Reaction norms of life history traits in response to zinc in *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites

Caroline Dechamps, Claude Lefèbvre, Nausicaa Noret and Pierre Meerts
Université Libre de Bruxelles, Laboratoire de Génétique et Ecologie Végétales (Jardin Massart), Chaussée de Wavre 1850, 1160 Brussels, Belgium

**Summary**

- We examined phenotypic plasticity of fitness components in response to zinc (Zn) in the Zn hyperaccumulator, *Thlaspi caerulescens*.
- Two populations from Zn-enriched soils (M) and two populations from normal soils (NM) were grown in pots at three Zn concentrations (0, 1000, and 8000 mg kg$^{-1}$ Zn), for an entire life cycle. Growth, Zn accumulation and fitness components were assessed.
- Based on vegetative growth, M and NM populations had similar Zn tolerance at 1000 mg kg$^{-1}$ Zn. However, reproductive output was markedly decreased in NM at 1000 and 8000 mg kg$^{-1}$ Zn. In M populations, Zn did not affect fitness. However, low Zn status enhanced reproductive output in year 1 compared with year 2 and decreased survival after the first flowering season.
- M populations are able to achieve equal fitness across a broad range of Zn concentrations in soil by different combinations of fecundity and longevity. No cost of higher tolerance was demonstrated in M populations. Reproductive traits appeared to be a more sensitive indicator of tolerance than vegetative growth.

**Key words:** adaptation, fitness, genetic variation, heavy metal tolerance, life history traits, phenotypic plasticity, zinc (Zn).


**Introduction**

*Thlaspi caerulescens* is able to accumulate $> 10,000$ mg kg$^{-1}$ zinc (Zn) in its leaves without apparent signs of toxicity, and is therefore a heavy metal hyperaccumulator (Baker, 1981; Baker & Brooks, 1989). Compared with the physiology and molecular biology of heavy metal tolerance and accumulation, the ecology and evolution of *T. caerulescens* have been less intensively studied. It is now clear that *T. caerulescens* is a facultative metallophyte, occurring on both normal and metal-enriched soils (Reeves et al., 2001). Both types of populations show constitutively elevated metal tolerance (Zn, cadmium (Cd), nickel (Ni)) and accumulation (Zn/Cd) (Meerts & Van Isacker, 1997; Escarré et al., 2000; Assunção et al., 2003a,b; Frérot et al., 2003). Within-species variation in heavy metal tolerance and accumulation has been demonstrated (Pollard et al., 2002; Macnair, 2003). Higher Zn accumulation capacity and lower tolerance were consistently found in populations from normal soils compared with populations from metalliferous soils (Meerts & Van Isacker, 1997; Escarré et al., 2000; Assunção et al., 2003b; Frérot et al., 2003). Evolutionary relationships between the two types of populations are poorly known. In some regions of Europe, including Belgium, the two edaphic ecotypes are said to possess distinguishing morphological traits and, accordingly, they have sometimes been recognized as distinct taxonomic units (ssp. *calaminare* (Lej.) Dvořáková, and ssp. *caerulescens*) (Hegi, 1986; Lambinon et al., 2004). Allozymic markers revealed a clear differentiation between metallocolous populations from Belgium and nonmetallicolous populations.
from Luxembourg (Dubois et al., 2003). However, Ingrouille & Smirnoff (1986) considered that the pattern of morphological variation in the *T. caerulescens* complex was not amenable to taxonomic subdivision, which is in line with the pattern of isozyme variation in populations from Germany and Belgium (Koch et al., 1998).

Interestingly, some traits that are said to discriminate the two subspecies in Belgium and Luxembourg are related to life strategy and reproduction. The metallicolous ecotype would be an annual or a short-lived perennial plant with few seeds per fruit (four to eight) while the nonmetallicolous ecotype is said to be a short-lived semelparous plant (biennials) with more seeds per fruit (eight to 12) (Hegi, 1986; Lambinon et al., 2004). It has never been tested if these differences have a genetic basis or, alternatively, if they are merely phenotypic responses to contrasting metal concentrations in the local soil. Reaction norms in response to Zn must be characterized to better understand evolutionary responses to metal contamination (Ackerly et al., 2000).

It is striking that most studies investigating metal tolerance and accumulation in *T. caerulescens* have used materials grown for short periods (typically no longer than a few weeks), often in hydroponics (e.g. Assunção et al., 2003b; Roosens et al., 2003). Assessment of these characters has most often been based on juvenile plants grown under unnatural conditions and may arguably be irrelevant to determine the evolutionary ecology of heavy metal accumulation and tolerance. The response of fitness components to varying metal concentrations has never been investigated in *T. caerulescens*. Relevant fitness components include life history traits, the measurement of which requires long-term cultivation of plants.

Evolutionary scenarios of metal tolerance in plants often assume that tolerant genotypes suffer decreased fitness when growing on soils with low concentrations of metals (McNeilly, 1968; Hickey & McNeilly, 1975). For hyperaccumulating species, the ‘cost’ of tolerance might be the result of enhanced metal requirement inherent to tolerance mechanisms such as sequestration (Antonovics et al., 1971; Baker & Walker, 1990; Harper et al., 1997; Assunção et al., 2001). Then, on noncontaminated soil, essential micronutrient deficiency may occur. The evidence as to whether *T. caerulescens* has an elevated requirement for Zn is somewhat conflicting. Some authors (Mathys, 1977; Tolrà et al., 1996; Shen et al., 1997; Frérot et al., 2004) found growth stimulation or increased survival in response to Zn concentrations that are supraoptimal for normal species, while other studies did not (Lloyd-Thomas, 1995; Meerts & Van Isacker, 1997; Assunção et al., 2003b). However the ‘cost of tolerance’ in *T. caerulescens* was only tested during vegetative growth. The effect of suboptimal Zn supply on reproductive parameters has never been investigated.

In this paper, for the first time, we examine the response to Zn of life history traits in *T. caerulescens* grown in pots for 2 yr. We used four populations of contrasting ecological origins (two from metalliferous (M) and two from normal soils (NM)) to address the following questions:

- Do M and NM populations have constitutively contrasting life histories?
- Is reproduction more susceptible to Zn compared with vegetative growth?
- Do reaction norms of fitness components in response to Zn vary among populations of contrasting ecological origins?
- Is there evidence for a cost of increased metal tolerance in M populations?

### Materials and Methods

#### Plant material

Four populations of *Thlaspi caerulescens* J. & C. Presl (Brassicaceae) were studied: two metallicolous (M) populations (Prayon and Angleur, Province of Liège, Belgium) and two nonmetallicolous (NM) populations from Grand Duchy of Luxembourg (Lux 1 and Lux 2, population #29 and #28 in Molitor et al., 2004). The site of Prayon has been contaminated for c. 150 yr by dust from a Pb-Zn smelter (Meerts & Grommesch, 2001). The population of Angleur has established on a slagheap contaminated with Zn and Pb (waste from a zinc smelter). Luxembourg sites are described in Molitor et al. (2004). Seeds were randomly collected from six plants in each population to assess genetic variation within populations. The offspring from a single plant is referred to as a maternal family. In this study, the two groups of populations from contrasting soil origins (M and NM) are referred to as ecotypes.

#### Cultivation in controlled conditions

Seeds were sown in September 2003 in Petri dishes placed in a growth chamber. Two weeks after emergence, seedlings were transferred for a further 2 wk in a glasshouse in pots filled with arable soil. Twenty-four plants of each maternal family were then randomly selected and evenly distributed in three soil treatments (0, 1000, 8000 mg kg$^{-1}$ Zn). The Zn treatments were established by spiking arable soil with ZnO. This salt presents the advantage of being available without inducing soil acidification, as is the case with zinc sulphate (Meerts et al., 2003). The mineral composition of the substrate was measured before contamination (in mg kg$^{-1}$, extraction ammonium acetate-EDTA 1 N pH 4.65; Ca, 2147; Mg, 13; K, 170; Fe, 302; Mn, 27; Zn, 9; Cd, <1; Ni, 1). ZnO was thoroughly mixed with soil for each pot separately. Pots contained 350 g of dry soil (pH 6, organic matter, 7.5%). The soil was left to equilibrate for 4 wk before transplantation. One seedling was transplanted into each pot. The 576 pots (three soil treatments × two ecotypes × two populations × six families × eight replicates) were arranged in four blocks (two replicates per block). Within each block, pots were randomized every 2 months. Pots were placed in an unlit
Assessment of Zn concentration in plants

In May 2004, half of plants were harvested (above-ground parts only). The plant material was rinsed in deionized water, dried at 60°C for 48 h, and weighed to the nearest 0.001 g. Afterwards, shoots were mineralized in a mixture of nitric and perchloric acid with a Tecator digestor. The concentration of Zn was determined by ICP-OES (Varian Vista MPX).

Results

Figure 1 presents reaction norms of shoot mass, Zn accumulation and reproductive traits in response to Zn concentrations in the soil. Reaction norm is the set of phenotypes expressed by a single genotype across a particular environmental range. The reproductive traits presented in this figure are mean values calculated on the whole life cycle, for each population.

Shoot mass

The two ecotypes have similar values of shoot mass in the low and intermediate Zn treatments (1–1.3 g) (Fig. 1a). Shoot mass is slightly increased in the intermediate compared with the lowest Zn treatment (post-hoc test: +0.2 g in M, F = 6.2*; +0.1 g in NM, F = 2.4 (ns)) (Fig. 1a). The two ecotypes have contrasting responses to the highest Zn treatment (8000 mg kg⁻¹ Zn) (Fig. 1a), which explains the highly significant ecotype × treatment interaction in the full model ANOVA (Table 1, F = 25.4***). Shoot mass decreases (~50%) in ecotype NM while it is not affected in ecotype M. Populations within ecotypes and families within populations also have varying responses to Zn (Fig. 1a, Table 1, population × treatment: F = 4.5**; family × treatment: F = 1.9**).

Zn accumulation

All populations show increased Zn accumulation in response to Zn concentration in the substrate (Fig. 1b, Table 1, treatment effect, F = 385.4***). At 1000 and 8000 mg kg⁻¹ Zn, NM populations accumulate on average 2.5 times more Zn than M populations (Fig. 1b, Table 1, ecotype effect: F = 238.5***). The pattern of increase is significantly different between ecotypes (Fig. 1b, Table 1, treatment × ecotype: F = 17.5***), because of a steeper increase from treatment 0 to treatment 1000 mg kg⁻¹ Zn in ecotype NM. No significant variation between populations within ecotypes and between families within populations was detected (Table 1). However, populations (within ecotype) responded differently to the soil treatment (Table 1, F = 6.1***), as well as families (within population) (Table 1, F = 1.7**).

Reproductive traits

The two ecotypes have significantly different responses from Zn for all reproductive traits (Fig. 1c–h, Table 1, significant ecotype × treatment interaction), except for the number of offspring produced per lifetime, no matter how long it takes to produce them.

\[ R_0 = \sum l_x m_x \]

\( l_x \) probability of surviving from birth to beginning of age \( x \); \( m_x \) number of offspring in age \( x \).

Statistical analyses

Shoot mass, Zn accumulation, survival and reproductive parameters were analysed by ANOVA after logarithmic transformation of data to respect the normality assumption. For data expressed as proportions, arcsin-transformation was carried out. Analyses were performed with Statistica 7 (Statsoft, 2005). Populations are nested within ecotypes and families were nested within populations. Ecotype, population and treatment were considered as fixed factors. Block and family are random factors. For reproductive parameters, family factor was not included in ANOVA, owing to the high mortality registered for NM populations in the highest Zn treatment (8000 mg kg⁻¹). Differences in the proportion of different life cycles were compared with chi-square tests. The level of significance is noted, as follows: ***, \( P < 0.001 \); **, \( P < 0.01 \); *, \( P < 0.05 \); ns, not significant.
Fig. 1 Reaction norms of shoot mass (a), zinc (Zn) accumulation (b) and reproductive traits (c–h) in response to increased Zn in the soil for metallicolous (M) and nonmetallicolous (NM) populations of *Thlaspi caerulescens*. Continuous lines are for M populations: Prayon, circles; Angleur, squares. Dotted lines are for NM populations: Lux 1, diamonds; Lux 2, triangles. Values at each Zn concentration are means ± SE. Reproductive traits (c–h) are mean values calculated on the whole life cycle. The effect of Zn concentrations was tested for each population with ANOVA and results are included in the figure (***, *P* < 0.001; **, *P* < 0.01; *, *P* < 0.05; ns, not significant).

Table 1 ANOVAs of shoot mass, zinc (Zn) accumulation and reproductive traits of *Thlaspi caerulescens* populations from metallicolous and nonmetallicolous populations grown in three Zn treatments (0, 1000, 8000 mg kg\(^{-1}\)).

<table>
<thead>
<tr>
<th>Sources</th>
<th>Shoot mass</th>
<th>Zn accumulation</th>
<th>% of flowering plants</th>
<th>Number of flowering axes</th>
<th>Number of fruits</th>
<th>% of fertile fruits</th>
<th>Number of seeds/fruit</th>
<th>Seed yield</th>
<th>Fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>0.2 ns</td>
<td>0.3 ns</td>
<td>0.1 ns</td>
<td>2.3 ns</td>
<td>0.7 ns</td>
<td>0.5 ns</td>
<td>0.9 ns</td>
<td>0.7 ns</td>
<td>1.7 ns</td>
</tr>
<tr>
<td>Treatment</td>
<td>15.6***</td>
<td>385.4***</td>
<td>24.9***</td>
<td>0.4 ns</td>
<td>0.8 ns</td>
<td>26.6***</td>
<td>14.1**</td>
<td>16.9**</td>
<td>12.1**</td>
</tr>
<tr>
<td>Ecotype</td>
<td>16.7***</td>
<td>238.5***</td>
<td>–</td>
<td>0.6 ns</td>
<td>15.7***</td>
<td>35.0***</td>
<td>0.4 ns</td>
<td>57.1***</td>
<td>148.8***</td>
</tr>
<tr>
<td>Population (Eco)</td>
<td>5.1*</td>
<td>1.0 ns</td>
<td>75.6***</td>
<td>8.2***</td>
<td>14.3***</td>
<td>0.9 ns</td>
<td>11.3***</td>
<td>0.7 ns</td>
<td>27.7***</td>
</tr>
<tr>
<td>Family (Pop (Eco))</td>
<td>1.3 ns</td>
<td>1.6 ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tr × Block</td>
<td>2.2*</td>
<td>1.1 ns</td>
<td>0.1 ns</td>
<td>0.3 ns</td>
<td>1.7 ns</td>
<td>0.5 ns</td>
<td>0.9 ns</td>
<td>1.5 ns</td>
<td>1.2 ns</td>
</tr>
<tr>
<td>Tr × Eco</td>
<td>25.4***</td>
<td>17.5***</td>
<td>–</td>
<td>1.5 ns</td>
<td>2.4 ns</td>
<td>5.1***</td>
<td>9.9***</td>
<td>19.0***</td>
<td>27.7***</td>
</tr>
<tr>
<td>Tr × Pop (Eco)</td>
<td>4.5**</td>
<td>6.1***</td>
<td>10.0***</td>
<td>3.4*</td>
<td>0.7 ns</td>
<td>3.2*</td>
<td>1.1 ns</td>
<td>1.5 ns</td>
<td>1.9 ns</td>
</tr>
<tr>
<td>Tr × Fam (Pop (Eco))</td>
<td>1.9*</td>
<td>1.7**</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
</tbody>
</table>

Family effect was tested only for shoot mass and Zn accumulation. For the percentage of flowering axes, the M population of Angleur was excluded from the ANOVA, because of absence of variation. For this last parameter, populations could not be nested in ecotype. ***, *P* < 0.001; **, *P* < 0.01; *, *P* < 0.05; ns, not significant.
flowering axes and the number of fruits. Zn never influences reproductive traits of ecotype M (treatment effect not significant in one-way ANOVA for each population; Fig. 1c–h) except a slight decrease in the percentage of fertile fruits in Prayon at 1000 mg kg\(^{-1}\) Zn in the soil. In contrast, both NM populations have strongly decreased values of four reproductive traits in response to increased Zn in the soil: the proportion of flowering plants, which drops from c. 70% to c. 20% (Fig. 1c), the number of seeds per fruit (Fig. 1f; Zn 0, 7; Zn 8000, 3.5), the percentage of fertile fruits (Fig. 1g; Zn 0, c. 45%; Zn 8000, c. 15%) and the total seed yield (Fig. 1h; Zn 0, c. 500; Zn 8000, c. 60). For these four traits, the negative effect of Zn in ecotype NM is already apparent at the intermediate Zn treatment. For instance, total seed yield decreases from c. 500 in the lowest Zn treatment to 100–200 in the intermediate Zn treatment. Differences in the response of populations within ecotypes are observed for the number of flowering axes and for the number of seeds per fruit (Table 1).

Variation of life cycles

Figure 2 shows the relative proportions of the different life cycles according to populations and Zn concentrations in the substrate. Notice that the plants that died without flowering are also mentioned in this figure. A majority of M plants (69%) growing on 0 and 1000 mg kg\(^{-1}\) Zn showed a semelparous annual life cycle (Fig. 2a,b). On 8000 mg kg\(^{-1}\) Zn, the proportion of annuals decreased (~25%) in favour of iteroparous perennials. A chi-square test showed that these changes of proportions between the two extreme treatments (0 and 8000 mg kg\(^{-1}\) Zn) were significant for Prayon (\(\chi^2 = 5.77\)) and marginally significant for Angleur (\(\chi^2 = 3.63, P = 0.056\)). For both M populations, increased life span in response to Zn was associated with decreased reproductive output in year 1 (Fig. 3a,b). Notice that across all Zn treatments, the proportion of semelparous annuals was higher for Angleur (55%) than for Prayon (35%) (\(\chi^2 = 3.79, P = 0.051\)).

For NM populations, a majority (65%) of plants that flowered during the experiment were semelparous biennials (Fig. 2c,d). For all these plants, flowering was initiated in the second year and was followed by death. The other flowering plants were either semelparous annuals (20%) or iteroparous perennials (15%). Finally, the proportion of NM plants dead before flowering significantly increased with increasing Zn concentration in the substrate (chi-square between the two extreme treatments, \(\chi^2 = 32.02***\)). Notice that at 0 mg kg\(^{-1}\) Zn, 25% of NM plants died in summer 2004 before flowering.

Estimation of fitness

Despite phenotypic variation in life cycles, M populations kept fitness constant across Zn treatments (Fig. 4, \(F = 1.5\) (ns))
for Prayon and \( F = 1.4 \) (ns) for Angleur). For NM populations, the drop in fitness is already obvious at 1000 mg kg\(^{-1}\) Zn (from 430 to 26 for Lux 1*** and from 289 to 110 for Lux 2*, mean values and post hoc test), and is even more pronounced at 8000 mg kg\(^{-1}\) Zn (from 26 to 4 for Lux 1 (ns), and from 110 to 12** for Lux 2, means values and post hoc test) (Fig. 4).

### Discussion

#### Variation in life cycle between and within ecotypes

Our study highlights large variations in the life cycles between and within the two ecotypes of *Thlaspi caerulescens*. Most NM plants (65%) that flowered during the experiment were semelparous biennials. Habitat conditions of both NM populations may favour a biennial life cycle (Molitor et al., 2004): the open plant cover (two mowings per year) and the soil instability (microscrees resulting from a steep slope > 30\(^\circ\)) of road banks represent favourable habitat conditions for the establishment of semelparous biennial plants (Silvertown & Charlesworth, 2001). In contrast, almost all M plants (except four plants from Prayon) flowered in the first year with a mixture of semelparous annuals and iteroparous perennials. Angleur showed a significantly higher proportion of semelparous annual plants compared with Prayon. This may result from contrasting selection regimes owing to different habitat conditions. Angleur is established on a slagheap, with excessive drainage and low N and P status. The site of Prayon was contaminated by atmospheric fallout and has better water regime and nutrient status. In Angleur, earlier reproduction might have evolved as a response to a lower survival probability. However, reciprocal transplant experiments are needed to demonstrate local adaptation of these populations convincingly. Our results confirm the life cycles observed in the field by Lambinon et al. (2004) for the M and NM ecotypes (annual or short-lived perennial plants for M and semelparous biennial plants for NM). However, our results must be interpreted with caution, because of the relatively unnatural climate in the greenhouse. Most of our plants died after 2 yr, while many plants in the Prayon site obviously live for a longer time and eventually reach a larger size compared with nonmetallicolous populations (C. Dechamps, unpublished). A demographic monitoring of natural populations is needed to confirm our results.

#### Survival and seed yield are more sensitive to Zn than vegetative growth in nonmetallicolous populations

A striking result is that Zn tolerance assessed from vegetative growth is similar for both ecotypes at 1000 mg kg\(^{-1}\) Zn. In contrast, survival and components of reproductive output were strongly depressed at that Zn concentration in the NM ecotype. In particular, the decrease of seed yield results from
the impact of Zn on several reproduction steps: (i) the decrease of flowering plants; (ii) the increase of the proportion of aborted fruits; and (iii) the decrease of the number of seeds per fruit. There are surprisingly few published data on the impact of heavy metals on plant reproduction. Ryser & Sauder (2006) noted higher sensitivity to Zn of reproduction compared with growth in the nontolerant species *Hieracium piloselloides*. In our study, fruit and ovule abortion can result either from high sensitivity of the female function to Zn or from pollen limitation. Further investigations are needed to determine which of the male or female function is most affected by Zn in this ecotype. Izmailow (2002) showed that Cu had a negative impact on several components of reproductive success in *Ranunculus repens*, including flower production, pollen viability, endosperm development and seed maturation.

Our results therefore indicate that the Zn tolerance assessed from vegetative growth in short-term experiments may be overestimated and would not be entirely relevant to ecological and evolutionary perspectives. Ernst & Nelissen (2000) also concluded that long-term experiments are more reliable as bioassays of risk assessment of metal-rich soil.

Surprisingly, mortality rate in 0 mg kg\(^{-1}\) Zn was higher for NM (25%) than for M plants. This may be a by-product of their contrasting life cycle. Thus, most NM plants delayed flowering until year 2 and therefore had a higher risk of dying before flowering.

**Trade-off between survival and fecundity regulated by Zn in metallicolous populations**

Another striking result is that the M ecotype achieved homeostasis of fitness across all Zn treatments through a shifting balance between reproduction and survival. Fecundity during the first year was higher and survival after first flowering was lower in the lowest Zn treatment compared with the highest Zn treatment. For M plants growing on the highest Zn treatment, enhanced reproduction during the second year compensated for the low reproductive output of the first year. Therefore, enhanced reproduction in year 1 in the lowest Zn treatment could arguably be interpreted as a symptom of Zn deficiency, triggering early shift of resources from vegetative growth to reproduction. However, homeostasis of fitness in all Zn treatments rather points to broader niche width in that ecotype. Those results illustrate that plasticity may contribute to fitness homeostasis in variable environments, and thus support the notion that plastic responses can be adaptive (Bradshaw, 1965; Ackerly *et al*., 2000). Homeostasis of fitness by change of life strategy in response to heavy metals has, to our knowledge, never been demonstrated. In M populations of *T. caerulescens*, the selection pressure that could favour life cycle plasticity may be fine scale heterogeneity in metal pollution. This disruptive selection in space is known to favour plasticity (Bradshaw, 1965). This adaptive hypothesis should be tested by direct observations in natural populations.

**No evidence for strong fitness costs of elevated Zn tolerance in the metallicolous ecotype**

The M ecotype did not show important signs of decreased growth or reproduction in the lowest Zn treatment. Therefore, our results do not provide evidence for antagonistic pleiotropy or strong fitness costs of higher Zn tolerance in the M ecotype. However, to characterize ‘the cost of tolerance’ correctly, the response of fitness components to Zn should be compared between genetic lines selected for their contrasted degrees of tolerance (Harper *et al*., 1997, 1998).

**Conclusion**

*Thlaspi caerulescens*, a constitutively Zn-tolerant species, has large genetic variation for reaction norms of fitness-related traits in response to Zn. The Zn status of the native soil of populations accounted for much of this variation. Populations originating from low Zn soil evidently show maladaptive responses to high Zn concentrations, in terms of lower survival and decreased reproductive output. In contrast, populations originating from Zn-rich soil have equal fitness on low and high Zn concentrations in the soil. This is achieved through presumably adaptive alterations in the pattern of resource allocation to reproduction and survival. Future works should test for local adaptation using reciprocal transplantation experiments *in situ*. It would be interesting to test if the M/NM contrast revealed for populations from Belgium/Luxembourg holds true for other parts of the distribution range where M and NM populations exist in parapatry (e.g. southern France). Finally, our results also demonstrate that vegetative traits assessed in short-term experiments may not reveal the whole range of genetic variation for Zn tolerance existing in *Thlaspi caerulescens*.

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