Seasonal pattern of germination and seed longevity in the invasive species *Heracleum mantegazzianum*

Sezónní průběh klíčení a životnost semen invazního druhu *Heracleum mantegazzianum*

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We investigated the effects of different temperature regimes and dry storage on germination of *H. mantegazzianum* (*Apiaceae*, native to Caucasus) seeds in the laboratory and linked the results with studies of seasonal seed bank depletion in a common garden experiment and under field conditions. Seeds were collected at seven sites in the Slavkovský les region, Czech Republic, cold-stratified for 2 months and germinated at seven temperature regimes. Under all temperature regimes, fresh seeds germinated to significantly higher percentages than older (1, 2, 3 years) seeds. For all storage lengths, seeds germinated best at alternating day/night temperatures of 20/5 °C. The length of the germination period had a significant effect only at low constant temperatures of 2 and 6 °C, where germination percentage increased between 2 and 6 months. Seasonal germination exhibited a distinct pattern, with rapid depletion of seed bank by the first spring after seed burial. Non-dormant seeds were present in the soil early in spring and late in autumn. The higher summer temperatures prevented dormancy breaking and another cold period of at least two months below 10 °C was needed to bring non-germinated seeds out of dormancy. The results suggest that (1) seed dormancy of *H. mantegazzianum* was not completely broken until the first spring, but that some seeds re-enter or retain dormancy during high summer temperatures and that (2) the threshold needed for breaking the dormancy was achieved gradually during the cold autumn and winter months. However, in a small fraction of seeds the dormancy breaking process took several years. Of seeds buried in 10 different regions of the Czech Republic, on average 8.8% survived 1 year, 2.7% 2 years and 1.2% remained viable and dormant after 3 years of burial. The ability of even small fraction of *H. mantegazzianum* seeds to survive for at least 3 years can result in re-invasion of this species into controlled sites.

Keywords: alien plant, *Apiaceae*, biological invasions, burial experiment, cold stratification, Czech Republic, *Heracleum mantegazzianum*, morphophysiological dormancy, seed bank, seed longevity, temperature regime

Introduction

Biological invasions have been intensively studied over the last few decades (Pimentel 2002, Mandák et al. 2004, Mooney et al. 2005, Chytrý et al. 2005) and the question of what makes a species invasive is an important focus of much research (Rejmánek et al. 2005, Richardson & Pyšek 2006). Reproductive traits have been repeatedly recognized as important determinants of the success of invasive species (see Pyšek & Richardson 2007 for a review). High fecundity (e.g. Pyke 1990, Radford & Cousens 2000, Goergen &

Daehler 2001, Morris et al. 2002), efficient dispersal (Honig et al. 1992, Sallabanks 1993, Vila & D'Antonio 1998) and ability to germinate across a wide range of conditions (Dreyer et al. 1987, Van Clef & Stiles 2001, Callaway & Josselyn 1992) were shown to promote invasiveness; these factors are especially important for successful invasion of species that lack vegetative propagation. Some studies have also addressed the soil seed bank in relation to invasiveness and available data, yielded by comparison of native/alien and invasive/non-invasive congeners or confamilials, suggest that aliens and successful invasive species often have larger and longer-persisting seed banks (Pyke 1990, Honig et al. 1992, Radford & Cousens 2000, Van Clef & Stiles 2001). For other species pairs, however, no significant difference was found (Radford & Cousens 2000, Van Clef & Stiles 2001), but none of the studies indicates the opposite, i.e. invasive alien having smaller and less persistent seed bank (Pyšek & Richardson 2007). This suggests that not only fecundity and dispersal, but also the fate of seeds after they have reached the new regions or sites, are important in determining the outcome of invasion.

The present paper deals with *Heracleum mantegazzianum*, an important invasive species in Europe (Nielsen et al. 2005). For such a species relying on generative reproduction by seeds, a detailed knowledge of its seed ecology is crucial for understanding its invasive behaviour. Previous studies report that seeds of *H. mantegazzianum* do not germinate after dry storage (Grime et al. 1981) and cold stratification is necessary for germination (Nikolaeva et al. 1985, Tiley et al. 1996, Moravcová et al. 2005). The requirement of cold stratification for dormancy breaking was confirmed by germination experiment with freshly harvested non-stratified seeds, where none of the seeds germinated (L. Moravcová, unpublished). Under natural conditions in the Czech Republic, seeds germinate and seedlings establish early in spring (March to April) after snow melting (Krinke et al. 2005, Pergl et al. 2007). Seeds of *H. mantegazzianum* exhibit morphophysiological dormancy (further MPD) in the sense of Nikolaeva et al. (1985) and Baskin and Baskin (1998, 2004); ripe seeds have an underdeveloped embryo, which is physiologically dormant. For a seed to germinate, the growth of embryo needs to be completed and its physiological dormancy broken. Both these processes occur in cold and wet conditions of autumn and winter stratification in the field; corresponding laboratory temperatures range from 1–6 ºC (Moravcová et al. 2005). Another research on reproductive ecology of *H. mantegazzianum* concerned the pattern of germination of seeds produced at different positions on the mother plant (Moravcová et al. 2005) and the size, structure and seasonal dynamics of soil seed bank (Krinke et al. 2005). However, the factors affecting seed germination, in particular the temperature regime during the year, have not been studied yet. Also, nothing has been known about the long-term dynamics of soil seed bank. Thus, the purpose of this study was to (1) investigate the effects of temperature regimes and dry storage on germination of *H. mantegazzianum* seeds under laboratory conditions; (2) monitor seasonal seed bank depletion in an experimental garden over 2 years, and (3) monitor long-term seed longevity under field conditions. Results from these studies are combined with those from previous work on seed banks of *H. mantegazzianum* to provide more complex understanding of seed ecology of this invasive species.

Material and methods

Study species

Heracleum mantegazzianum Sommier et Levier (giant hogweed, *Apiaceae*) is native to the Western Greater Caucasus where it occurs in the upper belt of mixed forest on southern slopes, mainly in meadows, clearings and forest margins (Mandenova 1950). Its exotic appearance and large size (flowering stem reaches 200–500 cm in height; leaves up to 300 cm long; large umbels with terminal up to 80 cm in diameter) made it a popular garden ornamental in many European countries. It has become naturalized or invasive in Europe (Nielsen et al. 2005), Canada and in the United States (Morton 1978, Kartesz & Meacham 1999). In the Czech Republic, the species was first recorded as planted in 1862 (chateau Lázně Kynžvart, W Bohemia; Pyšek 1991). *Heracleum mantegazzianum* is a monocarpic perennial herb that flowers in its 3rd to 5th year under natural conditions, but taking up to 12 years to flower in some cases (Pergl et al. 2006). The species reproduces exclusively by seeds (Tiley et al. 1996, Krinke et al. 2005, Moravcová et al. 2005); an average plant was reported to bear 20,671 seeds, with a maximum of 46,470 (Perglová et al. 2006). Fruits are oval-elliptical broadly winged mericarps (hereafter called seeds), which are connected into pairs by carpophore and split when mature (Holub 1997). Mericarps are 6–18 mm long and 4–10 mm wide, and contain one seed. The embryo is rudimentary (Martin 1946) and surrounded by oily endosperm.

Seed collection

Ripe seeds were collected in August 2001, 2002, 2003 and 2004 from extensive stands of *H. mantegazzianum* at seven sites in the Slavkovský les Protected Area in the western part of the Czech Republic: Žitný I, Potok, Dvorečky, Krásná Lípa I and II, Litrbachy and Rájov (see Krinke et al. 2005, Moravcová et al. 2005, Müllerová et al. 2005 for the detailed description of sites and populations). Since previous studies showed that percentage of germinated seeds is not affected by their position on the mother plant (Moravcová et al. 2005) and about half of the seed is produced by the terminal umbel (Perglová et al. 2006), seeds were collected only from terminal umbels. Further, variation in percentage germination among sites was much smaller than that of individual plants within site (Moravcová et al. 2005). Thus a mixed sample of seeds from the seven sites collected in 4 following years was used for the experiments. Seeds were dried for 5 days at room temperature and stored in paper bags in an unheated room (ca 10–15 °C) until the beginning of the experiments.

Effect of different temperature regimes and the length of dry storage on seed germination

Seeds from samples collected in August 2001–2004 (four seed batches) were dry-stored for 38 months (henceforth referred to as 3 years), 26 months (2 years), 14 months (1 year) and 2 months (fresh seeds), respectively. After each storage period, the seeds were cold-stratified in the dark at the temperature of 2–6 ºC for two months prior to the experiment and tested for germination at 2/2, 6/6, 10/5, 20/5, 15/10, 25/10 and 22/22 ºC (12/12 h daily alternating temperature regimes or constant temperature and 12/12 h light/dark at all temperatures). Seeds were stratified and germinated in plastic Petri dishes filled with heat sterilized river sand. Each treatment consisted of four replicates with 25 seeds that were weighed before stratification. All experiments started on 1 November 2004 and stratification lasted 2 months. Germination of fresh seeds was assessed after 2 and 6 months, since most seeds germinate in the field within the first 2 months (March and April), when the temperatures are low and then the germination markedly declines (Pergl et al. 2007). If seeds ceased to germinate at higher temperature regimes after 2 months, it is suggested that higher temperatures prevent further dormancy breaking. If the seeds would continue to germinate at higher temperatures even after 2 months, later germination in the field would not be constrained by high soil temperatures but rather by the developing vegetation cover. Seeds were kept continuous moist by adding tap water weekly. Decayed seeds at the end of the experiment were considered as dead, non-germinated viable seeds as dormant. Seed viability at the end of experiment was tested by staining with tetrazolium.

Seed bank depletion and the seasonal timing of germination in an experimental garden

Seeds collected in August 2002 were placed in nylon bags, buried in the soil to the depth of 5–10 cm at the Experimental Garden of the Institute of Botany in Průhonice (N 49°59', E 14°34') in October 2002, and monitored for two germination seasons until March 2005. Each bag contained 100 seeds. Ten sets (replicates located in different parts of the experimental bed) of 23 bags were used, for a total of 230 bags. Each month, with exception of the winter period when soil was frozen, 10 replicate bags were exhumed from the soil, and numbers of seeds in the following categories counted: (1) germinated or decayed in the soil, taken as a measure of seed bank depletion; (2) germinated in laboratory at 10/5ºC (mean early spring temperature) during the first month, considered as non-dormant; (3) did not germinate in the laboratory but gave a positive response to stain with tetrazolium, considered as dormant.

Long-term survival of buried seeds

Seeds collected in August 2002 were buried in the soil to the depth of 5–10 cm at 10 localities in the Czech Republic; in each locality, 15 nylon bags containing 100 seeds each were used. The localities were selected in order to cover a range of geographic conditions in the country (Table 1). Starting in 2003, five bags (replicates) were exhumed in each October. The exhumed seeds were tested for viability. Climatic locality data were obtained from Quitt (1992) (Table 1).

Statistical analysis

Data on the effect of different temperature regime and the length of dry storage on seed germination were analysed with the germination percentage of 25 seeds from each Petri dish as the dependent variable. The overall difference in germination among the dry storage lengths was assessed by a split-plot ANOVA. Climate chambers with the seven different temperature regimes were blocks, and the differences in germination among the dry storage lengths were compared by one-way ANOVA nested within the blocks, using the (block) × (dry storage length) interaction to test the significance of the between dry storage lengths differences (Crawley 2002, p. 65–66). The a-priori hypothesis that fresh seeds germinate better than those after the varying dry storage lengths was tested by or-

Table 1. – Localities used in the long-term survival study of seeds of *Heracleum mantegazzianum* buried in the Czech Republic in November 2002. Seeds were exhumed in October of the following years and tested for viability by tetrazolium. Numbers are means of five replicates. Regions: C – central, N – northern, W – western, S – southern, E – eastern; B – Bohemia. Percentage of viable seeds (mean, range) after 1, 2 and 3 years of burial is shown.

Locality	Region	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Mean annual temperature (°C)	Precipitation (mm/yr)	% of viable seeds after		
							1 yr	2 yrs	3yrs
Černolice	CB	49°55'	14°19'	400	7.5	525	3.0 (0–5)	1.2 (0–2)	1.2 (0–2)
Vroutek	NB	50°11'	13°23'	337	7.5	475	2.0 (1–3)	0	0
Plzeň	WB	49°42'	13°19'	337	7.5	525	13.2 (2–18)	0.4 (0–1)	0.6 (0–2)
Kralupy	CB	50°14'	14°19'	175	8.5	475	2.8 (1–5)	0.8 (0–2)	0
Kyjov	NB	50°55'	14°28'	376	7.5	850	2.2 (0–5)	2.4 (1–4)	2.2 (1–4)
Žitný	WB	50°03'	12°37'	787	5.5	850	6.0 (2–9)	2.0 (1–3)	0.8 (0–1)
Koda	CB	49°56'	14°07'	299	8.5	525	3.0 (1–5)	2.2 (1–5)	0.8 (0–2)
Lužnice	SB	49°05'	14°45'	419	7.5	625	1.4 (0–6)	2.0 (1–4)	1.0 (0–2)
Průhonice	CB	49°59'	14°34'	303	8.5	625	19.8 (6–30)	1.8 (0–6)	2.2 (0–4)
Broumov	EB	50°38'	16°16'	520	6.5	850	34.8 (13–68)	7.8 (5–12)	2.8 (1–6)

thogonal contrasts (e.g. Sokal & Rohlf 1995), after the use of seven independent one-way ANOVAs at the seven different temperature regimes (Crawley 2002, p. 66). For fresh seeds, an additional analysis of the germination after 2 and 6 months were carried out using, independently for each of the seven temperature regimes, a t-test for paired comparison (Sokal & Rohlf 1995).

Data on the long-term survival of buried seeds were analysed with the percentage of viable seeds of 100 seeds from each bag as the dependent variable. In a mixed model ANOVA (e.g. Sokal & Rohlf 1995), the date of exhumation (October 2003, 2004, 2005) was a fixed factor, while the individual localities with the buried seeds a random factor. The effect of altitude, mean annual temperature and precipitation at these localities on long-term survival of buried seeds was analysed using a multiple linear regression, with a backward elimination (e.g. Crawley 1993) of the individual explanatory variables from the model.

To normalize the error distribution and to homogenize the variance, all percentages were angular transformed (e.g. Sokal & Rohlf 1995). The homogeneity of variance was checked by Cochran's test (e.g. Underwood 1997), and the fitted models by plotting standardized residuals against fitted values, and by normal probability plots (e.g. Crawley 1993).

Results

Effect of different temperature regimes and the length of dry storage on seed germination

The period of dry storage significantly affected germination (Table 2). Under all temperature regimes, fresh seeds (i.e. after two months of dry storage) germinated to significantly

Table 2. – Split-plot ANOVA on the differences in germination (angular transformed percentages) of *Heracleum mantegazzianum* seeds among dry storage lengths (fresh, 1, 2, 3 years) at varying temperature regimes (day/night temperatures in °C: 2/2, 6/6, 10/5, 20/5, 15/10, 25/10, 22/22).

Source of variation	df	MS	F	
Dry storage length	3	3.473	61.11	< 0.00001
Temperature regime	6	0.512		
Dry storage length × temperature regime	18	0.057		

Table 3. – One-way ANOVAs and orthogonal contrasts on the differences in germination (angular transformed percentages) of *Heracleum mantegazzianum* seeds among four dry storage lengths (fresh, 1, 2, 3 years). Seven independent analyses at seven temperature regimes (day/night temperatures in °C). *** P < 0.001.

Source of variation		2/2 °C		6/6 °C		10/5 °C		20/5 °C		15/10 °C		25/10 °C		22/22 °C	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Dry storage length	3	0.557	21.89 ***	1.074	97.72 ***	0.454	24.15 ***	0.799	42.70 ***	0.347	29.02 ***	0.321	17.64 ***	0.262	30.22 ***
Fresh vs 1, 2, 3 yrs	1	1.52	59.79 ***	2.81	243.00 ***	0.91	48.44 ***	1.73	92.41 ***	0.84	70.53 ***	0.72	39.56 ***	0.68	78.75 ***
Error	12	0.025		0.012		0.019		0.019		0.012		0.018		0.0087	

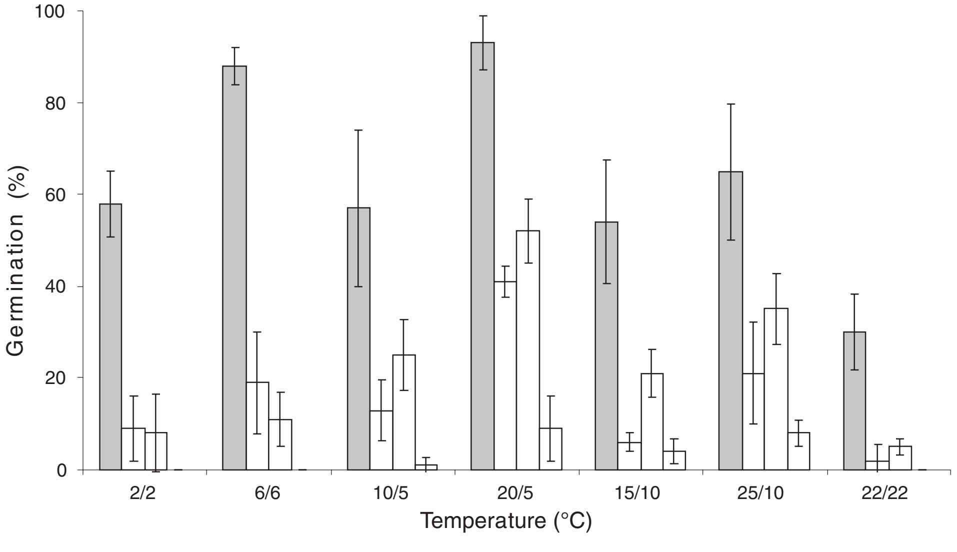

Fig. 1. – Germination percentage of *Heracleum mantegazzianum* seeds (mean ± standard deviation) for the dry storage lengths (fresh, 1, 2, 3 years) at the varying temperature regimes (day/night temperatures in °C). Shaded vs. transparent bars indicate significant differences between fresh and longer stored seeds at individual temperature regimes in orthogonal contrasts. Statistics are given in Table 3.

(Table 3) higher percentages than older seeds (Fig. 1). In the majority of temperature regimes, the high germination of fresh seeds (range 30–93%) markedly decreased after the first year of storage (2–41%), sustained at similar levels after the second year (5–52%) and decreased again (0–9%) after three years of storage. For all storage lengths, seeds germinated best at the temperature regime of 20/5 °C. In contrast, consistently lowest germination percentages were recorded at 22/22 ºC. Seeds stored for 3 years and germinated at 2/2, 6/6 and 22/22 °C were the only that did not germinate at all; in all other temperature regimes/storage lengths some germination occurred (Fig. 1).

The germination of fresh seeds was highest (93%) at 20/5 °C and at 6/6 °C (88%). As longer stored seeds, fresh seeds germinated to lowest percentages at 22/22 °C (Fig. 1).

In fresh seeds, where the percentages of germinated seeds were assessed after the two germination periods (2 and 6 months), the length of germination period significantly affected the percentage of germinated seeds only at low constant temperatures. From months 2 to 6, germination increased from 37 to 58% at 2/2 °C ($t_3 = 11.19$, $P = 0.001$) and from 45 to 88% at 6/6 °C ($t_3 = 8.99$, $P = 0.003$). At all other combinations with day temperatures of at least 10 °C, percentage of germinated seeds did not increase significantly from month 2 to month 6 (Fig. 2).

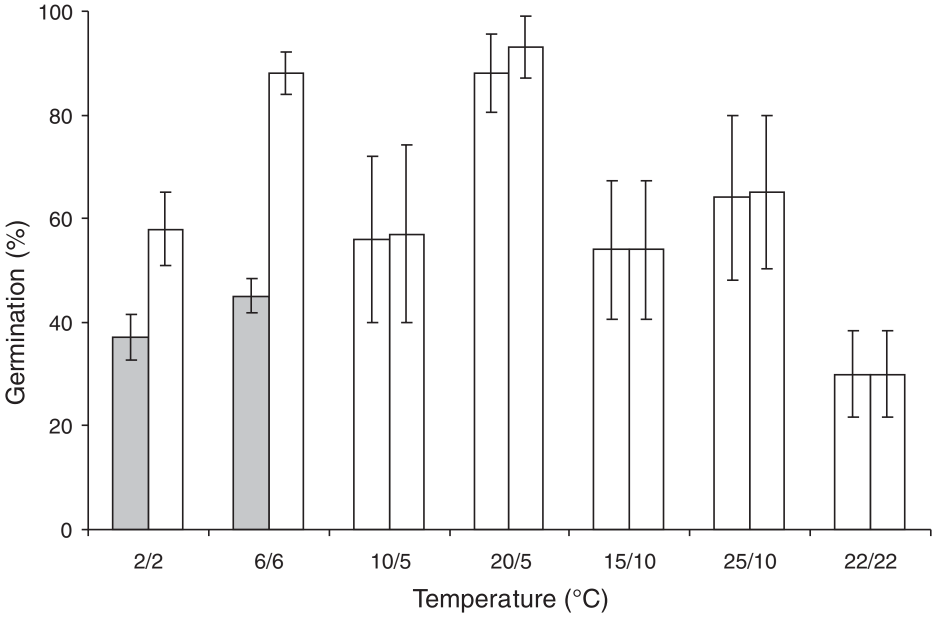

Fig. 2. – Germination percentage of *Heracleum mantegazzianum* fresh seeds (mean ± standard error) for two germination periods of 2 and 6 months. Shaded vs. transparent bars indicate significant differences within the temperature regimes (day/nigh temperatures in °C). Statistics are given in the text.

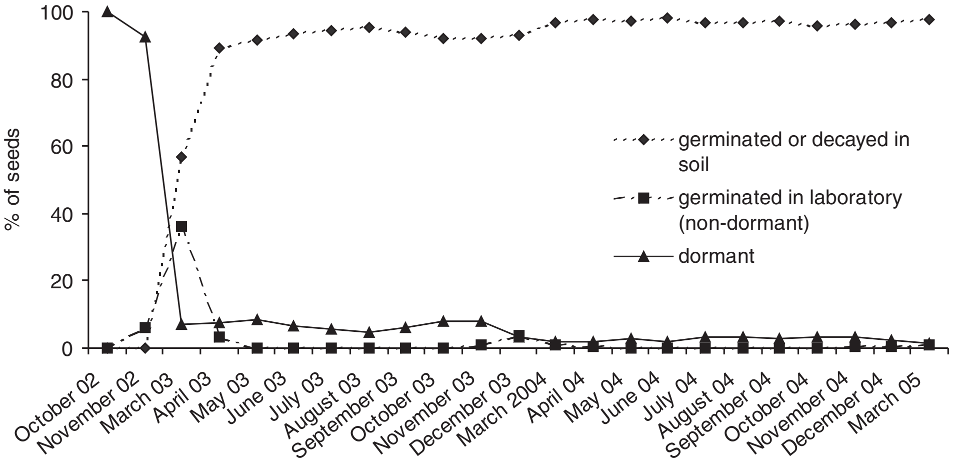

Fig. 3. – Seasonal pattern of seed bank depletion studied over two germination seasons. Changes in the percentage of dormant and nondormant seeds of the total number buried at the experimental garden of the Institute of Botany, Průhonice, Czech Republic, in autumn 2002 are shown. Each number is a mean of 10 replicates.

Seed bank depletion and the seasonal timing of germination in an experimental garden

The seasonal pattern of seed bank depletion over two germination seasons is illustrated in Fig. 3. Of the seeds buried in autumn 2002, 91.4% (range 76–98%) germinated or decayed by May and 91.8% (76–98%) by October of the following year. Over the winter 2003/2004, the proportion of germinated or decayed seeds increased to 97.4% (88–99%) in May 2004. In May and October 2003, dormant seeds made up 8.4% (2–24%) and 8.2%, respectively, of the total. A year later, there were 2.6% (0–7%) of dormant seeds in May 2004 and 3.3% (0–6%) in October 2004. Non-dormant seeds were present in the soil early in the spring or at the end of autumn (see Fig. 3). Numbers of non-dormant seeds that germinated in the laboratory after they were exhumed from the soil is closely related to the mean air temperature at the respective months. Visual inspection of the relationship indicates that non-dormant seeds start to germinate approximately 2 months after air temperature decreased below 10 °C (Fig. 4).

Long-term survival of buried seeds

Of the seeds buried in different regions of the Czech Republic on average 8.8% survived 1 year, 2.7% 2 years and 1.2% 3 years (Table 1). The differences among survival of the buried seeds among years were significant, and significantly varied among different regions of the Czech Republic (Table 4). However, as indicated by insignificant multiple regression of germination on altitude and climatic factors of the examined localities, the differences in survival of buried seeds were not explicable by these factors. As the analysis did not reveal significant results, the data are not presented here.

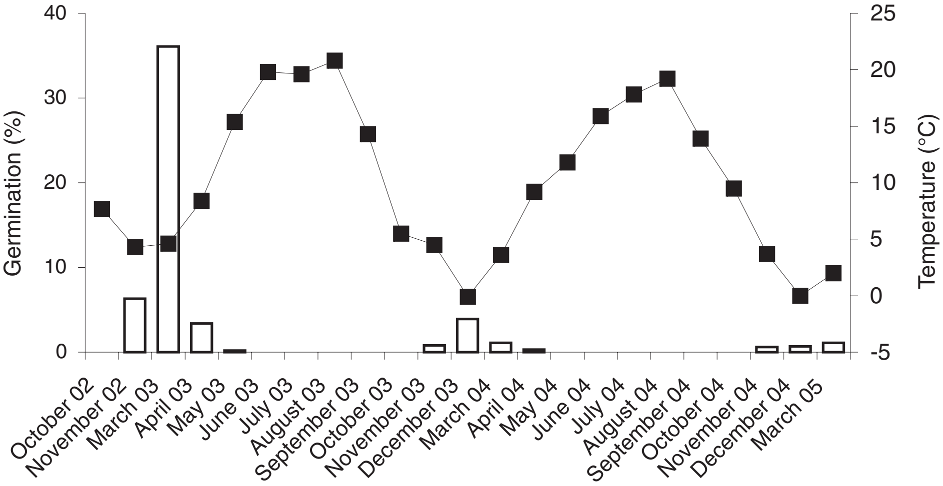

Fig. 4. – Percentage of non-dormant seeds (bars) of *Heracleum mantegazzianum* that germinated in the laboratory at 10/5°C temperature regime. Seeds were tested for germination immediately after the removal from the soil at particular sampling days. The pattern recorded over two germination seasons is related to the mean air temperature (solid squares) in the months when seeds were tested for germination.

Table 4. – Mixed model ANOVA on the differences in percentage of viable seeds (angular transformed percentages) of *Heracleum mantegazzianum* among different dates of exhumation (October 2003, 2004 and 2005) of buried seeds (fixed factor) at 10 different localities of the Czech Republic (random factor).

Source of variation	df	SS	MS	F	P
Date of exhumation	2	0.5804	0.29020	7.02	0.006
Locality	9	1.006	0.11178	17.50	< 0.0001
Date × locality	18	0.7443	0.04135	6.47	< 0.0001
Error	120	0.76659	0.006388		

Discussion

Breaking of dormancy and the course of germination over the year

Results of our study make it possible to outline the seasonal pattern of seed germination in *Heracleum mantegazzianum*. Seeds of this species at the experimental garden in central Bohemia and at field sites in the western part of the Czech Republic, 170 km apart (Krinke et al. 2005) start to germinate in early spring (March) and germination continues until late April or early May. No seedlings or germinating seeds were recorded later on. The next germination event occurs in spring of the following year, after another cold autumn and winter period. Although seeds in the field do not germinate in the autumn, they do so if tested in the laboratory. This indicates that unsuitable autumn conditions are the reason for lack of field germination. Buried seeds exhumed from soil start to germinate in the laboratory approximately 2 months after the outside air temperature has dropped below 10 °C

(Fig. 4). None of the buried seeds was capable of germination after they were exhumed in late spring, summer and early autumn, when the day temperatures were above 10 °C. Thus, although stratified seeds of *H. mantegazzianum* are able to germinate at a broad range of temperatures (Fig. 1), germination ceases if they are exposed to temperatures higher than 6 °C for a longer time (up to 2 months) and another cold period is needed before they can germinate (Fig. 4). It appears that seeds that have not germinated or decayed in spring (ca 9% of those buried in previous autumn) re-enter or retain dormancy during the high summer temperatures and this dormancy is broken again during the following cold autumn and winter (Fig. 3). It is difficult to decide whether seeds in the soil go through annual dormancy/nondormancy cycles which are reported for many annuals (Baskin & Baskin 1998) and also some perennials. Conditional dormancy/nondormancy cycles are reported for perennial *Rumex obtusifolius* (*Polygonaceae*; Van Assche & Vanlerberghe 1989), biennials *Verbascum blattaria*, *V. thapsus* (*Scrophulariaceae*; Baskin & Baskin 1981, Vanlerberghe & Van Assche 1986) and *Oenothera biennis* (*Onagraceae*; Baskin & Baskin 1991), while real dormancy/nondormancy cycles are reported for perennials such as *Lychnis flos-cuculi* (*Caryophyllaceae*; Milberg 1994a), *Primula veris* (*Primulaceae*) and *Trollius europaeus* (*Ranunculaceae*; Milberg 1994b), *Rhexia mariana* var. *interior* (*Melastomataceae*; Baskin et al. 1999a) or *Carex* ssp. (Schütz 1998). In *Heracleum mantegazzianum* the question of dormancy/nondormancy cycles seems to be more complex. In our burial experiments, the majority of seeds found dormant after 1 year in the soil had a morphologically fully developed embryo, which indicates that they are only physiologically dormant; the morphological dormancy must have been broken after first winter. However, some buried seeds with underdeveloped or partially developed embryo that retained morphological (or morphophysiological) dormancy were also found. Two winter annual species of *Apiaceae* are known to have MPD and dormancy cycles: *Chaerophyllum tainturieri* (Baskin & Baskin 1990) and *Ptilimnium nuttallii* (Baskin et al. 1999b). Further study is needed to determine whether the seeds of *H. mantegazzianum* go through annual dormancy/nondormancy cycles. It seems more likely that the dormancy of seeds in *H. mantegazzianum* is not broken completely in the first spring. Thus dormancy breaking happens gradually and this process can take place only in the months with sufficiently cold temperatures. This would explain why the seeds that remain dormant in soil do not germinate until the next spring. The small numbers of seeds (about 1%) surviving dormant in the soil for at least three years (Table 1) suggests that in a small fraction of seeds the dormancy-breaking processes can take quite a long time and that the threshold is gradually achieved through accumulation of active temperatures during cold months.

All results are consistent in suggesting that MPD in *Heracleum mantegazzianum* seeds is broken by cold stratification only. The processes of embryo growth and breaking of physiological dormancy both occur during the cold autumn and winter period when temperature is below 10 ºC. To classify the type of MPD sensu Baskin and Baskin (2004) would require more detailed information about growth of the embryo and its response to gibberellic acid stimulation. Nonetheless, our results support the findings of Nikolaeva et al. (1985), who classified the type of dormancy of *H. mantegazzianum* as a deep complex MPD (sensu Baskin & Baskin 2004). The same type of MPD was found in another species of *Apiaceae*, e.g. *Heracleum sphondylium* (Stokes 1952a,b, Nikolaeva et al. 1985) or *Anthriscus sylvestris* (Lhotská 1978, Baskin et al. 2000).

Survival of seeds in the soil

Depletion of the seed bank during the first winter is very fast, but a small proportion of dormant seed survived for one (8.4%) or two (2.6%) winters (Fig. 3). Very similar results were obtained with seeds buried at 10 sites in the Czech Republic: 8.8% of seeds survived 1 year, 2.7% 2 years and 1.2% remained viable and dormant after 3 years. Although the rate of seed decomposition was not taken into account, these results suggest that the pattern of long-term seed survival in the soil found in our studies is generally valid for Central Europe. The long-term burial experiment continues in order to determine the maximum persistence of seeds of *H. mantegazzianum* in the soil (L. Moravcová, unpublished). Nevertheless, the results obtained so far allow to conclude that seeds of *H. mantegazzianum* do not persist in the soil for as long as reported by Lundström (1989). Species with seeds persisting in the soil for at least 1 year, but less than 5 years, form a short-term persistent seed bank (sensu Thompson et al. 1997). This type of seed bank is indicated in *H. mantegazzianum* (Krinke et al. 2005). In previous studies, this species was considered to have a transient soil seed bank, i.e. missing from the seed bank or present only in the surface layer (Thompson et al. 1997). Also, Tiley et al. (1996) note that the seed biology of *H. mantegazzianum* is very similar to that of the native *H. sphondylium* in that once seeds are adequately chilled, they germinate quite well at 5 °C in the dark – a persistent seed bank is thus unlikely. Further indirect evidence that seed longevity is less than previously thought was provided by Andersen and Calov (1996), who sampled the soil beneath a stand of *H. mantegazzianum* where seed production was eliminated by sheep grazing. They found no viable seeds 7 years after the elimination of seed production. Although the results of individual authors may partly reflect the variation due to different climatic conditions, the presumption of Lundström (1989) that seeds remain viable for 15 years seems very unlikely in the light of the results presented here. Moreover, it is not clear whether Lundström (1989) was referring to dry seeds or to seeds in the soil. Our experiments demonstrate that dry-stored seeds are also able to germinate at least 38 months after the collection but their germination dramatically decreases compared to fresh seeds.

Implication of seed longevity for the invasion

The role of the seed bank in plant invasions is rarely studied, presumably because it requires a long-term commitment. Although previous reports on very long-term survival of *H. mantegazzianum* seeds in the soil are not confirmed by the present study, there are few examples in the literature illustrating that subtle, short-term differences in the release of seeds from seed bank can contribute to the invasiveness of alien species. The evidence comes from the comparisons of invasive aliens with their native congeners in the invaded regions. In the W USA, alien *Agropyron desertorum* (*Poaceae*) is reported, in contrast to native *Agropyron spicatum*, to form a seed bank persisting for longer that 1 year (Pyke 1986). In the NE USA, the seed bank of the invasive *Polygonum perfoliatum* (*Polygonaceae*) persisted to at least the third year, while the seeds of the native *Polygonum sagittatum* had lost their viability by then (Van Clef & Stiles 2001). Other studies report a larger seed bank in invasive aliens compared to native congeners or confamilials. This has been documented for invasive *Senecio madagascariensis* and native *S. lautus* (*Asteraceae*) in Australia (Radford & Cousens 2000) and for invasive *Banksia ericifolia* and native *Leucadendron laureolum* (*Proteaceae*) in South Africa (Honig et al. 1992). Larger seed banks of aliens with extended germination period do not operate alone but are usually associated with other features related to reproduction, e.g. higher fecundity, extended period of seed release, more efficient dispersal (Pyke 1990, Honig et al. 1992), longer flowering period, high seed germination and higher survival of seedlings (Radford & Cousens 2000). Although these studies did not find, in terms of seed bank characteristics, significant difference between other pairs of native and alien congeners (Radford & Cousens 2000, Van Clef & Stiles 2001), they provide a strong hint that ability to extend germination from seed bank over at least several years may be an important co-determinant of a successful invasion. The bet-hedging ability (sensu Venable 1985) of *H. mantegazzianum* increases the invasion potential of this species by spreading the germination over time and minimizing the risk of total failure of population recruitment from seeds.

Finally, a note needs to be made on the ecological relevance of the absolute number of seeds found to survive in the soil over several years. Our study shows that 1.2% of seeds that enter the seed bank during the autumn seed release remain viable after 3 years: These seeds represent the potential for re-invasion of sites subjected to the control of *Heracleum mantegazzianum*, which is especially relevant since preventing populations from seed production is one of the convenient control measures (Pyšek et al. 2006). In the study sites in the western part of the Czech Republic, the total number of seeds present in the soil in autumn was 6719 ± 4119/m^2 (mean from seven sites ± S.D.) (Krinke et al. 2005). This implies that after three years, there are on average 80.6 (0.012 × 6719) viable seeds per m^2 in the soil. Given the high germination of *H. mantegazzianum* seeds (on average 91% in the laboratory study of Moravcová et al. 2005), ability to self and high number of seeds produced by individual plants (Perglová et al. 2006), it is evident that even a single plant can start re-invasion. As such, it will remain difficult to control by usual measures.

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Souhrn

V práci byl studován vliv různých teplotních režimů a suchého skladování na klíčení semen *Heracleum mantegazzianum* (*Apiaceae*, původní na Kavkaze) v laboratorních podmínkách. Získané výsledky byly propojeny s výsledky studia sezónního vyčerpávání půdní banky na pokusném pozemku a v terénu. Semena byla sebrána na 7 lokalitách ve Slavkovském lese a stratifikována po dobu 2 měsíců v chladných a vlhkých podmínkách, aby byla překonána morfofyziologická dormance. Poté bylo studováno klíčení v 7 teplotních režimech; ve všech klíčila čerstvá semena průkazně více než semena starší, skladovaná 1, 2 a 3 roky v suchu. Semena ve všech skladovacích režimech klíčila nejlépe při střídavém teplotních režimu den/noc: 20/5 °C. Vliv doby klíčení se významně projevil pouze při nízkých stálých teplotách 2 a 6 °C, kdy procento vyklíčených semen mezi druhým a šestým měsícem klíčení průkazně vzrostlo. Sezónní klíčení semen v půdě vykazovalo zřetelný trend; převážná většina semen zakopaných na podzim byla vyčerpána do následujícího jara. Nedormantní semena byla v půdě přítomna pouze na

jaře a pozdě na podzim, díky nepříznivým podzimním klimatickým podmínkám však klíčila pouze na jaře. Vyšší letní teploty bránily dalšímu odbourávání dormance, a proto bylo k přerušení dormance nevyklíčených semen nutné další období chladu (nejméně 2 měsíce s teplotou nižší než 10 °C). Získané výsledky ukázaly, že: (1) Dormance semen *H. mantegazzianum* nebyla zcela odstraněna v průběhu chladného období prvního podzimu a zimy, takže určitá část semen buď zůstává dormantní, nebo do dormance znovu vstupuje v období vyšších letních teplot. (2) Proces odbourávání dormance probíhá postupně pouze v průběhu chladných a vlhkých období a u malého procenta semen může trvat i několik let. Ze semen, která byla zakopána na 10 různých lokalitách v České republice, přežilo v průměru 8.8 % semen 1 rok, 2.7 % 2 roky a 1.2 % semen zůstalo živých a dormantních po dobu tří let od zakopání. Schopnost malého procenta semen přežít v půdě nejméně 3 roky vede k tvorbě půdní banky semen, která představuje potenciál k obnově populací *H. mantegazzianum* na místech, kde již došlo k jejich likvidaci.

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