Ganges populations, local adaptation of mine ecotypes to soils highly contaminated with Zn, Pb and Cd seems to have occurred to such a degree that moderate concentrations of Cd are actually required for optimal growth, a response that may involve increased root allocation into Cd-enriched patches of soil (Whiting *et al.* 2000). Metallicolous populations of *T. caerulescens* are known to have an elevated requirement for Zn (Mathys 1977; Shen, Zhao & McGrath 1997), which may be a reflection of constitutive expression of highly effective sequestration mechanisms

that reduce the availability of Zn in the cytoplasm. There is, however, no known biological function for Cd in vascular plants, although Cd can stimulate the growth of the marine diatom *Thalassiosira weissflogii* under low-Zn conditions by incorporation into a specific isoform of carbonic anhydrase (Cullen *et al*. 1999; Lane & Morel 2000). Given that the growth stimulation by Cd is specific to plants from the Ganges region – so that this is not simply a general case of 'hormesis' (Stebbing 1982; Calabrese & Baldwin 2003) – it is possible that Cd has acquired a biological role in some populations of *T. caerulescens*.

All seven populations of *T. caerulescens* showed the ability to accumulate Cd to concentrations above the hyperaccumulation threshold of 0.01% of shoot dry biomass (Chaney *et al*. 1997; Brooks *et al*. 1998), even after only 14 d of exposure to 3 μM Cd (Fig. 2b). In the most tolerant population, *T.c.* St Félix-de-Pallières, shoot Cd concentration reached 0.7% (i.e. 7 g kg^{-1}) at 30 μM Cd without any impairment of growth compared with the zero-Cd control, a value over three orders of magnitude higher than the critical tissue concentration for Cd toxicity in crop plants (Beckett & Davis 1977; Pence *et al*. 2000). Although the exceptional capacity for Cd accumulation can thus be said to be a species-wide property of *T. caerulescens*, there were also pronounced differences between populations in their mean shoot Cd concentration, which varied approximately 3.5-fold (compared with a 2.0-fold variation in shoot Zn concentration). In fact, hyperaccumulation of Cd and Zn showed a strong positive correlation across the seven populations, both for plants grown at 3 and at 30 μM Cd (Figs 2 & 3), which might indicate that Cd and Zn share certain components of the uptake and accumulation mechanisms.

A rather complex picture emerges of the relationship between Cd tolerance (Fig. 1) and hyperaccumulation (Fig. 2) in this species. For the five populations in which 30 μM Cd was inhibitory to growth, there was a clear inverse relationship between tolerance and hyperaccumulation: *T.c.* Prayon was the most tolerant and *T.c.* Puente Basadre the least tolerant of these populations, showing low and high shoot Cd concentrations, respectively, whereas the three British populations were intermediate in their responses. Given the characteristics of the parent sites (cf. Table 1), this concurs with the observation that Zn-hyperaccumulation potential in *T. caerulescens* tends to be greater in populations from non-mine sites compared with mine populations (Ingrouille & Smirnoff 1986; Lloyd-Thomas 1995; Meerts & Van Isacker 1997; Escarré *et al*. 2000; Assunção *et al*. 2001). Such a relationship has also been suggested for the Zn/Cd-hyperaccumulator *Arabidopsis halleri* (Bert *et al*. 2000, 2002, 2003), although there appear to be sources of considerable between-population genetic variation in this species that are not related to differences in bulk soil metal content (Macnair 2002). Indeed, there is good genetic evidence for independent variation in Zn tolerance and hyperaccumulation from crosses between *A. halleri* and its non-tolerant, non-hyperaccumulating relative *Arabidopsis lyrata* ssp. *petraea* (Macnair *et al*. 1999). But the populations of *T. caerulescens* from the Ganges region – especially *T.c.* St Félix-de-Pallières and the material studied by Lombi *et al*. (2000) – are distinct in being both highly Cd-tolerant and having a high hyperaccumulation potential, as also implied by the pot studies of Robinson *et al*. (1998) and Escarré *et al*. (2000). This suggests that the Ganges populations would be valuable for more detailed studies of the biochemical basis of Cd detoxification and sequestration.

Hyperaccumulator plants in general are characterized by concentration ratios of the accumulated metal in shoot to root biomass exceeding 1, a trait indicative of limited retention of the metal in the root, efficient xylem loading and long-distance translocation, and effective sequestration in non-toxic form within the shoot (Baker 1981; Lasat, Baker & Kochian 1998; Baker *et al*. 2000; Salt & Krämer 2000; Schat, Llugany & Bernhard 2000). Shoot : root concentration ratios for Zn above 1 have been reported frequently for *T. caerulescens* (Vázquez *et al*. 1992; Baker *et al*. 1994; Lasat, Baker & Kochian 1996; Shen *et al*. 1997; Assunção *et al*. 2001), but the present work demonstrates considerable (nearly seven-fold) variation in this ratio between populations. Shoot : root concentration ratios for Cd were somewhat lower on average, but were greater than 1 for all populations (Table 2). Shoot : root ratios for Cd above 1 have been observed less consistently in *T. caerulescens* (Vázquez *et al*. 1992; Baker *et al*. 1994; Brown *et al*. 1995; Lloyd-Thomas 1995; Knight *et al*. 1997; McGrath *et al*. 1997; Lombi *et al*. 2000), a fact that may be related to differences in Cd treatments and the procedures used to desorb metals from the root surface on harvesting. Shoot : root concentration ratios for Cd did not show any obvious correlation with either Cd tolerance or Cd hyperaccumulation (Table 2), suggesting that other growth parameters might be influencing this trait. But the highly Cd-tolerant Ganges populations from France were again distinctive in showing either no decrease (*T.c.* Les Malines) or even an increase (*T.c.* St Félix-de-Pallières) in shoot : root concentration ratio for Cd at the higher treatment concentration.

The strong positive correlation between Zn and Cd concentrations in shoot dry biomass (Fig. 3) suggests there might be common influences on the degree of hyperaccumulation of these two elements. In fact, there was a highly significant negative correlation between shoot metal concentrations and shoot : root biomass ratio (Fig. 4), implying that a simple dilution effect – that is, of metals delivered per unit biomass of roots into different volumes of shoot biomass – could explain much of the variation in metal hyperaccumulation. Indeed, to a first approximation, the 2.0-fold variation between populations in mean shoot Zn concentrations after 14 d exposure to 100 μM Zn (Fig. 2a) could be entirely accounted for by the 2.0-fold variation in shoot : root biomass ratio (Fig. 1a), assuming that the populations did not differ significantly in the net flux of Zn from root to shoot. This trend agrees with the general observation that shoot : root biomass ratios tend to be lower under conditions of mineral nutrient deficiency but

higher at toxic levels of mineral supply, consistent with theories of biomass allocation based on optimal partitioning of resources between root and shoot (Wilson 1988; Poorter & Nagel 2000; Shipley & Meziane 2002). The extent to which such allometric relationships contribute to within- and between-population variation in metal hyperaccumulation would be worthy of further study. Lombi *et al*. (2000) also noted that the Ganges material has a lower shoot : root ratio than *T.c.* Prayon, and suggested that this could contribute to the difference in Cd hyperaccumulation between the two populations. However, the somewhat greater variation in Cd hyperaccumulation (3.5-fold) than Zn hyperaccumulation (Figs 2 & 3) suggests there are additional genetic components to between-population variation for this element, possibly related to the marked differences in Cd-tolerance. For example, when grown at 30 μM Cd, the highly tolerant population from St Félix-de-Pallières was characterized by relatively high shoot Cd concentrations for a given shoot : root ratio (Fig. 4b), and by a higher Cd : Zn concentration ratio in the shoot than any other population (Table 3). The scatter of data for shoot Zn and Cd concentrations in individual plants (Figs 3 & 4) also emphasizes the considerable within-population genetic variability in this species noted by other workers (Lloyd-Thomas 1995; Pollard & Baker 1996; Escarré *et al*. 2000; Whiting *et al*. 2000). This has hampered previous attempts to detect significant relationships between metal hyperaccumulation and plant growth traits, which the broader range of populations included in the present study has helped to reveal.

Although correlations between the degree of hyperaccumulation of Zn and Cd imply that components of the uptake and translocation mechanisms may be shared by different metals (Baker *et al*. 1994), the notable differences between populations in their responses to Cd remain to be explained. In a kinetic analysis of metal uptake by *T. caerulescens*, Lombi *et al*. (2001) and Zhao *et al*. (2002) have shown that the exceptional capacity of the Ganges population for Cd hyperaccumulation is associated with expression – at high activity levels compared with the Prayon population – of a high-affinity uptake system for Cd (with a K_m value in the submicromolar range), whereas the two populations show similar kinetics of Zn uptake. Based on ion-competition experiments, in which Ca, Zn, Mn and La were found to inhibit Cd uptake by the Prayon population but not the Ganges population, Zhao *et al*. (2002) suggested that Cd uptake in the former may be mediated partly via Ca channels or transporters for Zn and Mn, whereas in the latter it may be dominated by the more selective high-affinity Cd uptake system. Our results agree with this interpretation, as elevated Zn concentration reduced Cd hyperaccumulation (Fig. 6) and xylem Cd concentration (Table 4) in *T.c.* Prayon but not in *T.c.* St Félix-de-Pallières. Moreover, accumulation of the macronutrient Ca in the shoot was inhibited by 30 μM Cd in the five non-Ganges populations (by 30% on average), but was unaffected by Cd in the two Ganges populations (Fig. 5b). This again suggests a mechanistic link between Cd transport and the major uptake system for Ca in the non-Ganges populations. A number of transport systems are thought to contribute to Ca uptake in plants, most notably the hyperpolarization-activated calcium channels and voltage-independent non-selective cation channels located in the root epidermis (Miedema *et al*. 2001; Demidchik *et al*. 2002a). Such ion channels typically have a finite permeability for, and are competitively inhibited by, other divalent metal ions (White 2000; Demidchik, Davenport & Tester 2002b), so they may present a significant permeation pathway for Cd or simply be blocked by Cd. Additionally, the LCT1 transporter from wheat can mediate uptake of both Cd and Ca when expressed in yeast (Clemens *et al*. 1998), although no homologue of this transporter has been identified in the *Arabidopsis thaliana* genome (Mäser *et al*. 2001).

Although 30 μM Cd reduced Ca accumulation in the five non-Ganges populations of *T. caerulescens* (Fig. 5b), it had no significant effect on Zn accumulation in these plants (Fig. 2a), implying that Zn was taken up through a pathway distinct from Ca. In contrast, 30 μM Cd substantially reduced the accumulation of both Zn (Fig. 2a) and Fe (Fig. 5a) in the shoots of the two Ganges populations (by 30 and 38%, respectively, for the two populations averaged). Various members of the ZIP and Nramp transporter families are able to mediate uptake of Cd in addition to Zn and/or Fe (Mäser *et al*. 2001), and indeed the ZIP-family member ZNT1 cloned from the Prayon population of *T. caerulescens* can mediate high-affinity Zn transport and low-affinity transport of Cd (Pence *et al*. 2000). Considerable interest has focused on the possible role of high-affinity Fe transporters in metal uptake, since Fe-deficiency is known to lead to increased uptake of various divalent metals, including Cd (Cohen *et al*. 1998). Genetic evidence suggests that the ZIP-family member IRT1 is the major high-affinity root Fe uptake system in *Arabidopsis thaliana*, possibly also playing a significant role in Zn transport (Henriques *et al*. 2002; Vert *et al*. 2002), and that enhanced expression of this transporter can account for the increased uptake of Cd observed under Fe-deficiency conditions (Connolly, Fett & Guerinot 2002; Vert *et al*. 2002). Moreover, in a Ganges population of *T. caerulescens*, Lombi *et al*. (2002) have shown that Fe-deficiency strongly enhances Cd uptake and dramatically increases transcript levels of the IRT1 orthologue cloned from this species, whereas these responses are not observed in the Prayon population. Our finding that 3 μM Cd reduced shoot Fe accumulation in the two Ganges populations but not in the other five populations (Fig. 5a), and similarly that 30 μM Cd reduced Zn accumulation only in the Ganges populations (Fig. 2a), is fully consistent with these results. Indeed, the critical deficiency content of Fe in leaves is typically in the range 0.05–0.15 g kg^{-1} dry biomass (Marschner 1995), and a chlorotic phenotype is detectable in *A. thaliana* at a shoot Fe content of 0.08 g kg^{-1} (Connolly *et al*. 2002), suggesting that the Ganges plants are bordering on incipient Fe-deficiency when exposed to Cd. The constancy under these conditions of shoot K con-

centration (Fig. 5c) reflects the precise feedback control to which accumulation of some nutrients can be subject (Pitman 1988) and emphasizes the specificity of these responses to metal exposure.

In view of the risks posed by Cd as an environmental pollutant (Sanità di Toppi & Gabbrielli 1999), there has been interest in the potential use of *T. caerulescens* as a Cd-hyperaccumulating plant to extract this element from contaminated soils (Brown *et al*. 1995; Robinson *et al*. 1998; Escarré *et al*. 2000; Lombi *et al*. 2000). The total amount of Cd phyoextracted from the substrate will be given by the product of plant biomass and Cd content per unit biomass. If sufficient variation exists in these traits, it may be possible to develop lines with improved phytoextraction efficiencies using traditional breeding techniques. So it is of interest to ask whether plant metal tolerance (as reflected in biomass production) or hyperaccumulation (metal content per unit biomass) is more important in determining differences in phytoextraction between populations. The answer in the present case is the former: total shoot Cd content (Fig. 7b) was closely related to Cd tolerance, being highest for the two Ganges populations, intermediate for *T.c.* Prayon, and lowest for *T.c.* Puente Basadre (Fig. 1b), but did not correlate with shoot Cd concentration (Fig. 2b). This emphasizes the importance of plant metal tolerance as a prerequisite for sustainable phytoextraction (Chaney *et al*. 1997; Salt *et al*. 1998; Lombi *et al*. 2000; McGrath *et al*. 2002), and suggests that selection for high shoot metal concentration (e.g. Macnair *et al*. 1999) would not necessarily lead to maximal rates of metal removal. The two Ganges populations are notable in combining an exceptional degree of Cd tolerance with both comparatively high biomass and high shoot metal concentrations, so this material will be valuable in developing a better understanding of the genetic basis of Cd homeostasis in plants.

In summary, this study has revealed widely different degrees of Cd tolerance within *T. caerulescens*, ranging from populations in which growth is strongly inhibited by Cd to those in which this element is apparently required for optimal growth. All populations are able to hyperaccumulate Cd, but the relationship between hyperaccumulation and tolerance is complex. For those populations in which Cd is inhibitory, there is an inverse relationship between hyperaccumulation and tolerance, as observed previously for Zn. A major contribution to this quantitative variation in hyperaccumulation is made by the allometry of biomass partitioning between shoot and root, which varies with Cd tolerance in a manner expected as a response to accumulation of a toxic element. The Ganges populations of *T. caerulescens*, however, combine a high level of Cd accumulation with an exceptional degree of tolerance, suggesting that Cd hyperaccumulation may be of adaptive significance in these plants. Whether this reflects a role for Cd as a micronutrient in the Ganges populations, or perhaps an involvement in defence against herbivores and pathogens as suggested for hyperaccumulation of other elements, should become clearer as the genetic basis of these traits is further explored.

ACKNOWLEDGMENTS

This work was supported by the Fondation Philippe Wiener – Maurice Anspach, Brussels (N.R), by 'IAP 5/13 Plant Growth & Development' from the Federal Office for Scientific, Technical and Cultural Affairs, OSTC (N.V), by a Short-Term Scientific Mission under EU COST Action 837 (P.X.-E), and by the Biotechnology and Biological Sciences Research Council and the Natural Environment Research Council (J.A.C.S). We thank A.J.M. Baker, C. Lefèbvre, A.J. Pollard and R.D. Reeves for providing seeds, C. Camara for her support, and R.S. Leighton, F.A. Harper, R.A. Ingle and A.J. Pollard for helpful discussions.

REFERENCES

Assunção A.G.L., Da Costa Martins P., De Folter S., Vooijs R., Schat H. & Aarts M.G.M. (2001) Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant, Cell and Environment* **24,** 217–226.

Baker A.J.M. (1981) Accumulators and excluders – strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3,** 643–654.

Baker A.J.M. & Brooks R.R. (1989) Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* **1,** 81–126.

Baker A.J.M., McGrath S.P., Reeves R.D. & Smith J.A.C. (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In *Phytoremediation of Contaminated Soil and Water* (eds N. Terry & G. Bañuelos), pp. 85–107. Lewis Publishers, Boca Raton, FL, USA.

Baker A.J.M., Reeves R.D. & Hajar A.S.M. (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New Phytologist* **127,** 61–68.

Beckett P.H.T. & Davis R.D. (1977) Upper critical levels of toxic elements in plants. *New Phytologist* **79,** 95–106.

Bert V., Bonnin I., Saumitou-Laprade P., de Laguérie P. & Petit D. (2002) Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytologist* **155,** 47–57.

Bert V., Macnair M.R., de Laguérie P., Saumitou-Laprade P. & Petit D. (2000) Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). *New Phytologist* **146,** 225–233.

Bert V., Meerts P., Saumitou-Laprade P., Salis P., Gruber W. & Verbruggen N. (2003) Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant and Soil* **249,** 9–18.

Boyd R.S. (1998) Hyperaccumulation as a plant defensive strategy. In *Plants that Hyperaccumulate Heavy Metals* (ed. R.R. Brooks), pp. 181–201. CAB International, Wallingford, UK.

Brooks R.R., Chambers M.F., Nicks L.J. & Robinson B.H. (1998) Phytomining. *Trends in Plant Science* **3,** 359–362.

Brown S.L., Chaney R.L., Angle J.S. & Baker A.J.M. (1995) Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. *Soil Science Society of America Journal* **59,** 125–133.

Calabrese E.J. & Baldwin L.A. (2003) Toxicology rethinks its central belief. *Nature* **421,** 691–692.

Chaney R.L., Malik M., Li Y.M., Brown S.L., Brewer E.P., Angle J.S. & Baker A.J.M. (1997) Phytoremediation of soil metals. *Current Opinion in Biotechnology* **8,** 279–284.

Clemens S., Antosiewicz D.M., Ward J.M., Schachtman D.P. & Schroeder J.I. (1998) The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. *Proceedings of the National Academy of Sciences of the USA* **95,** 12043–12048.

Clemens S., Palmgren M.G. & Krämer U. (2002) A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science* **7,** 309–315.

Cohen C.K., Fox T.C., Garvin D.F. & Kochian L.V. (1998) The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiology* **116,** 1063–1072.

Connolly E.L., Fett J.P. & Guerinot M.L. (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell* **14,** 1347–1357.

Cullen J.T., Lane T.W., Morel F.M.M. & Sherrell R.M. (1999) Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. *Nature* **402,** 165–167.

Dahmani-Muller H., van Oort F., Gélie B. & Balabane M. (2000) Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environmental Pollution* **109,** 231–238.

Demidchik V., Bowen H.C., Maathuis F.J.M., Shabala S.N., Tester M.A., White P.J. & Davies J.M. (2002a) *Arabidopsis thaliana* root non-selective cation channels mediate calcium uptake and are involved in growth. *Plant Journal* **32,** 799–808.

Demidchik V., Davenport R.J. & Tester M. (2002b) Non-selective cation channels in plants. *Annual Review of Plant Biology* **53,** 67–107.

Denaeyer-De Smet S. & Duvigneaud P. (1974) Accumulation de métaux lourds toxiques dans divers écosystèmes terrestres pollués par des retombées d'origine industrielle. *Bulletin de la Société Royale de Botanique de Belgique* **107,** 147–156.

Escarré J., Lefèbvre C., Gruber W., Leblanc M., Lepart J., Rivière Y. & Delay B. (2000) Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *New Phytologist* **145,** 429–437.

Grafen A. & Hails R. (2002) *Modern Statistics for the Life Sciences*. Oxford University Press, Oxford, UK.

Henriques R., Jásik J., Klein M., Martinoia E., Feller U., Schell J., Pais M.S. & Koncz C. (2002) Knock-out of *Arabidopsis* metal transporter gene IRT1 results in iron deficiency accompanied by cell differentiation defects. *Plant Molecular Biology* **50,** 587–597.

Hewitt E.J. & Smith T.A. (1975) *Plant Mineral Nutrition.* The English Universities Press, London, UK.

Hoagland D.R. & Arnon D.I. (1950) The water-culture method for growing plants without soil. *California Agricultural Experimental Station Circular* **347,** 1–32.

Ingrouille M.J. & Smirnoff N. (1986) *Thlaspi caerulescens* J. & C. Presl (*Thlaspi alpestre* L.) in Britain. *New Phytologist* **102,** 219–233.

Knight B., Zhao F.J., McGrath S.P. & Shen Z.G. (1997) Zinc and cadmium uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution. *Plant and Soil* **197,** 71–78.

Koch M., Mummenhoff K. & Hurka H. (1998) Systematics and evolutionary history of heavy metal tolerant *Thlaspi caerulescens* in Western Europe: evidence from genetic studies based on isozyme analysis. *Biochemical Systematics and Ecology* **26,** 823–838.

Küpper H., Lombi E., Zhao F.-J. & McGrath S.P. (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* **212,** 75–84.

Lane T.W. & Morel F.M.M. (2000) A biological function for cadmium in marine diatoms. *Proceedings of the National Academy of Sciences of the USA* **97,** 4627–4631.

Lasat M.M., Baker A.J.M. & Kochian L.V. (1996) Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiology* **112,** 1715–1722.

Lasat M.M., Baker A.J.M. & Kochian L.V. (1998) Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens*. *Plant Physiology* **118,** 875–883.

Lloyd-Thomas D.H. (1995) Heavy Metal Hyperaccumulation by *Thlaspi caerulescens* J. & C. Presl. PhD Thesis. University of Sheffield, Sheffield, UK.

Lombi E., Tearall K.L., Howarth J.R., Zhao F.-J., Hawkesford M.J. & McGrath S.P. (2002) Influence of iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology* **128,** 1359–1367.

Lombi E., Zhao F.J., Dunham S.J. & McGrath S.P. (2000) Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. *New Phytologist* **145,** 11–20.

Lombi E., Zhao F.J., McGrath S.P., Young S.D. & Sacchi G.A. (2001) Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *New Phytologist* **149,** 53–60.

Macnair M.R. (2002) Within and between population genetic variation for zinc accumulation in *Arabidopsis halleri*. *New Phytologist* **155,** 59–66.

Macnair M.R., Bert V., Huitson S.B., Saumitou-Laprade P. & Petit D. (1999) Zinc tolerance and hyperaccumulation are genetically independent characters. *Proceedings of the Royal Society, London, Series B* **266,** 2175–2179.

Marschner H. (1995) *Mineral Nutrition of Higher Plants*, 2nd edn. Academic Press, London, UK.

Mäser P., Thomine S., Schroeder J.I., *et al.* (2001) Phylogenetic relationships within cation transporter families of *Arabidopsis*. *Plant Physiology* **126,** 1646–1667.

Mathys W. (1977) The role of malate, oxalate and mustard oil glucosides in the evolution of zinc-resistance in herbage plants. *Physiologia Plantarum* **40,** 130–136.

McGrath S.P., Shen Z.G. & Zhao F.J. (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant and Soil* **188,** 153–159.

McGrath S.P., Sidoli C.M.D., Baker A.J.M. & Reeves R.D. (1993) The potential for the use of metal-accumulating plants for the *in situ* decontamination of metal-polluted soils. In *Integrated Soil and Sediment Research: a Basis for Proper Protection* (eds H.J.P. Eijsackers & T. Hamers), pp. 673–676. Kluwer Academic Publishers, Dordrecht, The Netherlands.

McGrath S.P., Zhao F.J. & Lombi E. (2002) Phytoremediation of metals, metalloids, and radionuclides. *Advances in Agronomy* **75,** 1–56.

Meerts P. & Van Isacker N. (1997) Heavy metal tolerance and accumulation in metallicolous and non-metallicolous populations of *Thlaspi caerulescens* from continental Europe. *Plant Ecology* **133,** 221–231.

Miedema H., Bothwell J.H.F., Brownlee C. & Davies J.M. (2001) Calcium uptake by plant cells – channels and pumps acting in concert. *Trends in Plant Science* **6,** 514–519.

Pence N.S., Larsen P.B., Ebbs S.D., Letham D.L.D., Lasat M.M., Garvin D.F., Eide D. & Kochian L.V. (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumu-

lator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences of the USA* **97,** 4956–4960.

Persans M.W. & Salt D.E. (2000) Possible molecular mechanisms involved in nickel, zinc and selenium hyperaccumulation in plants. *Biotechnology and Genetic Engineering Reviews* **17,** 389–413.

Pitman M.G. (1988) Whole plants. In *Solute Transport in Plant Cells and Tissues* (eds D.A. Baker & J.L. Hall), pp. 346–391. Longman Scientific & Technical, Harlow, Essex, UK.

Pollard A.J. & Baker A.J.M. (1996) Quantitative genetics of zinc hyperaccumulation in *Thlaspi caerulescens*. *New Phytologist* **132,** 113–118.

Pollard A.J., Powell K.D., Harper F.A. & Smith J.A.C. (2002) The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21,** 539–566.

Poorter H. & Nagel O. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology* **27,** 595–607.

Reeves R.D. & Baker A.J.M. (2000) Metal-accumulating plants. In *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* (eds I. Raskin & B.D. Ensley), pp. 193–229. John Wiley & Sons, Inc, New York, USA.

Reeves R.D. & Brooks R.R. (1983) European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. *Journal of Geochemical Exploration* **18,** 275–283.

Reeves R.D., Schwartz C., Morel J.L. & Edmondson J. (2001) Distribution and metal-accumulating behavior of *Thlaspi caerulescens* and associated metallophytes in France. *International Journal of Phytoremediation* **3,** 145–172.

Robinson B.H., Leblanc M., Petit D., Brooks R.R., Kirkman J.H. & Gregg P.E.H. (1998) The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. *Plant and Soil* **203,** 47–56.

Salt D.E. & Krämer U. (2000) Mechanisms of metal hyperaccumulation in plants. In *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* (eds I. Raskin & B.D. Ensley), pp. 231–246. John Wiley & Sons, Inc, New York, USA.

Salt D.E., Smith R.D. & Raskin I. (1998) Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology* **49,** 643–668.

Sanità di Toppi L. & Gabbrielli R. (1999) Response to cadmium in higher plants. *Environmental and Experimental Botany* **41,** 105–130.

Schat H., Llugany M. & Bernhard R. (2000) Metal-specific patterns of tolerance, uptake, and transport of heavy metals in hyperaccumulating and nonhyperaccumulating metallophytes. In *Phytoremediation of Contaminated Soil and Water* (eds N. Terry & G. Bañuelos), pp. 171–188. Lewis Publishers, Boca Raton, FL, USA.

Shen Z.G., Zhao F.J. & McGrath (1997) Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. *Plant, Cell and Environment* **20,** 898–906.

Shipley B. & Meziane D. (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. *Functional Ecology* **16,** 326–331.

Sokal R.R. & Rohlf F.J. (1995) *Biometry*, 3rd edn. W.H. Freeman, New York, USA.

Stebbing A.R.D. (1982) Hormesis – the stimulation of growth by low levels of inhibitors. *Science of the Total Environment* **22,** 213–234.

Vázquez M.D., Barceló J., Poschenrieder C., Mádico J., Hatton P., Baker A.J.M. & Cope G.H. (1992) Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. *Journal of Plant Physiology* **140,** 350–355.

Vert G., Grotz N., Dédaldéchamp F., Gaymard F., Guerinot M.L., Briat J.-F. & Curie C. (2002) IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* **14,** 1223–1233.

White P.J. (2000) Calcium channels in higher plants. *Biochimica et Biophysica Acta* **1465,** 171–189.

Whiting S.N., Leake J.R., McGrath S.P. & Baker A.J.M. (2000) Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **145,** 199–210.

Wilson J.B. (1988) A review of evidence on the control of shoot: root ratio, in relation to models. *Annals of Botany* **61,** 433–449.

Zhao F.-J., Hamon R.E., Lombi E., McLaughlin M.J. & McGrath S.P. (2002) Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany* **53,** 535–543.

Received 24 January 2003; received in revised form 28 May 2003; accepted for publication 4 June 2003