Life history traits of the pseudometallophyte *Thlaspi caerulescens* in natural populations from Northern Europe

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**Keywords**

Adaptation; drought; heavy metals; life cycle; Noccaea.

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**ABSTRACT**

We examined recruitment, survival, life cycle and fecundity of two metallicolous (M, on metalliferous calamine soils) and two non-metallicolous (NM, on normal soils) populations of *Thlaspi caerulescens* in Belgium and Luxemburg. In each population, permanent plots were monitored over two reproductive seasons. In M populations, plots were located in two contrasting environments (grass versus grove) in order to test the influence of vegetation cover on life strategy. Our results show that the monocarpic life cycle is dominant in all populations of *T. caerulescens*. However the length of the pre-reproductive period varies from several months (winter annuals) to 1 year or more (perennials), and it is partly related to plant origin (M versus NM). Most plants growing in metallicolous environments were annuals, whereas NM plants were mostly perennials. These differences in life cycle were related to differences in survival during summer, which was better in NM than in M populations. Within each M population, different survival conditions and life cycles were observed according to vegetation cover. Plants growing in grass areas were mostly annuals and had a low survival rate in summer whereas grove plants were mostly perennials and survived better in summer. Our results suggest the selection of stress avoiders (shortening of life cycle) in M populations of *T. caerulescens* but only for individuals growing in grass areas. Summer survival seems to play a key role in selection of life strategy in *T. caerulescens*.

**INTRODUCTION**

Sites contaminated with heavy metals are very stressful environments that are hostile to most organisms. Among plants, a few species, called metallophytes, have acquired the capacity to grow in such environments. To be able to grow and reproduce on these sites, species are adapted to metal stress, as well as to a series of other abiotic constraints such as drought, intense sun exposure, low soil fertility and the heterogeneity of stress conditions (Antonovics et al. 1971; Denaeyer-De Smet & Duvigneaud 1974; Simon 1975, 1978; Kruckeberg & Kruckerberg 1990; Wu 1990; Macnair 1997; van Oort et al. 2002). Pseudometallophytes, i.e. species occurring on both metallicolous and normal soils, constitute a relevant model to study microevolutionary adaptive processes occurring within a species. More generally, observing phenotypic divergence at the within-species level allows inference on speciation processes (Linhart & Grant 1996; Lexer & Fay 2005). Two edaphic types are thus recognised in pseudometallophytes, referred to as metallicolous (M) and non-metallicolous (NM) ecotypes. Up to now, studies using pseudometallophytes as model organisms mainly focused on the metal tolerance trait and not on other associated adaptive traits, such as life history strategies (e.g. Gibson & Risser 1982; Al-Hiyaly et al. 1988; Pauwels et al. 2006).

Generally, theoretical models predict that abiotic stress such as heavy metal contamination exerts selection pressure either for stress tolerance or stress avoidance (Grime 1977; Southwood 1988; Hoffmann & Parsons 1991; Stanton et al. 2000). Stress tolerators are described as poor competitors, characterised by a slow growth rate, delayed reproduction and reduced phenotypic plasticity (Grime 1977; Stanton et al. 2000), whereas stress avoiders are plants with rapid development and early reproduction (Rice & Mack 1991; Aronson et al. 1992). Based on experiments characterising growth and competitive ability of M and NM populations of pseudometallophytes, it is generally accepted that the stress tolerance strategy is selected in metallicolous environments (McNeilly 1968; Cook et al. 1972; Hickey & McNeilly 1975; Lefèbvre & Vernet 1990). However, the very few studies comparing life cycles of metallicolous and non-metallicolous populations of pseudometallophytes revealed an opposite trend, suggesting the selection of stress avoiders in metallicolous environments (e.g. *Anthoxanthum odoratum* in Antonovics 1972; *Silene vulgaris* in Wierzbicka & Panufnik 1998). Interestingly, contrasting conclusions are drawn on life strategies selected in metallicolous environments, depending on the traits considered (vegetative or reproductive traits).

The pseudometallophyte and metal hyperaccumulator (Zn, Cd, Ni), *Thlaspi caerulescens*, represents a first-choice model...
to study life strategy selection in metalliferous environments. Together with *Arabidopsis halleri*, *T. caerulescens* is the pseudometallophyte that has been the focus of most studies in recent decades. However, compared to metal tolerance and metal accumulation traits, life history traits have been totally neglected. Knowing the life history of M and NM populations of these pseudometallophytes would allow better characterisation of the general pattern of adaptation to metalliferous environments. Only a few recent studies have characterised life history variations between M and NM populations of *T. caerulescens* (Dechamps et al. 2007; Jiménez-Ambriz et al. 2007). These studies, conducted in controlled conditions, highlighted large variations in life cycles between northern (Belgium and Luxemburg) and southern populations (south of France), as well as between and within the two edaphic ecotypes (Dechamps et al. 2007; Jiménez-Ambriz et al. 2007). Irrespective of edaphic origin, southern populations have lower life cycle variability than northern populations; most southern M and NM plants are annuals (Jiménez-Ambriz et al. 2007), whereas in Northern Europe, a large variation in life cycles is observed between and within edaphic ecotypes (Dechamps et al. 2007). In northern populations, most plants of the NM ecotype are monocarpic perennials, whereas the M ecotype is characterised by a mixture of monocarpic annuals and polycarpic perennials in controlled conditions (Dechamps et al. 2007). These results suggest that drought would represent a more important selective pressure than heavy metals for modelling life histories in *T. caerulescens*. However, no demographic monitoring *in natura* has ever confirmed these results.

Plastic variation in life cycle in response to Zn concentration in soil was observed in M populations from Northern Europe, with a shift from an annual cycle on normal soil to a short perennial cycle on Zn-contaminated soil (Dechamps et al. 2007). Such life cycle plasticity in M plants was also expressed in a reciprocal transplantation experiment using the same populations (Dechamps et al. 2008). In this latter study, most M plants transplanted into a non-metalliferous environment expressed a monocarpic biennial strategy, similar to that of native transplanted NM plants (in the metalliferous environment M plants were mostly annuals). The life cycle plasticity of the M ecotype might be due to the fact that it grows in heterogeneous habitats. The heterogeneity of life conditions in metalliferous sites has been demonstrated in several studies (Denaeyer-De Smet & Duvigneaud 1974; Simon 1975, 1978; Lefèbvre 1982; Lefèbvre & Vernet 1990; Meerts & Grommesch 2001; van Oort et al. 2002; Dubois 2005; Dechamps et al. 2008). Grove areas often alternate between open and closed grass areas. Open grass areas are characterised by higher soil toxicity and more severe drought conditions compared to closed grass and underbrush areas (Simon 1978; Lefèbvre 1982). In open grassland, drought is largely determined by unfavourable soil structure and texture. Soil toxicity is due to high concentrations of heavy metals, but is also influenced by calcium and organic matter content (Simon 1978; Lefèbvre 1982). *T. caerulescens* grows in open as well as in closed vegetation areas (Denaeyer-De Smet & Duvigneaud 1974; Dubois 2005). In this particular context, it is relevant to test the influence of contrasting growth conditions occurring within metalliferous sites on plant life histories.

In this study, we assessed phenotypic variation of life history traits in two M and two NM populations of *T. caerulescens* from Northern Europe (Belgium and Luxemburg). We compared life history traits of M and NM ecotypes to better understand evolutionary processes in pseudometallophytes and the evolutionary direction of life histories in metal-contaminated sites. The life history traits studied are recruitment, length of the pre-reproductive period and number of reproductive seasons (one or two). We investigated (i) whether the diversity of life history traits recorded in controlled conditions (Dechamps et al. 2007) is expressed in the field; (ii) the relative contribution of autumn and spring recruitment; and (iii) how environmental heterogeneity (grass versus grove areas) influences life histories in M populations.

### MATERIAL AND METHODS

#### Biology of *Thlaspi caerulescens*

*Thlaspi caerulescens* J. & C. Presl (Brassicaceae, Group: Nocceae) is described in floras as annual, biennial or perennial, generally flowering in early spring (Tutin et al. 1993; Lambinon et al. 2004). This species requires a vernalisation period of at least 1 month to induce flowering (Assunção et al. 2003). Plants produce one to several raceme(s) of many flowers. Flowers are hermaphrodite, self-compatible and protogynous (Riley 1956). Insects seem to play an important role in pollination (Riley 1956). Fruits (siliqua: dry dehiscent fruits) mature at the end of the spring and each fruit releases a maximum of 12 seeds through barochory. This species is able to form a short-lived seed bank (Meerts & Grommesch 2001; Elvinger 2007).

#### Populations studied

For each ecotype (Table 1; metallicolous, M, and non-metallicolous, NM) of *T. caerulescens*, two populations were studied. The metallicolous populations were in the Province of Liège (Belgium) in Prayon (Pr) and Angleur (Ang). The population at Pr occurs on a site contaminated for about 150 years by fallout from a lead/zinc/cadmium (Pb/Zn/Cd) smelter. The population at Ang is situated on a slag heap contaminated with Zn and Pb (waste from a smelter). The populations at Pr and Ang occupy in areas of 75 ha and 3 ha, respectively (Graitson et al. 2005). In these sites, *T. caerulescens* occurs in vegetation typical of calamine soils (Bizoux et al. 2004) composed of a central grassland area (~60%) surrounded by groves (~40%). The herbaceous stratum of the groves mainly consists of calamine species like *T. caerulescens* and *Viola calaminaria*. The woody stratum is dominated by *Betula pendula*, *Acer platanoides* and *Sambucus nigra* in Angleur and *Acer campestre* and *Quercus robur* in Prayon. In order to compare soil composition between grass and grove areas, soil samples (depth 0–10 cm) were collected adjacent to each plot (for design of plots, see below) in the two metalliferous sites. Soil concentrations of heavy metals (Zn, Cd, Pb, Ni) and biogenic cations (Mg, K, Ca, Fe) were determined (Table 2; extraction with ammonium acetate-EDTA 1N pH 4.65; for more details on the procedure, see Dechamps et al. 2008). The Zn/Ca and Pb/Ca ratios were used as metal toxicity indicators (Simon 1978). Analyses...
show that in both metalliferous sites, soils from grass areas have higher concentrations of Ca, K and Mg than those of grass areas (Table 2). All grove areas were characterised by lower Zn/Ca and Pb/Ca than grass areas (Table 2). The non-metallicolous populations (Lux 1 = population #28 = Goeblesmühle, and Lux 2 = population #33 = Hoscheid, in Molitor et al. 2005) are located in the Sûre valley in Luxembourg, and grow on steep roadside banks with a SW aspect (Table 1). These NM populations occur in open plant communities at the edge of the forest. The communities have relatively low productivity (for more details, see Molitor et al. 2005) and are mown twice a year (at the end of spring and summer). The areas occupied by populations Lux 1 and Lux 2 are 50 m² (~150 flowering plants) and 200 m² (~500 flowering plants), respectively. These areas are homogeneous for soil properties and sun aspect (Molitor et al. 2005). Furthermore, herbivore pressure on *T. caerulescens* is known to be higher in non-metallicolous than in metallicolous sites (Noret et al. 2007).

### Field data collection

Five censuses were performed in each population, including in two reproductive seasons: May 2006 (=spring 1) – October 2006 – December 2006 (=autumn 1) – April 2007 – June 2007 (=spring 2). At each census, the position of all living plants in each plot was recorded (handmade cartography with 5 × 5 cm and 10 × 10 cm grid systems, adapted to M and NM plots, respectively). At each census, each plant was ascribed to one of the four following size classes: seedling (SL, 2 cotyledons–5 leaves), small rosette (S, 6–10 leaves), medium rosette (M, 11–20 leaves), large rosette (L, >20 leaves). Because of the strong correlation with rosette dry weight, the number of leaves was used as a biomass estimator (verified in Ang population: $r^2 = 0.95^{***}$, n = 40). Small, medium and large rosettes were either at vegetative (V) or at reproductive (R) stages. For each reproductive season, population structure was evaluated as the proportion of individuals in each of the above size classes for each population. To simplify the

### Field experimental design

In May 2006, we selected seven plots of 0.5 × 0.5 m in each M population and six plots of 1 × 1 m in each NM population. Plots were larger in NM populations to compensate for the lower population density. In M populations, plots were distributed in two groups: located in open grassland (grass area; Pr: n = 4 and Ang: n = 5) and those in underbrush (grove area; Pr: n = 3 and Ang: n = 2). Plots were deliberately placed where *T. caerulescens* was present. At the beginning of the census, the number of individuals in each population was: 565 in Pr, 369 in Ang, 375 in Lux 1 and 606 in Lux 2.

### Table 1. Characteristics of the four sites where *Thlaspi caerulescens* populations occur.

<table>
<thead>
<tr>
<th>characteristics</th>
<th>metallicolous (M) populations</th>
<th>non-metallicolous (NM) populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>sites</td>
<td>Prayon (Belgium)</td>
<td>Angleur (Belgium)</td>
</tr>
<tr>
<td>acronyms</td>
<td>Pr</td>
<td>Ang</td>
</tr>
<tr>
<td>geographic coordinates</td>
<td>50°35'3&quot;N, 5°40'23&quot;E</td>
<td>50°36'46&quot;N, 5°36'34&quot;E</td>
</tr>
<tr>
<td>pollution origin</td>
<td>Dust fallout from smelter</td>
<td>Solid waste from smelter</td>
</tr>
<tr>
<td>surface (m²)</td>
<td>750,000</td>
<td>30,000</td>
</tr>
<tr>
<td>altitude (m)</td>
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<td>60</td>
</tr>
<tr>
<td>slope (°)</td>
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<td>0</td>
</tr>
<tr>
<td>aspect</td>
<td>SE</td>
<td>–</td>
</tr>
<tr>
<td>substrate</td>
<td>Limestone</td>
<td>Furnace slag</td>
</tr>
<tr>
<td>flowering plants</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

### Table 2. Soil mineral element concentrations in two metalliferous sites where *Thlaspi caerulescens* plots were monitored. In each site, the soil under two different types of vegetation (grass versus grove areas) was sampled separately (Prayon: n = 4 for grass area, n = 3 for grove area; Angleur, n = 5 for grass area, n = 2 for grove area). Data are means (±SE). The results of the *ANOVA* highlight the sources of variation in mineral concentration.

<table>
<thead>
<tr>
<th>mineral element concentrations (mg kg⁻¹)</th>
<th>Prayon</th>
<th>Angleur</th>
<th>sources of variation in the <em>ANOVA</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>calcium (Ca)</td>
<td>1545 (128)</td>
<td>4248 (1046)</td>
<td>site; vegetation cover; site x vegetation cover</td>
</tr>
<tr>
<td>potassium (K)</td>
<td>152 (11)</td>
<td>263 (54)</td>
<td>ns</td>
</tr>
<tr>
<td>magnesium (Mg)</td>
<td>96 (3)</td>
<td>122 (11)</td>
<td>ns</td>
</tr>
<tr>
<td>iron (Fe)</td>
<td>426 (0.1)</td>
<td>219 (37)</td>
<td>ns</td>
</tr>
<tr>
<td>zinc (Zn)</td>
<td>45918 (7707)</td>
<td>15161 (182)</td>
<td>ns</td>
</tr>
<tr>
<td>cadmium (Cd)</td>
<td>205 (39)</td>
<td>287 (19)</td>
<td>F₁₀ = 97.6***</td>
</tr>
<tr>
<td>lead (Pb)</td>
<td>5190 (548)</td>
<td>3350 (335)</td>
<td>F₁₀ = 7.7</td>
</tr>
<tr>
<td>Pb/Ca ratio</td>
<td>3.4 (0.6)</td>
<td>0.9 (0.25)</td>
<td>ns</td>
</tr>
<tr>
<td>Zn/Ca ratio</td>
<td>1.4 (0.1)</td>
<td>0.7 (0.1)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Field data collection

Five censuses were performed in each population, including in two reproductive seasons: May 2006 (=spring 1) – October 2006 – December 2006 (=autumn 1) – April 2007 – June 2007 (=spring 2). At each census, the position of all living plants in each plot was recorded (handmade cartography with 5 × 5 cm and 10 × 10 cm grid systems, adapted to M and NM plots, respectively). At each census, each plant was ascribed to one of the four following size classes: seedling (SI, 2 cotyledons–5 leaves), small rosette (S, 6–10 leaves), medium rosette (M, 11–20 leaves), large rosette (L, >20 leaves). Because of the strong correlation with rosette dry weight, the number of leaves was used as a biomass estimator (verified in Ang population: $r^2 = 0.95^{***}$, n = 40). Small, medium and large rosettes were either at vegetative (V) or at reproductive (R) stages. For each reproductive season, population structure was evaluated as the proportion of individuals in each of the above size classes for each population.
presentation of results in Fig. 1, several size classes were merged. Thus, all plants with more than five leaves (including small, medium and large rosettes) are simply referred to as adults (in contrast to seedlings). In Fig. 1, three developmen-
tal stages are defined in order to simplify the results: seed-
lings, vegetative adults and reproductive adults.
To assess survival and recruitment, we compared cartogra-
phies of successive censuses. Summer and winter survivors
RESULTS AND DISCUSSION

Figure 1 illustrates life histories of M populations in grass areas (a), M populations in grove areas (b) and of non-metallicolous (NM) populations (c). Transitions show the proportion of plants that survived from spring 1 (2006) to autumn (transition 1), and from autumn to spring 2 (2007; transition 2). Metallicolous populations: Prayon [Pr] and Angleur [Ang], non-metallicolous populations: Goebelmühle [Lux1] and Hoscheid [Lux2]. Plants in three development classes were considered (seedlings, vegetative adults and reproductive adults). Pie charts on the right of the figure represent the relative proportions of vegetative (V, in white) and reproductive (R, in black) plants during spring 2. The histogram under each pie chart shows the different life cycles (monocarpic annuals, monocarpic perennials and polycarpic perennials) recorded amongst reproductive plants. The fecundity (mean number of seeds per plant) of each life cycle class in spring 2007 is given above bars (mean ± SE).

### General observations

**Recruitment**

In all populations, seedling recruitment peaked at the beginning of autumn (mainly in October). Annual plant contribution from autumnal germination was 89% (226/254) for Pr (M), 96% (852/887) for Ang (M), 87% (1178/1349) for Lux 1 (NM) and 78% (489/634) for Lux 2 (NM). Annual plant contribution from spring germination was generally low (from 10 to 22%) but varied among populations. The existence of two seedling cohorts (autumn and spring) may play a key role in the variations in life cycle within a population.

Bender et al. (2000) showed that spatial variation in life cycles in populations of Polymnia canadensis (monocarpic Asteraceae from North America) was explained by differences in germination rate and seedling survival between autumn and spring cohorts. The autumn cohort was more successful in dry sites than in mesic sites, and the reverse was true for the spring cohort. As this species requires vernalisation (as does T. caerulescens), this contrast in behaviour between the two cohorts leads to a higher proportion of winter annuals in dry sites and a higher proportion of perennials in mesic sites. The relative importance of autumn and spring cohorts could be responsible for a part of the life cycle variation observed among populations of T. caerulescens (see below, on variations within M populations).

**Length of the pre-reproductive period and number of reproductive seasons**

The length of the pre-reproductive period varied from 6 months (winter annuals) to 1 year or more (perennials), and was partly explained by plant origin (ecotype and population) and by vegetation cover within the population (grass versus grove areas in M populations). These differences are further described below. The results show that the monocarpic...
life cycle (one reproduction season following by death) is predominant (95–100%) in all populations of *T. caerulescens* (Fig. 1).

Size-dependent fecundity

As expected, fecundity (mean number of seeds per plant) significantly increased with rosette size in all populations (Fig. 2; Table 3: significant ‘rosette size’ effect for both years). For M and NM populations, fecundity of monocarpic perennials (vegetative in spring 1 and flowering in spring 2) was, on average, four-times higher than fecundity of winter annuals (plants that germinated in autumn 1 and flowered in spring 2) (Fig. 1). The higher fecundity of monocarpic perennials was associated with a higher proportion of large and medium reproductive plants (Fig. 1). In contrast, winter annuals were mainly composed of small and medium reproductive plants in M and NM populations (Fig. 1).

Size-dependent survival

Rosette survival tends to increase with an increase in rosette size (Fig. 3), but this relation varies both among populations (see below) and between seasons (summer versus winter survivals). Seedling survival was lower than survival of the three other size classes (S, M, L) in NM populations in summer and in winter (Fig. 3). However, in M populations, seedling survival was not significantly different from the survival of small rosettes (in three out of four cases; Fig. 3). The three size classes of vegetative adults were generally not significantly different for summer and winter survivals (Fig. 3).

Differences between M and NM ecotypes

NM populations were characterised by a longer pre-reproductive period than M populations. In spring 2, the percentage of flowering plants developing from seedlings recruited in autumn 1 was lower in NM than in M populations (∼7 versus ∼40%; $\chi^2 = 202.6$, $P = 0.001$; proportions inferred from Fig. 1). Consequently, in both NM populations, most reproductive plants in spring 2 were monocarpic perennials (∼60%), a low proportion were monocarpic annuals (∼30%), and a few were polycarpic perennials (∼10%) (Fig. 1c). During spring 2, the predominance of vegetative plants (∼70%) in NM populations reflects the protracted pre-reproductive period. Moreover, ∼10% of the NM vegetative adults censused in spring 1 remained vegetative in spring 2 (proportion inferred from Fig. 1).

Moreover, M plants were mainly annuals (∼50–95%) except in grove areas of Prayon (Fig. 1). In contrast to the observation in NM populations, all M plants that were vegetative in spring 1 flowered in spring 2. For monocarpic plants, a longer pre-reproductive period is selected for in environments favouring adult survival between two reproductive seasons (de Jong et al. 1987; Stearns 1992). In the present study, this was confirmed by the significantly higher survival of seedlings, vegetative and reproductive adults in summer (transition 1) in NM compared to M populations (Fig. 1; Table 4a). Surviving more than 1 year seems thus more difficult in M than in NM environments. In addition, in contrast to the situation in NM populations, being an adult plant (i.e. having more than six leaves) in the M environment did not guarantee better survival during summer than survival of seedlings (survival of small vegetative rosettes was not significantly different from seedling survival; Fig. 3). Poor survival in the M environment has already been demonstrated in reciprocal transplantation experiments of M and...
NM populations of *T. caerulescens* (Dechamps et al. 2008). During summer, drought and soil temperature is higher in the M than in the NM environment (unfavourable soil texture and structure, dark soil, lack of vegetation cover). At the end of spring, NM plants are thus better protected from temperature and humidity variations due to the growth of surrounding vegetation (tall grasses, shade from the surrounding forest). The crucial role of summer drought in the selection of life strategy in *T. caerulescens* was initially proposed by Dubois (2005). In the Mediterranean climate, most individuals from M and NM populations are annuals. Furthermore, this influence of drought on pre-reproductive time scale has already been demonstrated in several monocarpic species with wide north–south distribution (annuals in the south and biennials in the north; Klinkhamer et al. 1987).

**Variations within M populations**

One of the most striking results of this study was the contrast between life histories of plants growing in grass versus those in grove areas within both M populations. Reproductive plants growing in grove areas produced three- to seven-times more seeds than individuals growing in grass areas, whatever their life cycle (Fig. 1a and b; Table 3b: significant effect of ‘vegetation cover’ for both years). This higher fecundity was related to larger reproductive rosettes (Fig. 1a and b), both in winter annuals and monocarpic perennials, and was probably the consequence of better growth conditions in grove versus grass areas. Interestingly, this increase in *T. caerulescens* fecundity was also observed in grove areas of the Mediterranean (M populations: Dubois 2005) and Switzerland (NM populations: Basic et al. 2006) populations. It is difficult to specify which of the three possibilities – drought, higher metal availability or lower nutrient fertility – is most limiting in open grassland. A negative relation between fecundity and soil metal concentration was found in the metallophyte *Viola calaminaria* (Bizoux 2006). In controlled conditions, for *T. caerulescens* there was no effect of soil Zn concentration on M plant fecundity (Dechamps et al. 2007). The influence of other factors on life history traits should be tested in future experiments.

Besides this increase in fecundity, there was a significantly higher proportion of monocarpic perennial plants in grove areas compared to grass areas, but only in the M population of *Pr* (78% in grove areas versus 42% in grass areas, \(\chi^2 = 8.4**;\) Fig. 1a and b). For the *Ang* population, this trend was not observed in spring 2. However, the majority of plants (~70%) growing in grove areas in *Ang* during spring 2 was vegetative and only 17% of winter annuals grew in grove areas (83% grew in grass areas; proportions inferred from Fig. 1a and b). The vegetative plants growing in grove areas in *Ang* in spring 2 were probably monocarpic perennials that would have reproduced during the following spring(s). Furthermore, in spring 1, 15 large reproductive plants were present in grove plots of *Ang* (data not shown), which represented 100% of the grove reproductive plants and ~56% of all plants growing in grove plots (proportion inferred from Fig. 1b). These large reproductive plants with high fecundity (seed output: 4740 ± 1100; mean ± SE) that died after reproduction in spring 1 were probably monocarpic perennials. We think that these plants were perennials because plants reaching this size and fecundity level in other populations were mostly perennials. In the *Ang* population, we can explain the absence of flowering monocarpic perennials in spring 2 by lack of overlap between successive cohorts of monocarpic biennials at the plot scale. Consequently, in grove areas of both M populations we observed an asynchronous oscillation in density of reproductive and vegetative plants between the two reproductive seasons (Fig. 1b). Such a density fluctuation is known in populations of strict monocarpic biennials (e.g. *Pedicularis sylvatica*, Petru 2005). This delayed reproduction in grove areas was related to better survival of seedlings and adults in these areas (Table 4), supporting life history theory predicting an increase in threshold.

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**Fig. 3.** Summer and winter survival as a function of size classes in M populations (Prayon, Angleur) and NM populations (Lux1 and Lux2) of *Thlaspi caerulescens*. The different size classes are seedlings (<5 leaves), small vegetative rosettes (6–10 leaves), medium vegetative rosettes (11–20 leaves) and large vegetative rosettes (>20 leaves). Bars topped with similar letters are not significantly different (Chi-square test with Bonferroni correction).

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<table>
<thead>
<tr>
<th>M populations</th>
<th>NM populations</th>
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<tr>
<td></td>
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<tr>
<td><strong>Prayon</strong></td>
<td></td>
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<tr>
<td><strong>Angleur</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Lux1</strong></td>
<td></td>
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<tr>
<td><strong>Lux2</strong></td>
<td></td>
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<tr>
<td><strong>Seedlings</strong></td>
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<tr>
<td><strong>Small rosettes</strong></td>
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<tr>
<td><strong>Medium rosettes</strong></td>
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<td><strong>Large rosettes</strong></td>
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<tr>
<td>Summer survival</td>
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size for reproduction in environments more favourable to survival (Stearns 1992). Growth rate during the pre-reproductive period is an important parameter in the control of flowering in some facultative monocarpic perennials (e.g. Daucus carota, Lacey 1986; Cynoglossum officinale, Wesselingh et al. 1997). In these species, a decrease in relative growth increases the probability of flowering in the next reproductive season. Therefore, the higher proportion of annuals in grass areas might be a result of lower relative growth during the pre-reproductive period. Although our results show better growth in grove than in grass areas, further experiments measuring growth rate are necessary to test the influence of recent growth rate on flowering probability. In Cynoglossum officinale, a better growth rate and a higher threshold size for flowering were found in a grove habitat compared to an open one (Wesselingh et al. 1997).

The plastic or genetic origin of this variation in life strategies will have to await results of further experiments. However, as flowering times of both types of plant (grass versus grove) are not different, thus allowing pollen exchange, we can support the hypothesis of a plastic origin for the life strategy variation. Furthermore, life cycle plasticity of the M population of T. caerulescens has already been highlighted in controlled conditions (Dechamps et al. 2007) and in a transplantation experiment (Dechamps et al. 2008). The influence of various environmental factors (light, temperature, drought, soil fertility) on length of the pre-reproductive period has also been demonstrated previously (Bender et al. 2000;
conclusions and perspectives

First, the present study shows large variation in life strategy of _T. caerulescens_, and the diversity of life strategies recorded in the field is similar to that observed in controlled conditions (Dechamps et al. 2007). NM plants are mostly monocarpic perennials whereas most M plants are winter annuals, except those growing in grove areas. Furthermore, our study demonstrates a strong relationship between survival conditions during summer and the length of the pre-reproductive period. NM plants thus delay reproduction and store resources for future reproduction because their environment is more favourable for survival. In contrast, in M populations, our results suggest the selection of stress avoiders (shortening of life cycles) but only for individuals growing in grass areas. Our results do not fit in well with Grime's (1977) model that supports the selection of a longer life cycle under stress conditions. However, our results support the few empirical studies on other pseudometallophytes, suggesting the selection of stress avoiders in metalliferous environments (Anthoxanthum odoratum, Antonovics 1972; Silene vulgaris, Wierzbicka & Panufnik 1998).

A second striking result is the contrast between the life history of plants growing in grass and those in grove areas within M populations. M plants growing in grove areas grow, survive and reproduce better than M plants growing in grass area. Life cycles preferentially expressed in these two contrasting areas are also different. The perennial strategy is favoured in grove areas, whereas most plants are winter annuals in grass areas. Plastic or genetic origins of this life strategy variation will have to be examined in further experiments. Life cycle plasticity would represent an advantage to colonise heterogeneous habitats such as metalliferous sites.

It will be interesting to broaden this type of study to the whole ecogeographical distribution range of _T. caerulescens_ in order to relate life history traits to the large diversity of ecological conditions in habitats occupied by _T. caerulescens_. Finally, a phylogeographical approach would allow inference of reliable evolutionary hypotheses accounting for the observed quantitative variations in life history traits.

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