

RESEARCH PAPER

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

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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 Luxembourg. 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 is partly related to plant origin (M versus NM). Most plants growing in metalliferous 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; Denaeher-De Smet & Duvigneaud 1974; Simon 1975, 1978; Kruckeberg & Kruckeberg 1990; Wu 1990; Macnair 1997; van Oort *et al.* 2002). Pseudometallophytes, *i.e.* species occurring on both metalliferous 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 metalliferous 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 metalliferous environments (*e.g.* *Anthoxanthum odoratum* in Antonovics 1972; *Silene vulgaris* in Wierzbicka & Panufnik 1998). Interestingly, contrasting conclusions are drawn on life strategies selected in metalliferous 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: *Noccaea*) 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 (silicula: 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-metalliferous, 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 slagheap 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

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

characteristics	metallicolous (M) populations		non-metallicolous (NM) populations	
	Prayon (Belgium)	Angleur (Belgium)	Goebelsmuhle (Luxembourg)	Hoscheid (Luxembourg)
acronyms	<i>Pr</i>	<i>Ang</i>	<i>Lux 1</i>	<i>Lux 2</i>
geographic coordinates	50°35'3"N, 5°40'23"E	50°36'46"N, 5°36'34"E	49°55'23"N, 6°03'45"E	49°55'06"N, 6°04'38"E
pollution origin	Dust fallout from smelter	Solid waste from smelter	no pollution	no pollution
surface (m ²)	750,000	30,000	50	200
altitude (m)	160	60	255	320
slope (°)	20	0	40	60
aspect	SE	–	SW	SW
substrate	Limestone	Furnace slag	Shale	Shale
flowering plants	>1000	>1000	150	500

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 (\pm SE). The results of the ANOVA highlight the sources of variation in mineral concentration.

mineral element concentrations (mg kg ⁻¹)	Prayon		Angleur		sources of variation in the ANOVA		
	grass areas	grove areas	grass areas	grove areas	site	vegetation cover	site \times vegetation cover
calcium (Ca)	1545 (128)	4248 (1046)	761 (178)	3916 (1513)	ns	F _{1,9} = 13.9**	ns
potassium (K)	152 (11)	263 (54)	72 (21)	228 (53)	ns	F _{1,9} = 12.1**	ns
magnesium (Mg)	96 (3)	122 (1)	106 (12)	267 (95)	ns	F _{1,9} = 8.4*	ns
iron (Fe)	426 (0.1)	219 (37)	268 (69)	100 (11)	F _{1,9} = 7.9*	F _{1,9} = 10.6**	ns
zinc (Zn)	39918 (7707)	15161 (182)	18812 (632)	24969 (6895)	ns	F _{1,9} = 7.2*	F _{1,9} = 18.3**
cadmium (Cd)	205 (39)	287 (19)	18 (4)	26 (4)	F _{1,9} = 97.6***	ns	ns
lead (Pb)	5190 (548)	3530 (335)	1531 (395)	2310 (1101)	F _{1,9} = 8.7*	ns	ns
Pb/Ca ratio	3.4 (0.6)	0.9 (0.25)	2.2 (0.49)	1.2 (0.9)	ns	F _{1,9} = 7.1*	ns
Zn/Ca ratio	1.4 (0.1)	0.7 (0.1)	1.5 (0.1)	0.9 (0.1)	ns	F _{1,9} = 29.8***	ns

show that in both metalliferous sites, soils from grove 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 = Goebelsmühle, 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 m⁻² (~150 flowering plants) and 200 m⁻² (~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-metalliferous than in metalliferous sites (Noret *et al.* 2007).

Field experimental design

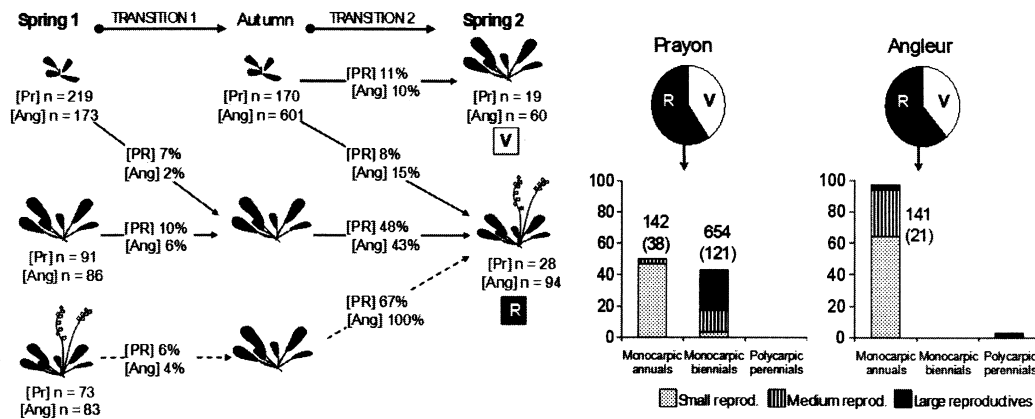
In May 2006, we selected seven plots of 0.5 \times 0.5 m in each M population and six plots of 1 \times 1 m in each NM population. Plots were larger in NM populations to compensate for the lower population density. In M populations, plots were

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*.

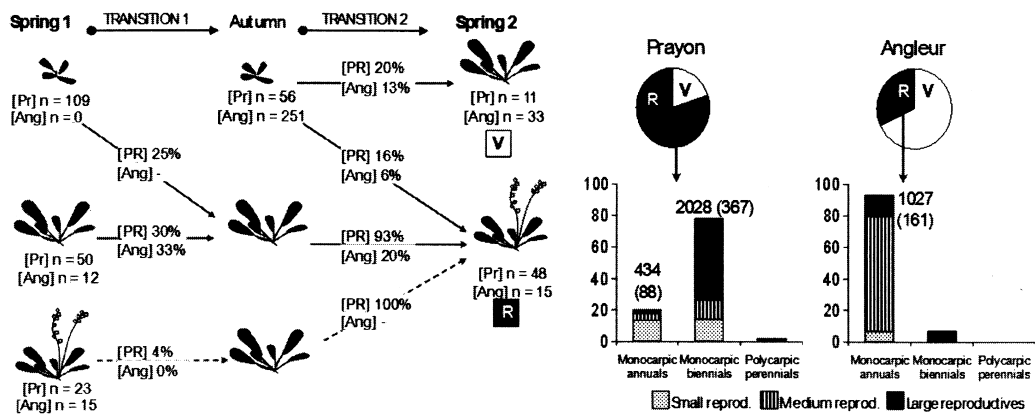
Field data collection

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 \times 5 cm and 10 \times 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 (S1, 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

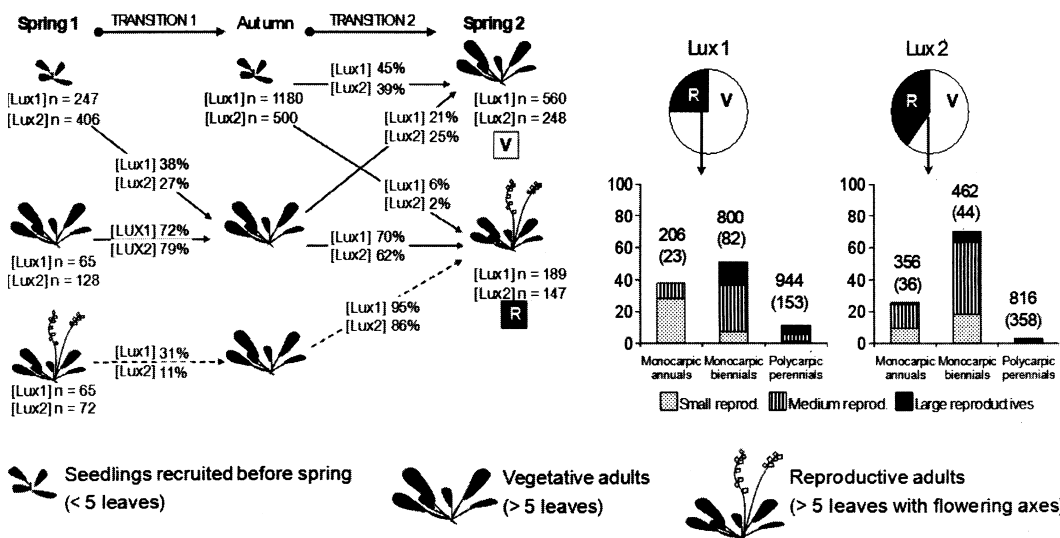
(a) Metallicolous populations in grass areas



(b) Metalicolous populations in grove areas



(c) Non metallicolous populations



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: seedlings, vegetative adults and reproductive adults. To assess survival and recruitment, we compared cartographies of successive censuses. Summer and winter survivors

Fig. 1. Life transitions of metallicolous (M) populations of *Thlaspi caerulescens* 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: Goebelsmühle [*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 \pm SE).

were, respectively, assessed in October 2006 and April 2007. Recruitment was assessed in autumn and spring 2; seedlings censused in spring 1 were not considered because the exact time of germination was not certain. Each reproductive plant in spring 2 (2007) was ascribed to one of the following life cycles: monocarpic winter annual (germination in autumn 1 and flowering in spring 2), monocarpic perennial (vegetative in spring 1 and flowering in spring 2), polycarpic perennial (flowering in spring 1 and 2). The monocarpic trait was validated by the last census of spring 2 (June). All plants that were ascribed to the monocarpic life cycle were fruiting, the rosette had disappeared in most cases or showed clear signs of senescence in other cases (without re-growth). For each reproductive plant, we assessed seed output by counting the number and length of flowering stems at fructification stage. Assessment of seed production was based on a linear relationship linking the cumulated length of flowering stems to the number of seeds. For the M ecotype, this relation was established on 20 individuals in each population (*Pr*: $r^2 = 0.97^{***}$; *Ang*: $r^2 = 0.96^{***}$). For the NM ecotype, we used 30 individuals in a third population (population #14 in Molitor *et al.* 2005; $r^2 = 0.97^{***}$) in order to limit the impact of harvesting on the small populations studied. In spring 2, we also assessed the proportion of reproductive plants producing sterile fruits only. For M populations, plants growing in grass areas were always compared to those growing in grove areas.

Statistical analyses

Differences in survival, flowering rate and life cycle proportions were analysed using Chi-square tests. Bonferroni corrections were applied to overcome problems in multiple comparisons. Fecundity (seed output per plant) was analysed with ANOVA for both reproductive seasons (spring 1, 2006, and spring 2, 2007). The factors tested were ecotype (M versus NM), rosette size (S, M, L), population (*Pr*, *Ang*, *Lux 1*, *Lux 2*). Populations were nested within ecotypes and were considered as a random factor (other factors were fixed). In M populations, the effect of vegetation cover (grass versus grove) on plant fecundity and on soil mineral concentration was also tested with ANOVAs with two fixed factors (population and vegetation cover). To fulfil the normality assumption of ANOVA, logarithmic transformations were applied to the data. ANOVAs were performed with STATISTICA 7 (Statsoft 2005, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Figure 1 illustrates life histories of M populations in grass areas (a), M populations in grove areas (b) and NM popula-

tions (c). Transitions, represented by arrows, describe the proportion of plants that survived from spring 1 (2006) to autumn 1 (TRANSITION 1), and from autumn 1 (2006) to spring 2 (2007; TRANSITION 2). Transitions are compared for plants belonging to three different developmental stages: seedlings, vegetative and reproductive plants. To simplify the results, vegetative and reproductive adults include different rosette sizes (small, medium and large). Proportions of reproductive and vegetative plants in spring 2 are shown in pie charts. For reproductive plants of spring 2, proportions of the different life cycles are presented in histograms. For each life cycle, mean fecundity and proportions of the different rosette sizes (small, medium and large) are given. Seedlings recruited in autumn include those recruited from October to December.

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

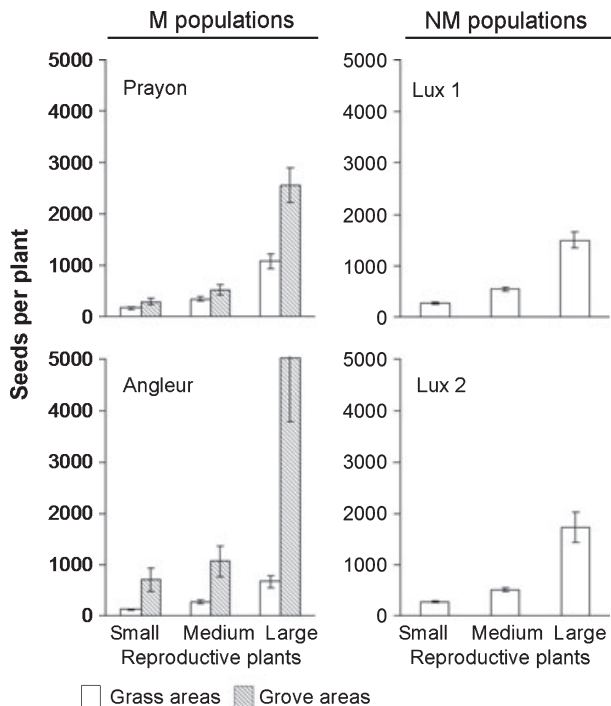


Fig. 2. Number of seeds produced per plant in two metallicolous (M) and two non-metallicolous (NM) populations of *Thlaspi caerulescens* according to growth class of reproductive plants. Data are means \pm SE. For M populations, data from plants growing in grove versus in grass areas are presented separately. Vegetation cover was homogeneous for all NM plants.

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).

Table 3. ANOVA results on fecundity data (number of seeds per plant) of *Thlaspi caerulescens* monitored in four field populations for 2 years (2006, spring 1; 2007, spring 2). (a) Populations belonged either to the metallicolous or the non-metallicolous ecotype (2 populations/ecotype). Plants were ascribed to one of three classes of rosette size. (b) In metallicolous (M) populations, plants growing in different vegetation cover (grove versus grass areas) were distinguished. Levels of significance are shown after F values (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant).

(a) fecundity		spring 1	spring 2
sources of variation	df	F	F
ecotype	1	17.2*	1.6 ns
population (Ecotype)	2	3.4 ns	4.7 ns
size class	2	95.3***	46.7**
ecotype \times size class	2	2.4 ns	0.01 ns
pop (Eco) \times size class	4	0.7 ns	3.2*
(b) fecundity within M populations		spring 1	spring 2
sources of variation	df	F	F
population	1	1.1 ns	5.8*
vegetation cover	1	75.7***	59.0***
population \times vegetation cover	1	11.8***	2.2 ns

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 $\sim 40\%$: $\chi^2 = 202.6$, $P = 0.001$; proportions inferred from Fig. 1). Consequently, in both NM populations, most reproductive plants in spring 2 were monocarpic perennials ($\sim 60\%$), a low proportion were monocarpic annuals ($\sim 30\%$), and a few were polycarpic perennials ($\sim 10\%$) (Fig. 1c). During spring 2, the predominance of vegetative plants ($\sim 70\%$) in NM populations reflects the protracted pre-reproductive period. Moreover, $\sim 10\%$ 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

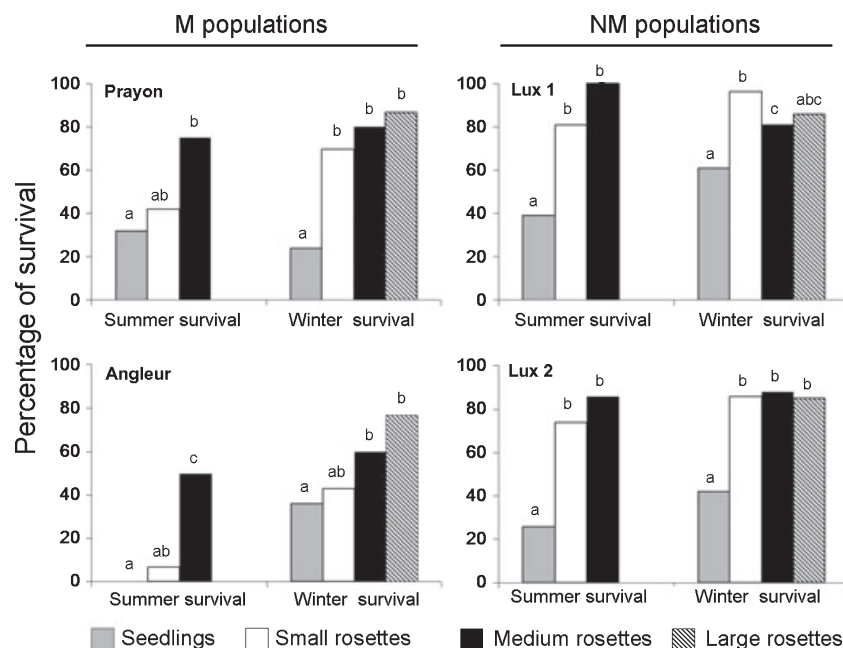


Fig. 3. Summer and winter survival as a function of size classes in M populations (Prayon, Angleur) and NM populations (*Lux1* and *Lux2*) of *Thlaspi caerulescens*. The different size classes are seedlings (<5 leaves), small vegetative rosettes (6–10 leaves), medium vegetative rosettes (11–20 leaves)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

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 \pm 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

Table 4. Results of Chi-square tests of (a) survival of *Thlaspi caerulescens* from spring to autumn (TRANSITION 1), of (b) survival from autumn to next spring (TRANSITION 2) and (c) flowering rate during spring 2. Chi-squares tests were used to answer the questions listed in the first column of the Table. These tests were performed separately for three different developmental stages (column 2).

	developmental stages		χ^2
(a) TRANSITION 1: survival from spring 1 to autumn			
between metalliferous <i>versus</i> normal environments?	seedlings	yes	73.1***
	vegetative adults	yes	135.4***
	reproductive adults	yes	25.5***
between metalliferous-grass <i>versus</i> normal environments?	seedlings	yes	113.5***
	vegetative adults	yes	172.6***
	reproductive adults	yes	17***
between metalliferous-grove <i>versus</i> normal environments?	seedlings	yes	n.s.
	vegetative adults	yes	41.6***
	reproductive adults	yes	n.s.
between grove <i>versus</i> grass within metalliferous environments?	seedlings	yes	42.5***
	vegetative adults	yes	19.9***
	reproductive adults	no	n.s.
(b) TRANSITION 2: survival from autumn to spring 2			
between metalliferous <i>versus</i> normal environments?	seedlings	yes	170.1***
	vegetative adults	yes	12.4***
	reproductive adults	no	n.s.
between metalliferous-grass <i>versus</i> normal environments?	seedlings	yes	129.6***
	vegetative adults	yes	13.9***
	reproductive adults	–	not determined
between metalliferous-grove <i>versus</i> normal environments?	seedlings	yes	70.6***
	vegetative adults	no	n.s.
	reproductive adults	–	not determined
between grove <i>versus</i> grass areas within metalliferous environments?	seedlings	no	n.s.
	vegetative adults	yes	n.s.
	reproductive adults	–	not determined
(c) proportion of flowering plants during spring 2			
between metalliferous <i>versus</i> normal environments?	seedlings	yes	202.6***
	vegetative adults	–	not determined
	reproductive adults	–	not determined
between metalliferous-grass <i>versus</i> normal environments?	seedlings	yes	214.9***
	vegetative adults	–	not determined
	reproductive adults	–	not determined
between metalliferous-grove <i>versus</i> normal environments?	seedlings	yes	37.9***
	vegetative adults	–	not determined
	reproductive adults	–	not determined
between grove <i>versus</i> grass areas within metalliferous environments?	seedlings	yes	9.2**
	vegetative adults	–	not determined
	reproductive adults	–	not determined

Levels of significance are shown after values ((*): $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant).

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;

Simons & Johnston 2000; Callahan & Pigliucci 2002; Johnson 2007; van Kleunen 2007). As Bender *et al.* (2000) showed in *Polymnia canadensis*, spatial variation in life cycle observed in a population may be partly explained by spatial differences in germination rate and seedling survival between autumn and spring cohorts. However, no differences in germination dates were observed between plants growing in grove and grass areas within metalliferous sites (data not shown).

In grass areas of both of the M populations, there was a low fecundity and a high sterility rate. A high proportion of flowering plants produced sterile fruits only (*Ang*: 57%, 40/70 flowering plants; *Pr*: 25%, 7/28 flowering plants). Fruit or seed abortion in stress conditions is known in hermaphroditic plants (Stephenson 1980; de Jong & Klinkhamer 1989). Some plants from grass areas might thus spread their genes only by pollen dispersal. These observations suggest differentiated sexual allocation between large grove reproductive plants and small grass reproductive plants. For the monocarpic species *Cynoglossum officinale*, sexual allocation type is a function of rosette size (Klinkhamer & de Jong 1987): small rosettes allocate more to male than to female function. In our study, the high female fecundity of large grove reproductive plants might support this hypothesis.

Despite the common trends described above, differences do exist between *Pr* and *Ang* plants. The most striking difference concerned the relative contribution of spring and autumn cohorts to the annual recruitment. The annual contribution from spring was significantly higher in *Pr* than in *Ang* (autumn 1 versus spring 2: *Pr*: 226 versus 28; *Ang*: 852 versus 35; $\chi^2 = 18.96$, $P < 0.001$). This difference may partly explain the lower occurrence of winter annuals in *Pr* compared to *Ang* (M population) because spring-germinated plants could not reproduce in the first year and therefore became perennials. This difference may result from the extreme ecological conditions occurring in the *Ang* site (Table 1), especially in grass areas, where the substrate is composed of dark scoria subject to excessive drainage and high temperatures in summer. These extreme conditions probably limit the survival of spring seedlings during summer and therefore limit the proportion of perennials. Our results support this hypothesis, as summer seedling survival was significantly lower in *Ang* than in *Pr* (Fig. 1; $\chi^2 = 5.30$, $P = 0.02$). Nevertheless, a longer observation period with several replicate of metalliferous sites will be necessary to confirm these results.

Variations within NM populations

Not all NM plants belonging to the same cohort reproduce at the same time, as observed in M populations. In contrast to M populations, no clear environmental factor seems to influence the life history of NM plants, as NM populations occur on small and homogeneous sites. However, micro-variations in environment (biotic: herbivory, competition; abiotic: fertility, drought) also might generate variations in life histories. In NM sites, herbivores represent a high selection pressure for *T. caerulescens* (Noret *et al.* 2007). In *Senecio jacobaea*, herbivore damage was shown to induce a flowering delay of 1 year or more (Van der Meijden & Van der Waals-Kooi 1979).

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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