



## Metal accumulation and competitive ability in metallicolous and non-metallicolous *Thlaspi caerulescens* fed with different Zn salts

P. Meerts<sup>1</sup>, Ph. Duchêne, W. Gruber & C. Lefèbvre

Laboratoire de Génétique et Ecologie végétales, Université Libre de Bruxelles, Chaussée de Wavre 1850, B-1160 Brussels, Belgium

<sup>1</sup>Corresponding author\*

**Key words:** competition, heavy metals, hyperaccumulation, phytoremediation, Zn availability, Zn–Mg interaction

### Abstract

A non-metallicolous (NM) ecotype of *Thlaspi caerulescens* from Luxembourg and a metallicolous (M) ecotype from Prayon (E Belgium) are compared for growth and Zn, Mg and Ca accumulation in shoot in a pot experiment in six soil conditions with contrasting Zn availability. The soils were spiked with 2000 mg kg<sup>-1</sup> Zn as monometallic salts of contrasting solubility. Both ecotypes were grown in pure and mixed culture in order to assess competitive ability. Both ecotypes had similar growth on all substrates except the one spiked with Zn-sulphate, where ecotype M grew better and had higher competitive ability than ecotype NM. Ecotype NM had higher Zn concentrations than M in all treatments and the difference varied with Zn availability, being largest with Zn-oxide (NM: 31 300 mg kg<sup>-1</sup> Zn; M: 5900 mg kg<sup>-1</sup> Zn). The results thus indicate that ecotype NM has constitutively higher Zn uptake capacity and may have a higher ability to obtain Zn from specific Zn salts. However, ecotype NM does not appear to be more efficient in obtaining Zn from little available forms. Mg concentration was also higher in ecotype NM. Zn mass per plant was higher in ecotype NM compared to ecotype M in all substrates except Zn-sulphate where the reverse was true. Accordingly, ecotype NM could prove to be a better phytoextractor of Zn for phytoremediation, except in substrates with low pH and high concentration of free Zn in the soil solution.

**Abbreviations:** ecotype M – metallicolous ecotype; ecotype NM – non-metallicolous ecotype

### Introduction

*Thlaspi caerulescens* J. & C. Presl is a well-known Zn hyperaccumulator able to tolerate up to 30,000 mg kg<sup>-1</sup> Zn in the shoot dry matter without growth reduction (Baker et al., 1994; Brown et al., 1995; Shen et al., 1997). The mechanisms by which *T. caerulescens* achieves such high accumulation of Zn (and other metals) have attracted considerable attention in the last few years. *T. caerulescens* has an unusually high rate of Zn influx in root cells (Lasat et al., 1996) probably due to a high expression of Zn transporters in roots (Lasat et al., 2000). Another root trait that probably contributes to its hyperaccumulation properties is its ability to forage actively Zn in soil by proliferating roots in Zn-rich patches (Schwartz et al.,

1999; Whiting et al., 2000). A further possibility is that *T. caerulescens* can access little soluble Zn forms (Knight et al., 1997; McGrath et al., 1997; Whiting et al., 2001). However, *T. caerulescens* does apparently not mobilise heavy metals in the rhizosphere by releasing acidifying compounds (Knight et al., 1997; McGrath et al., 1997; Luo et al., 2000) and its root exudates are not efficient at mobilising Zn from non labile forms (Zhao et al., 2001).

Although *T. caerulescens* is constitutively tolerant and hyperaccumulator of Zn, recent work has pointed out the existence of genetic variation in the ability to tolerate and accumulate Zn and other metals, both within and between populations (Assunção et al., 2001; Escarré et al., 2000; Meerts and Van Isacker, 1997; Pollard and Baker, 1996). In the few studies so far examining tolerance and accumulation properties of non-metallicolous populations, these were

\* FAX No: +32-2-6509170. E-mail: pmeerts@ulb.ac.be

found to accumulate Zn to much higher concentrations than metallicolous populations when growing on moderately Zn-rich soil (Assunção et al., 2001; Escarré et al., 2000; Meerts and Van Isacker, 1997). When growing in their natural, Zn-poor soils, non-metallicolous populations also achieve surprisingly high concentrations of Zn and their concentration factor (concentration ratio of Zn in shoots to Zn in soil) is higher compared to metallicolous populations on metal-rich soils (Lloyd-Thomas, 1995; Reeves et al., 2001). These observations might indicate that non-metallicolous populations have evolved a higher uptake capacity of Zn and/or a higher ability to obtain Zn from relatively little soluble Zn forms. Paradoxically, non-metallicolous populations might eventually prove to be more suitable to phytoremediation of Zn-polluted soil than their metallicolous relatives. Clearly, a deeper knowledge of variation in metal accumulation and tolerance existing among natural populations of *T. caerulescens* is desirable if this species is to be used for phytoremediation of metal-polluted soils.

In this paper, we assess Zn tolerance and accumulation in a metallicolous (M) ecotype of *T. caerulescens* from E Belgium and a non-metallicolous (NM) ecotype from Luxembourg. In a previous paper (Meerts and Van Isacker, 1997), the Luxembourg ecotype was shown to have higher Zn and Mg concentrations than ecotype M when grown on soil enriched with Zn-rich furnace slag. In this paper, the plants were grown in pots in soil spiked with monometallic Zn-salts of contrasting solubility. We test whether growth and Zn accumulation of the two ecotypes are systematically different irrespective of Zn availability and are similarly affected by the different chemical forms of Zn added to the soil. The tolerance to the different substrates is assessed in an experimental design allowing for competitive interactions between the two ecotypes. Mg and Ca were also analysed because a previous study suggested that M and NM had contrasting accumulation of these elements in shoots (Meerts and Van Isacker, 1997). Finally, the implications of the results for phytoremediation are briefly discussed.

## Materials and methods

Seeds were collected from the non-metallicolous population of Wilwerwiltz (Grand Duchy of Luxembourg) and the metallicolous population of Prayon (E Belgium) (for site characteristics see Meerts and Van Isacker (1997) and Reeves et al. (2001)). In a previous

study, no significant variation in Zn accumulation and tolerance was found between populations within either ecotype (Meerts and Van Isacker, 1997). Therefore, the two populations used in this study are assumed to be representative of their respective ecotype.

The seeds were sown in trays filled with a mixture of sand and garden compost. Three weeks after emergence, seedlings were transplanted into 750-mL pots and were assigned to six different soil treatments. The growth substrate consisted of a mixture of 65% Rhine sand, 20% loam and 15% home-made garden compost (fully decomposed compost of leaf litter from herbaceous plants and broad-leaved trees). Mineral element concentrations in the substrate are as follows (mean  $\pm$  SD,  $n = 6$ , extraction with ammonium acetate-EDTA 1M, pH 4.65):  $49 \pm 2$  mg  $\text{kg}^{-1}$  Zn,  $5234 \pm 531$  mg  $\text{kg}^{-1}$  Ca,  $186 \pm 26$  mg  $\text{kg}^{-1}$  Mg. Each pot contained 790 g dry soil. Five Zn treatments were established by spiking the growth substrate with monometallic Zn salts to obtain a total concentration of 2000 mg  $\text{kg}^{-1}$  Zn in all treatments. The sixth treatment was a control, unenriched soil. The five following Zn salts were chosen to span a wide range of solubility in water:  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (solubility in cold water: 96.5 g/100 ml), ZnO (0.00016 g/100 ml),  $\text{ZnCO}_3 \cdot 2[\text{Zn}(\text{OH})_2] \cdot \text{H}_2\text{O}$  (Zn-hydroxycarbonate; hereafter  $\text{ZnCO}_3$ ) (0.001 g/100 ml), ZnS (1–3-mm granules) (0.000065 g/100 ml), Zn (metallic Zn, 4–5-mm pellets) (insoluble). In the treatment with Zn-pellets, four pellets were put at the corners of a  $4 \times 4$ -cm square at 5 cm below ground level; the total Zn concentration in this treatment ranged from ca. 1800 to ca. 2200 mg  $\text{kg}^{-1}$  depending on pellet size.

The soil was moistened with tap water a few days before transplantation. Eight seedlings were transplanted into each pot. The seedlings were equally spaced in an 8-cm diameter circle. The experimental design consisted of a de Wit replacement series, comprising pure cultures of either ecotype and mixed equiproportional cultures with four M plants alternating with four NM plants. There were four replicates of either monoculture and 8 replicates of the mixed culture for each soil treatment. The 96 pots were randomly placed in an unlit greenhouse. Temperature was not allowed to drop below 5 °C but was not otherwise controlled. The plants were watered with tap water as necessary. Soil pH in the pots at the end of the experiment ranged from 7.3 to 7.8 in all treatments except in the Zn-sulphate treatment where it was  $6.1 \pm 0.2$ .

The plants were harvested 168 days after transplantation (aboveground parts only). Only half the

individuals were harvested (i.e., four plants in each pot for pure cultures; two M and two NM in each pot for mixed cultures), the other individuals were left to grow for further observations. The four (pure cultures) or two (mixed cultures) individuals of the same ecotype from each pot were pooled, oven-dried at 60°C and weighed to the nearest Mg.

Oven-dried aboveground parts were finely ground and mineralised in a mixture of nitric and perchloric acid. Zn, Ca and Mg were then determined through flame AAS; for Mg and Ca, lanthanum nitrate was added to the digests (for further details on analytical methods see Cottenie et al., 1982).

The data were analysed by means of two-way ANOVAs, with soil and ecotype as main effects. Post-hoc multiple comparison tests were used to compare the two ecotypes in each soil; Bonferonni-corrected probability levels were applied. Owing to heteroscedasticity and departure from normality, the data were log-transformed prior to statistical analysis.

## Results

### Shoot biomass

Average shoot mass ranged from 0.09 to 0.65 g depending on ecotype and soil (Figure 1). There was no significant soil effect in the two-way ANOVA, but the ecotype $\times$ soil interaction was highly significant (Table 1). Shoot mass was not statistically different between ecotypes except on Zn-sulphate, where ecotype M had on average 6-fold larger biomass than ecotype NM (Figure 1).

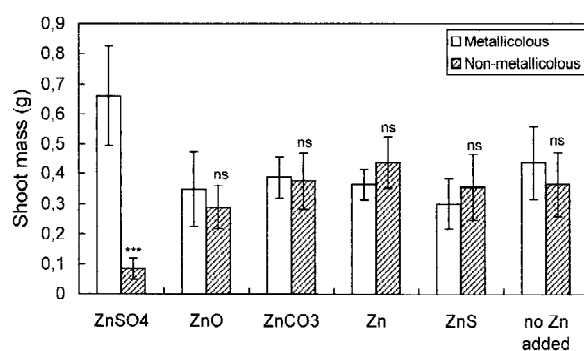


Figure 1. Shoot mass in metallicolous and non-metallicolous *Thlaspi caerulescens* grown in six soils with contrasting availability of Zn. For each soil, the statistical significance of the difference between ecotypes is indicated \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ , ns  $P > 0.05$ .

Table 1. Two-way analysis of variance of mineral element concentrations, biomass and Zn mass in shoots in two ecotypes of *Thlaspi caerulescens* grown in six soils with contrasting availability of Zn (data from pure and mixed culture pooled). \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ , ns not significant

	df	MS	F
<i>Zn concentration in shoot</i>			
Soil	5	10.18	643.1***
Ecotype	1	8.35	527.9***
Soil*Ecotype	5	0.19	12.2***
Error	132	0.016	
<i>Mg concentration in shoot</i>			
Soil	5	0.76	88.0***
Ecotype	1	0.85	98.2***
Soil*Ecotype	5	0.04	5.03***
Error	132	0.009	
<i>Ca concentration in shoot</i>			
Soil	5	0.056	9.55***
Ecotype	1	0.032	5.49*
Soil*Ecotype	5	0.143	24.34***
Error	132	0.006	
<i>Shoot biomass</i>			
Soil	5	0.031	1.02 ns
Ecotype	1	0.35	11.59***
Soil*Ecotype	5	0.35	11.49***
Error	132	0.03	
<i>Zn mass in shoot</i>			
Soil	5	8.27	108.9***
Ecotype	1	3.35	44.04***
Soil*Ecotype	5	1.14	14.97***
Error	132	0.076	

### Shoot Zn concentration

Average Zn concentration in shoots ranged from ca. 500 to 38 000 mg kg<sup>-1</sup> Zn, depending on ecotype and soil (Figure 2). In the two-way ANOVA the ecotype and soil effects as well as the ecotype $\times$ soil interaction were all highly significant (Table 1). Ecotype NM had significantly higher Zn concentrations in all treatments (Figure 2). The highest Zn concentrations were found in the Zn-sulphate treatment for both ecotypes, followed by Zn-oxide and Zn-hydroxycarbonate. Zn-sulphide, metallic-Zn and the unenriched treatment yielded similarly low shoot Zn concentrations (<2000 and <1000 mg kg<sup>-1</sup> in ecotype NM and ecotype M,

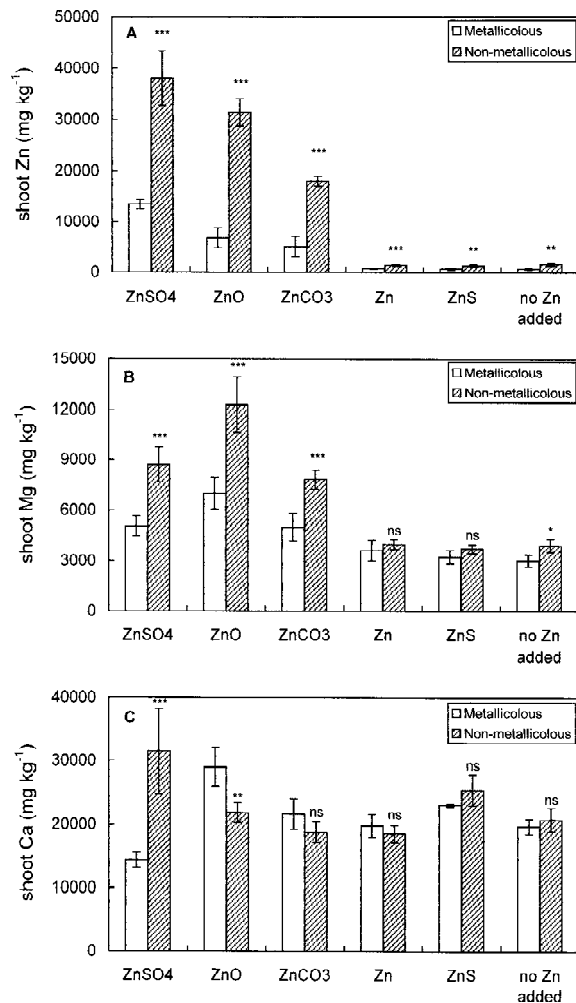


Figure 2. Shoot mineral element concentrations in metallicolous and non-metallicolous *Thlaspi caerulescens* grown in six soils with contrasting availability of Zn: (a) Zn; (b) Mg; (c) Ca. Mean and 95% confidence intervals. For each soil, the statistical significance of the difference between ecotypes is indicated \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ , ns  $P > 0.05$ .

respectively) (Figure 2). The ratio of Zn concentration in ecotype NM to Zn concentration in ecotype M was 5.3 on Zn-oxide, 3.6 on Zn-hydroxycarbonate, 2.8 on Zn-sulphate, 2.5 on the control, 2.1 on metallic-Zn and 2.0 on Zn-sulphide.

#### Shoot Mg and Ca concentration

Average Mg concentration ranged from ca. 3000 to 12000  $\text{mg kg}^{-1}$  depending on ecotype and soil (Figure 2). In the two-way ANOVA, the ecotype and soil main effects as well as the ecotype $\times$ soil interaction were all highly significant (Table 1). Mg concen-

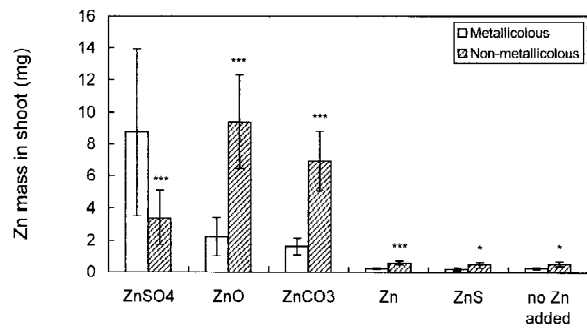


Figure 3. Zn mass in shoots in metallicolous and non-metallicolous *Thlaspi caerulescens* grown on six soils with contrasting availability of Zn. Mean and 95% confidence intervals. For each soil, the statistical significance of the difference between ecotypes is indicated \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ , ns  $P > 0.05$ .

tration was higher in ecotype NM on all substrates, but the difference was not significant for metallic Zn and Zn-sulphide. In both ecotypes Mg concentration was markedly higher on Zn-oxide, followed by Zn-sulphate and Zn-hydroxycarbonate compared to the three treatments with low Zn availability.

Ca concentration ranged from ca. 14500 to 32000  $\text{mg kg}^{-1}$  depending on soil and ecotype (Figure 2). The pattern of variation in relation to ecotype and soil was less clear than for Zn and Mg. Compared to the other treatments, Ca concentration was higher on Zn-sulphate in ecotype NM and lower on Zn-sulphate in ecotype M (Figure 2).

#### Zn mass in shoot

Average Zn mass accumulated in shoot (calculated as the product of Zn concentration by shoot mass) ranged from ca. 0.4 to ca. 10 mg, depending on ecotype and soil (Figure 3). Ecotype NM had significantly higher Zn mass than ecotype M in all treatments except Zn-sulphate, where the reverse was true. The difference was largest on Zn-oxide and hydroxycarbonate (4- to 5-fold difference) (Figure 3).

#### Zn-Mg correlation

Mg and Zn concentrations are strongly correlated in the whole data set ( $r = 0.832$ ,  $P < 0.001$ ,  $n = 144$ ) (Figure 4). This correlation has both a genetic and an environmental component, because ecotype NM had intrinsically higher Zn and Mg concentrations, and Mg concentration increased with Zn availability in both ecotypes (Figure 4).

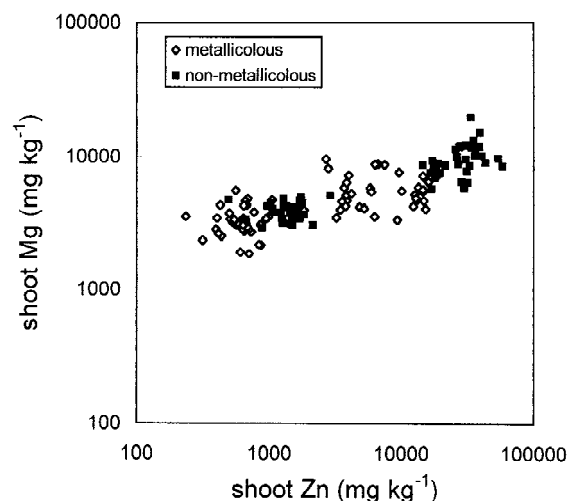


Figure 4. Correlation between shoot concentration of Mg and Zn in a metallicolous and non-metallicolous *Thlaspi caerulescens* grown in six soil treatments with contrasting availability of Zn.

### Competitive interactions

A two-way ANOVA with competition and ecotype as main effects was performed for shoot biomass and Zn concentration in each soil treatment. The competition effect and the soil  $\times$  competition interaction were mostly not significant (results not shown), indicating that Zn concentrations and growth were not different between pure and mixed cultures for either ecotype, except in very few cases. In the Zn-sulphate treatment, shoot mass of ecotype NM was negatively affected by competition (Figure 5). In the unenriched treatment, ecotype M had a higher Zn concentration in pure stands than in competition with ecotype NM, while the opposite result was found for ecotype NM (Figure 6).

## Discussion

### Zn availability

In a recent paper, Whiting et al. (2001) compared Zn accumulation in two metallicolous populations of *T. caerulescens* (one of which was Prayon) between soils with different Zn availability. Although their experimental conditions are somewhat different from ours (e.g., lower pH and lower total Zn concentration in soil in their study), our results share a number of similarities with theirs. In both studies, Zn-sulphide was less available than the other Zn salts, and *Thlaspi* achieved high Zn concentration when fed with the sparingly sol-

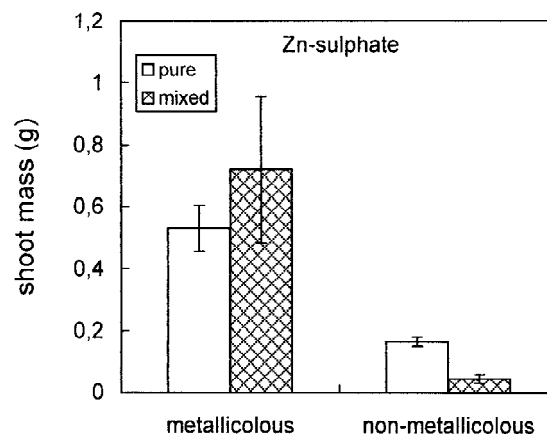


Figure 5. Shoot mass in metallicolous and non-metallicolous *Thlaspi caerulescens* grown with  $2000 \text{ mg kg}^{-1}$  Zn as Zn-sulphate in pure and mixed culture. Mean and 95% confidence intervals.

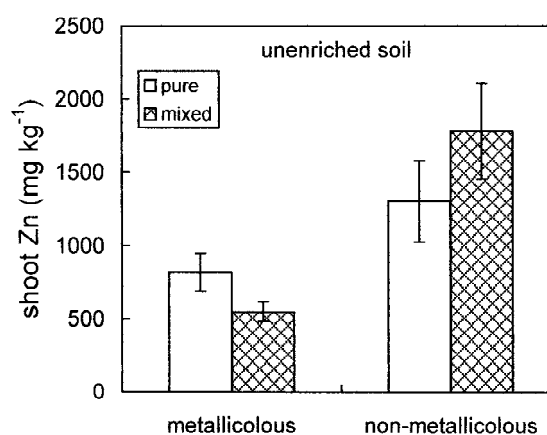


Figure 6. Shoot Zn concentration in metallicolous and non-metallicolous *Thlaspi caerulescens* grown on the control soil (no Zn added) in pure and mixed culture. Mean and 95% confidence intervals.

uble Zn-carbonate and Zn-oxide. In our study, neither ecotype could obtain significant amounts of Zn from Zn-pellets and Zn-sulphide granules. In addition to their intrinsically low solubility, unfavourable surface to volume ratio may have severely restricted mobilisation of Zn from these solids. In the treatments with little extractable Zn, Whiting et al. (2001) demonstrated that *T. caerulescens* could access Zn fractions that were not initially soluble. However, they concluded that the Zn solubilization mechanisms were not strong, since Zn concentration in shoots was highly responsive to increased Zn availability in soil; this conclusion may equally apply to our results as well. Both ecotypes are not markedly different in this re-

spect. In particular, there is no evidence that ecotype NM is much more efficient at mobilising Zn in the treatments with low Zn availability.

*Difference in Zn accumulation between metallicolous and non-metallicolous Thlaspi caerulescens*

The lower Zn concentration in shoots of ecotype M from Prayon, compared to ecotype NM from Luxembourg was verified on all substrates. It cannot be explained by a 'dilution' effect, because both ecotypes had similar shoot mass. Rather, the results point to constitutively higher Zn uptake capacity in ecotype NM compared to its metallicolous relative. Compared to non-accumulator species of *Thlaspi*, the hyperaccumulation property of *T. caerulescens* relies on a much higher Zn uptake rate due to a higher expression of Zn transporters in roots (Lasat et al., 2000). Our results suggest that there might exist genetic variation of that character between metallicolous and non-metallicolous populations of *T. caerulescens*. Assuming that *T. caerulescens* has constitutively high Zn requirements and strong Zn sequestration mechanisms (Shen et al., 1997), natural selection may conceivably have increased Zn uptake capacity in populations growing on Zn-poor soils to compensate for the low Zn availability in the substrate. Assunção et al. (2001) compared Zn uptake capacity in metallicolous and non-metallicolous ecotypes of *T. caerulescens* in hydroponics. They found a higher accumulation capacity in the non-metallicolous ecotype, which was obviously not due to a higher solubilisation capacity. However, they did not find enhanced transcription of the zinc transporters ZNT1 and ZNT2 in the non-metallicolous ecotype, suggesting that some other transporter(s) are involved. Apart from Zn transporters, other mechanisms could be involved in the higher Zn accumulation of ecotype NM, including higher root/shoot ratio, higher specific root length and enhanced Zn translocation from root to shoot (Whiting et al., 2000). Further investigations are needed to elucidate the physiological basis of the particular behaviour of ecotype NM.

The results suggest that ecotype NM has not evolved strongly enhanced capacity to obtain Zn from little available forms. The low shoot Zn concentration in ecotype NM in the three treatments with little extractable Zn is surprising considering the high Zn accumulation achieved by non-metallicolous populations growing in their natural Zn-poor soil (e.g., Lloyd-Thomas, 1995; Reeves et al., 2001). Thus, in its

natural habitat, in soil with 50 mg kg<sup>-1</sup> exchangeable Zn and 150–200 mg kg<sup>-1</sup> total Zn, the Luxembourg ecotype has foliar Zn concentration in the order of 3000–5000 mg kg<sup>-1</sup> (Reeves et al., 2001). Such a high accumulation factor might be achieved through the continuous reallocation of root growth towards soil pockets not yet depleted of readily available Zn (Schwartz et al., 1999; Whiting et al., 2000). In pot experiments, restricted rooting space may force roots to develop in Zn-depleted soil solution.

Interestingly, the ratio of Zn concentration in non-metallicolous and metallicolous plants varies strongly with Zn form in the soil. In the Zn-sulphate substrate, i.e., where Zn uptake rate is most likely not limited by concentration of Zn<sup>2+</sup> in the soil solution, the value of this ratio may be determined by a physiological difference in the Zn uptake rate between the two ecotypes. In the three treatments with low Zn availability, Zn uptake is primarily limited by low concentration of free Zn in solution and restricted rate of replenishment of soil solution from the solid phase. The large difference in Zn concentration between ecotypes fed with Zn-salts of intermediate solubility (Zn-oxide and Zn-hydroxycarbonate) is intriguing. In ecotype NM, Zn uptake from Zn-oxide is only slightly lower than from Zn-sulphate in spite of the vast difference in solubility between these salts and the much lower soil pH values (enhancing Zn mobility) in the Zn-sulphate treatment. Thus, ecotype NM apparently has higher capacity to obtain Zn from Zn-oxide compared to ecotype M. The recent literature provides little evidence for active Zn mobilisation by *Thlaspi caerulescens* through root exudates and/or rhizosphere acidification (Luo et al., 2000; Whiting et al., 2001; Zhao et al., 2001). However, all these studies have used metallicolous populations as experimental material. Our results suggest that non-metallicolous populations might be better candidates to isolate such mechanisms. However, Whiting et al. (2001) convincingly argue that high concentrations of labile Zn in soil amended with Zn-oxide and Zn-carbonate make Zn mobilisation mechanisms unnecessary. An alternative hypothesis is that a higher Zn uptake rate by roots of ecotype NM reduces the concentration of Zn in solution thereby enhancing the replenishment rate of soluble Zn from the solid phase. McGrath et al. (1997) and Knight et al. (1997) have shown that *T. caerulescens* can strongly deplete the soil solution in free Zn. Whiting et al. (2001) argue that the high root density typical of pot experiments can 'enhance the dissolution of buffered-Zn by more rapid depletion of the soluble pool due to plant up-

take'. Compared to ecotype M, the higher root uptake rate of Zn in ecotype NM could create steeper Zn concentration gradients in the rhizosphere, which could in turn enhance Zn release from the solid phase.

#### *Zn tolerance*

The results confirm that both ecotypes of *T. caerulescens* are highly tolerant of Zn. Ecotype NM grew less well than ecotype M only in the Zn-sulphate treatment. Foliar concentration of Zn in this treatment was also higher than in the other treatments, thus suggesting that some toxicity threshold was exceeded.

#### *Mg–Zn correlation*

The positive correlation between Mg and Zn concentration in shoot has apparently never been reported for *T. caerulescens*. This correlation has both a genetic and a phenotypic component. The genetic component arises from the fact that ecotype NM accumulates higher concentrations of both Zn and Mg. Might this indicate that  $Zn^{2+}$  and  $Mg^{2+}$  share the same transporters in root cell membranes, due to similar ionic radii and charge density? In support to this hypothesis, the only Mg transporter yet cloned from plants also has a strong affinity for Zn (Shaul et al., 1999).

The phenotypic component of the Zn–Mg correlation arises from the fact that Mg accumulation generally increases with Zn availability in either ecotype. Two mutually non-exclusive explanations can be put forward to explain this result. First, Zn cations may have competitively displaced magnesium sorbed on soil exchange sites, thereby increasing free Mg concentration in the soil solution (Olsen, 1972). Secondly, there may exist true synergy between Zn and Mg root absorption in *T. caerulescens*, even though this is not usually reported in the mineral nutrition literature (Marschner, 1995). Tolra et al. (1996) found no evidence for a correlation between Mg accumulation and Zn concentration in the nutritive solution in the Prayon population. The depressed Mg uptake in the  $ZnSO_4$  treatment (Figure 2b) suggests competitive uptake inhibition, which is expected at high external Zn concentration if Zn and Mg share the same transporters.

#### *Interactions between metallicolous and non-metallicolous plants*

Asymmetric competition between the two ecotypes was only observed in the Zn-sulphate treatment, in-

dicating that the lower Zn tolerance of ecotype NM results in decreased competitive ability in this treatment. There were only weak interactions between the two ecotypes in Zn uptake. In the control treatment, Zn concentration in ecotype M was strongly depressed (ca. 50%) when cocropped with the more strongly accumulating ecotype NM. This result points to asymmetric competition for Zn in this low Zn availability treatment, in line with the increased Zn concentration found in ecotype NM in the mixed culture. Whiting et al. (2001) have found that the non accumulator *T. arvense* cocropped with *T. caerulescens* accumulates less Zn, due to soil solution depletion of Zn by *T. caerulescens*.

#### *Implications for phytoremediation*

Total Zn content in shoot (calculated as Zn concentration  $\times$  shoot biomass) may arguably represent a useful criterion to select plants most suitable for phytoextraction of metal from polluted soils. In virtue of its higher Zn concentration, ecotype NM had markedly higher Zn mass in shoots than ecotype M in all substrates except Zn-sulphate. Therefore, the most suitable *Thlaspi caerulescens* accession for phytoremediation of polluted soil may depend on the dominant chemical form of Zn in the soil. Metallicolous populations could be more suitable to phytoremediate soils with high concentration of free Zn in soil solution, while non-metallicolous populations would be more efficient in soils polluted with less readily available Zn forms.

#### **Acknowledgements**

We thank A. Demoulin for assistance with seed collection and plant cultivation. This work was supported by the "Programme Environment, Vie et Société" (C.N.R.S., France).

#### **References**

- Assunção A G L, De Costa Martins P, De Folter S, Vooijs R, Schat H and Aarts M G M 2001 Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant Cell Environ.* 24, 217–226.
- Baker A J M, Reeves R D and Hajar A S M 1994 Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. et C. Presl (Brassicaceae). *New Phytol.* 127, 61–68.
- Brown S L, Chaney R L, Angle J S and Baker A J M 1995 Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. *Soil Sci. Soc. Am. J.* 59, 125–133.

- Cottenie A, Verloo M, Kiekens L, Velghe G and Camerlynck R 1982 Chemical analysis of plants and soils. Lab. Analytical and Agrochemistry State University, Ghent, B.
- Escarré J, Lefèbvre C, Gruber W, Leblanc M, Lepart J, Rivière Y and Delay B 2000 Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *New Phytol.* 145, 429–437.
- Knight B, Zhao F J, McGrath S P and 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 Soil* 197, 71–78.
- Lasat M M, Baker A J M and Kochian L V 1996 Physiological characterisation of root  $Zn^{2+}$  absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiol.* 112, 1712–1722.
- Lasat M M, Pence N S, Garvin D F, Ebbs S D and Kochian L V 2000 Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. *J. Exp. Bot.* 51, 71–79.
- Lloyd-Thomas D H 1995 Heavy metal hyperaccumulation by *Thlaspi caerulescens*. Ph.D. thesis, Univ. Sheffield, UK.
- Luo Y M, Christie P and Baker A J M 2000 Soil solution Zn and pH dynamics in non-rhizosphere soil and in the rhizosphere of *Thlaspi caerulescens*. *Chemosphere* 41, 161–164.
- Marschner H 1995 Mineral Nutrition of Higher Plants. Academic Press, London, UK.
- McGrath S P, Shen Z G and Zhao F J 1997 Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant Soil* 188, 153–159.
- Meerts P and Van Isacker N 1997 Heavy metal tolerance and accumulation in metallicolous and non-metallicolous populations of *Thlaspi caerulescens* from continental Europe. *Plant Ecol.* 133, 221–231.
- Olsen S R 1972 Micronutrient interactions. In *Micronutrients in agriculture*. Eds J J Mortvedt, P M Giordano and W L Lindsay. pp 243–264. Soil Science Society of America, Madison, WI.
- Pollard A J and Baker A J M 1996 Quantitative genetics of zinc hyperaccumulation in *Thlaspi caerulescens*. *New Phytol.* 132, 113–118.
- Reeves R D, Schwartz C, Morel J L and Edmondson J 2001 Distribution and metal-accumulating behavior of *Thlaspi caerulescens* and associated metallophytes in France. *Int. J. Phytoremediation*, 3: 145–172.
- Schwartz C, Morel J-L, Saumier S, Whiting S N and Baker A J M 1999 Root development of the zinc-hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localisation in soil. *Plant Soil* 208, 103–115.
- Shaul O, Hilgemann D W, De Almeida-Engler J, Van Montagu M, Inze D and Galili G 1999 Cloning and characterization of a novel  $Mg^{2+}/H^{+}$  exchanger. *EMBO J.* 18, 3973–3980.
- Shen Z G, Zhao F J and McGrath S P 1997 Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. *Plant Cell Environ.* 20, 898–906.
- Tolra R P, Poschenrieder C and Barcelo J 1996 Zinc hyperaccumulation in *Thlaspi caerulescens*. I. Influence on growth and mineral nutrition. *J Plant Nutr.* 19, 1531–1540.
- Whiting S N, Leake J R, McGrath S P, Baker A J M 2000 Positive response to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* 145, 199–210.
- Whiting S N, Leake J R, McGrath S P and Baker A J M 2001 Zinc accumulation by *Thlaspi caerulescens* from soils with different Zn availability: a pot study. *Plant Soil* 236, 11–18.
- Zhao E J, Hamon R E and McLaughlin M J 2001 Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilisation. *New Phytol.* 151, 613–620.