

SEED RAIN AND SEED PERSISTENCE OF *CALAMAGROSTIS EPIGEJOS* (L.) ROTH IN EXTREME ECOTOXICOLOGICAL CONDITIONS AT AN ABANDONED ORE-WASHERY SEDIMENTATION BASIN

PETR DOSTÁL^{1*} AND PAVEL KOVÁŘ²

¹*Institute of Botany ASCR, Zámek 1, CZ-252 43 Průhonice, Czech Republic*

²*Department of Botany, Faculty of Sciences, Charles University, Benátská 2, CZ-128 01 Praha 2, Czech Republic*

Received: 14th November 2013, **Accepted:** 17th December 2013

ABSTRACT

Clonal plants are very often among the first species to establish during primary succession. They may rapidly capture available space due to efficient vegetative propagation, but the question arises whether they may also rely on seed bank or seed rain during expansion and recovery after a possible disturbance. This question becomes increasingly important in extreme conditions of industrial deposit investigated in this study. We explored the two aforementioned seed sources in a clonal grass *Calamagrostis epigejos* within an ecotoxicologically stressed environment - abandoned tailings containment (former sedimentation basin) - from manganese-ore mining. Density of seeds found in seed rain was 617 seeds m⁻², and in soil seed bank 220 seeds m⁻², based on an autumn survey. Soil seed pool was almost depleted until the following spring as only 13 seeds m⁻² were present. This pattern contrasted with the capacity of *C. epigejos* to build a persistent soil seed bank as shown by a burial experiment. Seed rain was thus identified to be the sole reliable seed source that may assist in recovery of this species after a possible disturbance.

Keywords: clonal plants, *Calamagrostis epigejos*, human-made habitats, abandoned tailings containment, manganese-ore-mining, ecotoxicological stress, seed bank, seed dispersal, disturbance, primary succession of vegetation

INTRODUCTION

A lack of developed top-soil and the absence seed reserves are the attributes that determine the dynamics of primary succession, at least in its early phases. Establishment of vegetation largely depends on the capacity of species to reach the colonised site and also on their tolerance to harsh site conditions (Bradshaw 1993). The large proportion of easily-dispersed species and the presence of stress-tolerant species among colonising species indicate filtering by either dispersal or environmental filters (Tsuyuzaki 1987, Wood & del Moral 1987, Rydin & Borgegård 1991, McClanahan & Wolfe 1993, Chapin 1993, Chapin *et al.* 1994, Leishman & Westoby 1994, Kirner *et al.* 2008).

With the ongoing succession, however, the importance of both filters is fading away. Site conditions are being improved by early colonisers that accumulate nutrients and ameliorate water conditions (Chapin *et al.* 1994). Such changes have positive feedback on further expansion of colonisers that spread from originally patchily-distributed stands (Yarranton & Morrison 1974). In addition they may facilitate the establishment of poorly dispersed species that ultimately reach the site (Rydin & Borgegård 1991, Brown 1997, Franks 2003). Establishing vegetation that was originally the recipient of propagules from a donor vegetation in the surroundings is taking local control over future successional dynamics (Del Moral & Bliss 1993). However, seeds produced by the established vegetation may not only speed up vegetation development at the site, but they may equally stabilise this development. As initial successional phases very often experience disturbances such as fire (McClanahan & Wolfe 1993, Štefánek *et al.* 2012) or pest outbreak (Fagan *et al.* 2005), future development may largely depend on species capacity to buffer such interferences. Re-colonisation by seeds produced at untouched patches is one way how vegetation may recover after disturbances. Alternatively, this process can be implemented by seeds from a persistent soil seed bank accumulated prior to the disturbance. If developing vegetation is not resilient to disturbances because of the weak seed sources listed above, the future course of vegetation succession may differ from the original one, determined by recurrent dependency on the outer seed sources.

Clonal plants represent the dominant component of the vegetation cover in the early phases of primary succession within human-made habitats in Central Europe (Prach & Pyšek 1994). It was hypothesised that the ability of vegetative spread helps to capture available space (Prach 1988, Prach & Pyšek 1994). Clonally connected ramets sharing resources may even spread to microhabitats within a colonised area that are too harsh for recruitment by generative propagules (e. g. Pennings & Callaway 2000). Clonal growth is, however, assumed to be traded-off for reduced investment in sexual reproduction and poor build-up of persistent soil seeds bank (Eriksson 1992) which gained some support from experimental studies (Stöcklin & Fischer 1999, Hesse *et al.* 2007). Consequently, an establishing vegetation formed predominantly by clonal plants may have a reduced capacity to recover after disturbance (Štefánek *et al.* 2004, Jiráčková & Dostál 2004).

Two modes of prevailing dispersal strategy, sexual and vegetative reproduction, in clonal plant *Calamagrostis epigeios* are described from two compared localities, both the deposits of industrial material – waste ashes from power station in the first case, and ore-waste substrate in the second case (identical locality as that one described in this article) (Bryndová & Kovář 2004). Different adaptive types of *C. epigeios* could be distinguished in this way - in the sense of phalanx strategy typical for the ore-washery sediments and querilla-type associated with the ash substrate (Lowett Doust & Lowett Doust 1982). Vegetative reproduction is often predominant and efficient in clonal plants like *C. epigeios*, which is confirmed by its type of growth in the form of extensive polycormons with long ramets in sandy-like substratum (the first locality with ash-slag deposit). Recruitment from seeds is often rare in general, however, in our waste-sediment locality derived from manganese-ore mining, the clonal grass *C. epigeios* growing in a relatively unstressed environment is able to develop only very short ramets and create compact „tufts“, colonising the space extremely slowly (salinisation of the substrate surface during dry periods is high, concentration of heavy metals is of 1-3 orders higher and pH lower, but seasonally fluctuating broadly through wet and dry periods: within the range of 3.3-8.9, when comparing the second locality with the first one, Rauch 2004). This implies the phenomenon of a genetically diverse population of *C. epigeios* and causes surprisingly

high allelic differentiation of its genotypes, and thus higher resistance of this adaptively plastic grass against selective acting of ecotoxicological stress (Kovář *et al.* 2004).

In this study we surveyed seed rain and persistent soil seed bank of *C. epigejos*, which belongs to pioneer grasses establishing during primary succession within a human-made habitat. This habitat – abandoned sedimentation basin and/or tailings containment – was created as a deposition place of ore-washery by-products during the 1970's. Deposition activities terminated ca in 1980 until mid-1990's when this study was carried out, *C. epigejos* established here as one of the dominating species. However, within the surface of the abandoned sedimentation basin there were still some parts of the basin without any vegetation in 1995, most probably because of high salt concentration (Rauch 2004) and extreme fluctuations of microclimate conditions at the surface crust of substrate (Hroudová & Zákravský 2004). We used this two patch type system to assess the re-colonisation potential of seed rain and persistent soil seed bank after a possible disturbance.

In the first part of the study, by exploring seed rain at the basin with and without vegetation, we asked whether seeds may reach the bare part of the basin (that may be viewed also as a proxy to a disturbed site) and what is the difference in the density of landing seeds between the two patch types. We further surveyed whether the density of landing seeds is correlated with the cover of *C. epigejos* in the vegetation. In the second part of the study, we explored soil seed bank (in the patch with vegetation only) on two sampling dates, after seed release from mother plants in autumn and the following spring after germination. In this experiment we asked whether some seeds may become part of the persistent soil seed pool. Similarly as in the seed rain, we also explored the relationship between the soil seed density and the vegetation cover of *C. epigejos*. And finally, by an artificial burial experiment lasting for 13 months, we wanted to test directly the species capacity to build at least a short term-persistent seed bank (*sensu* Thompson *et al.* 1997; i. e. longevity at least one year). A weak pool of persistent soil seeds may be the result of multiple processes (Murdoch & Ellis 1992). Among others, it was repeatedly shown that site conditions such as nutrient or water availability may significantly influence seed longevity (Karsen & Hilhorst 1992, Bekker *et al.* 1998). By performing the burial experiment at the sedimentation basin (at both the parts with and without vegetation) and an adjacent meadow, we wanted to distinguish between seed persistence as a species biological characteristic, from seed persistence as a result of the specificity of the colonised habitat.

Table 1: Chemical composition of the pyrite substrate from manganese-ore mining (according to Kovář 2004)

	%
S	37.00-39.00
Fe	36.00
MnO	6.00
CaO	2.70
MgO	1.30
BaO	0.25
TiO ₂	0.35
P ₂ O ₅	1.50
Zn	traces
As	0.06
Cu	0.03
Al ₂ O ₃	2.50

Table 2: Chemical composition of the floatation wastes deposited in the sedimentation basin - apart the elements shown, traces of V, Co, Cr and Pb were also found (according to Kovář 2004)

	%
Fe ²⁺	4.03
Fe ³⁺	5.40
Al ₂ O ₅	5.70
SiO ₂	37.07
CaO	5.25
MgO	2.79
P ₂ O ₅	2.98
MnO	13.03
S	5.65
TiO ₂	0.71
H ₂ O	2.00
Cu	0.03
As	0.02
Ni	0.07
Na ₂ O	0.63
K ₂ O	0.48
C graphite	0.85
Loss by ignition	16.20

METHODS

Study site

Our study took place at an abandoned sedimentation basin near Chvaletice (200 m a. s. l., 50°02' N, 15°26' E). From the 1950's until the 1980's a system of three sedimentation basins serving as a deposition place of waste of pyrite-rich ore processing was set up in this area (Table 1). Two basins were engineered by piling up the top-soil and subsequent plantation, whereas the third one (area=33.5 ha, established in the 1980's) where we conducted our experiments was left to spontaneous succession (Table 2). The sediments here were characterised by high contents of sulphur (SO₄²⁻ up to 5014 mg l⁻¹), lower pH (minimum 3.7) and high conductance (up to 4.2 mS cm⁻¹), and the substrate texture was characterised by a high percentage of sandy particles and a low amount of clay as particles smaller than 0.002 mm were represented by 1.43 % (Rauch 2004).

During the vegetation survey in 1996, 35 taxa of vascular plants, 4 bryophyte species and 4 lichen species were found at the sedimentation basin (in the set of 32 relevés recorded at randomly selected plots, 25 m² each; Dostál unpubl.). The concentrations of selected elements in biomass of the organismal representatives living here are shown in Table 3 (the extreme values in some of them, e. g., Mn, Al or Zn indicate ecotoxicological exposure of the place, Kovář 2004). *C. epigejos* was shown to be one of the dominants at the basin with mean cover of 27.4 % and frequency of 94 %. Vegetation did not form a continuous cover, but was distributed in a patchy pattern, with ca 30 % of the basin area without any vegetation.

Table 3: Concentrations of elements in aboveground biomass in selected trees, grasses, mosses, lichens and fungi in abandoned sedimentation basin [mg/kg of dry mass] (according to Kovář 2004)

	Pb	Mn	Fe	Al	Zn	Cu	Cd	Ag	Hg
<i>Betula</i>	0.58	799.15	34.20	37.52	232.73	0.81	0.59	0.29	0.03
<i>pendula</i>									
<i>Populus</i>	1.49	1263.42	71.62	106.72	233.40	1.19	1.56	0.25	0.03
<i>tremula</i>									
<i>Calamagrostis</i>	1.15	1835.22	56.00	36.35	19.62	0.39	0.06	0.25	0.02
<i>epigejos</i>									
<i>Phragmites</i>	1.33	1845.66	118.80	58.56	25.63	0.58	0.06	0.25	0.02
<i>australis</i>									
<i>Ceratodon</i>	6.71	213.80	630.60	299.20	28.40	0.60	0.08	0.25	0.09
<i>purpureus</i>									
<i>Cladonia</i>	3.69	2294.10	3689.80	1053.80	34.40	2.23	0.23	0.25	0.12
<i>nemoxyna</i>									
<i>Lactarius</i>	0.23	114.57	87.80	72.32	94.25	2.44	0.17	2.11	0.62
<i>pubescens</i>									

Study species

C. epigejos is a polycarpic perennial with strong clonal growth, classified as geophyte or hemicryptophyte (Rebele & Lehmann 2001). New ramets of *C. epigejos* may produce tillers which grow both intra- and extravaginally from basal buds, however, the highest culms from tillers closely connected and shaping clumps (Bryndová & Kovář 2004) are mostly formed by intra-vaginal growth (Janczyk-Weglarska 1996, Jakrlová & Sedláková 1998). Culm height may be up to 200 cm. Rhizomes emerge from extravaginal growth; a single rhizome may elongate up to 140 cm per year (Dolečková & Osbornová 1990). Rhizomes are distributed within 0–40 cm from the surface and maximum rooting depth recorded was 200 cm (Burschel 1958). Vegetative propagation can be a very rapid and efficient way of spatial spread. Klebingat (1968) reported that 3 clones derived from seedlings covered an area of 10 m² within two years and their total rhizome length was 84.08 m.

Although a single ramet may produce up to 2142 plumed seeds, and a stand formed up to 135 ramets gives 289170 seeds m⁻² (Dolečková unpubl.) seedlings are rarely found in established vegetation (for more knowledge on the reproducing productivity see e. g. Lehmann & Rebele 1994 or Grüttner & Heinze 2003). *C. epigejos* may propagate to some degree by generative reproduction as shown by the analysis of clonal variation (Lehmann 1997). However, clonal expansion is assumed to be the predominant mode of reproduction in established vegetation. It seems most likely that recruitment from seeds occurs only in the colonisation of new open sites (Eriksson 1989, Rebele & Lehmann 2001). This feature is fulfilled in our locality in those parts of the abandoned deposit where topsoil was overburdened – it last occurred here in 1984 (Štefánek *et al.* 2012). Due very slow

expansion of the individual grass clumps, the long-term lasting (approx. 20 years) open patches of barren substrate stay in the mosaic, providing an opportunity for seed retention on disturbed surface and for seedling establishment (Kovář *et al.* 2004).

Native to Eurasia, *C. epigejos* is naturally occurring on sand dunes, river floodplains, steppes and subalpine grasslands. In recent decades its range expanded rapidly to human-maintained (pine forests) or human-made habitats, such as brown opencast mining areas or industrial wastelands (Rebele & Lehmann 2001). In their study on 15 successional seres starting on bare ground in human-made habitats, Prach & Pyšek (1999) indicated *C. epigejos* as the most successful species in terms of cover and frequency.

Seed rain monitoring

Seed input was monitored in the central part of the abandoned sedimentation basin within two habitat types – i) vegetation-covered areas and ii) bare areas with a rare occurrence of *C. epigejos* tussock-like assemblages (cover always < 1%). Eighteen seed traps, 20 m from each other, were set up along two transects 100 m apart within vegetation-covered areas. In addition, nine traps, 20 m from each other, were placed within bare areas of the basin, with average distance of ca 100 m from established vegetation. The trap trays, made of stainless metal sheet 50x50x3 cm in size, were leveled with the surrounding terrain and then filled with a mixture of sterilised peat and coarse sand on 2 May 1996. Trap trays were left exposed until 2 November 1996, when they were transported undisturbed to the laboratory and then stored for 14 days at a temperature of 7 °C to promote germination. Afterwards the trays were kept in 25°C/20°C temperature regime and corresponding light regime of 16 h light (400 W)/8 h darkness. The emerging seedlings were recorded and removed weekly for a total period 12 weeks. In the part with vegetation, the cover of *C. epigejos* was estimated in 18 plots of 5x5 m with trap trays in their centre on the day the traps were collected.

Soil seed bank survey

A 150 m transect was set up across a vegetation-covered part of the basin and 15 soil samples (each 10x10x5 cm in size), at a distance of 10 m from each other, were collected on 20 September 1996. On 30 March 1997, sampling was repeated along the same transect and 15 new soil samples of the same size were collected at positions close to the September sampling. In addition, vegetation cover of *C. epigejos* was estimated on 20 September 1996 in 15 plots of 2x2 m with sampling positions in their centre. Sampling on two different occasions was aimed at distinguishing between freshly dispersed seeds (September sampling) from those that remained non-germinated and thus could be integrated into the persistent soil seed bank (March sampling). Prior to further processing, soil samples from both sampling dates were kept refrigerated at a temperature of 7°C for 14 days to break dormancy of the present seeds. The soil was then spread onto plastic trays (each sample separately) that were then placed in a climate chamber with 25°C/20°C temperature regime and corresponding light regime of 16 h light/8 h darkness. Seedling emergence was monitored for a total period of 12 weeks and after six weeks the soil was disturbed in order to enable access to light to as many viable seeds as possible.

Seed persistence capacity

Persistence of seeds of *C. epigejos* was surveyed at three habitat types – i) at a part of the abandoned basin with vegetation, ii) at a part of the abandoned basin that was bare and iii) at an adjacent meadow approximately 1 km from the basin, dominated by *Arrhenatherum elatius*. Seeds of *C. epigejos*, collected at the sedimentation basin during September 1995,

were placed in nylon mesh bags (30 seeds per bag; mesh size=0.2 mm) that were sealed and buried at three depths (0, 5, 10 cm) in each of the above mentioned habitats. In total, 360 bags were placed in the field on 2 May 1996. In each habitat, bags were buried in 8 plots (2 x 2 m, 2 m apart from each other), 5 bags per each depth. Eight bags per each habitat type and depth (1 bag per plot and depth) were exhumed after two, four, six, ten and thirteen months. Excavated bags were then carefully opened and the contents were placed in Petri dishes. Damaged bags were discarded from further processing. After a cold stratification (14 days at a temperature of 7°C) seeds were kept in conditions as described for soil samples in the "Soil seed bank survey" for a period of 2 months. Initial seed viability used in this experiment was estimated by germination of 8 x 50 seeds, for a period of 1 month at room temperature after the cold stratification.

Statistical analyses

The relationship between seed density in traps and vegetation cover of *C. epigejos* (in the part with vegetation) was explored by Spearman's rank correlation. The difference between seed density in traps placed in vegetation and vegetation-free parts was tested by Wilcoxon rank test.

The relationship between soil seed density and vegetation cover of *C. epigejos* was explored by Spearman's rank correlation. The difference between seed density in the soil sampled in September 1996 and March 1997 was analysed by Generalised Linear Model with Poisson distribution as seeds were absent from most samples from March sampling.

Effect of habitat, burial depth and time on number of viable seeds was tested by Repeated-Measures ANOVA. Although the number of viable seeds in the mesh bags was scored just once (and the bags were not returned to the field) we assume that different sampling times are not independent; similarly we assumed dependency between different burial depths. We could not construct one model including both time and depth as repeated factor (S-Plus does not allow to do this). We therefore carried out two separate analyses: in the first one we analysed the effect of habitat, depth and their interactions and in the second one we explored the effect of habitat, time and their interactions on number (proportions) of surviving seeds. Prior to the analysis, proportions were arcsine square-root transformed to improve their normality.

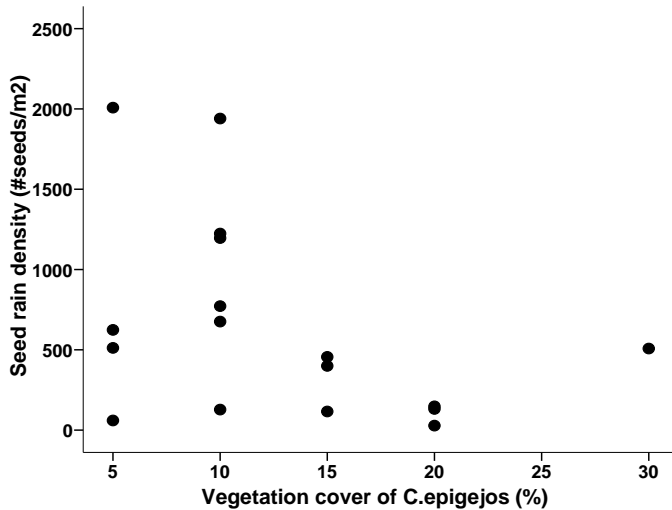
To describe seed decay over time, we applied two models to our data, log-linear (negative exponential) and log-logistic distribution models. Traditionally seed survivorship was described by the former model (Lonsdale 1989) but Rees and Long (1993) showed the latter one to be more appropriate. The negative exponential model describes number of seeds (N_t) at time t by $N_t = N_0 e^{-kt}$, where N_0 is the initial number of seeds and k is constant. Log-logistic model used e. g. by Cavieres and Arroyo (2001), describes the same process by $N_t = N_0 / (1 + (kt)^b)$, where N_0 is the initial number of seeds and k and b are constants.

After parameter estimation we further tested which of these two models better fits our data. We calculated the number of seeds surviving at respective time periods according to both models and then we regressed field-measured data against data predicted by the models. We finally chose the model that better explained variation in field-measured data (expressed by R^2).

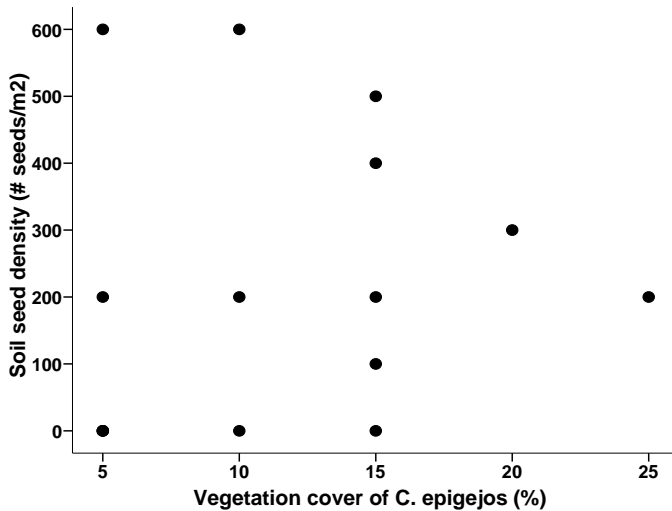
All calculations and analyses were done in S-Plus (2000).

Fig 1. Scatter plot of seed rain density (A) and soil seed density (B) of *C. epigejos* plotted against cover of *C. epigejos* in adjacent standing vegetation.

A)



B)



RESULTS

Seed rain and soil seed bank density

Mean density of seeds revealed in traps (in the part with vegetation) was 616.6 ± 609.4 seeds m^{-2} (mean \pm SD). Number of trapped seeds was negatively correlated with *C. epigejos* in standing vegetation, though this relationship was only weakly significant (Fig. 1A; $\rho = -0.44$; $z = -1.76$; $p = 0.079$). Density of seeds of *C. epigejos* trapped in the bare part of the abandoned basin was 496.9 ± 268.8 seeds m^{-2} (mean \pm SD) which was less than in the part with developed vegetation, but this difference was not significant ($Z = -1.29$; $p = 0.198$).

Density of soil seeds was 220.0 ± 217.8 seeds m^{-2} (mean \pm SD) as revealed in September 1996 sampling, but it declined dramatically to 13.3 ± 51.6 seeds m^{-2} (mean \pm SD) as found in samples from March 1997. All but one sample were without any seeds on the latter sampling term which indicated very weak seed persistence in the soil. Seed densities from September and March sampling thus differed significantly (Deviance=3318.80; d. f.=1, 28; $p < 0.001$). Standing vegetation had no effect on the size of soil seed reserves as no correlation was found between soil seed density (from September 1996) and vegetation cover of *C. epigejos* (Fig. 1B; $\rho = 0.26$; $z = 0.95$; $p = 0.34$).

Table 4: Results of repeated-measures ANOVA showing the effects of habitat, burial depth and time on seed survival of *C. epigejos*. Effects of burial depth and time were analysed by two separate models as only one factor with repeated-measurements is permitted in the analysis

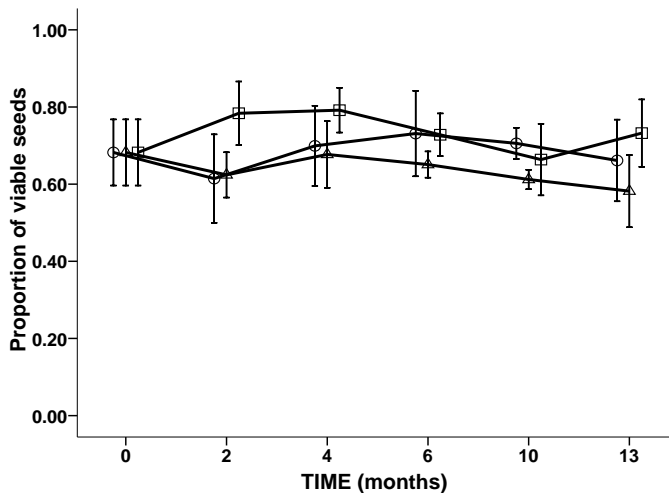
Model I			Model II		
Source of variation ¹	df	F	Source of variation ¹	df	F
1) Habitat ₂	2	30.33***	1) Habitat ₂	2	110.17***
2) Residuals	136		2) Residuals	63	
3) Depth ₅	2	11.46***	3) Time ₅	5	23.91***
4) Habitat*Depth ₅	4	8.26***	4) Habitat*Time ₅	10	12.10***
5) Residuals	269		5) Residuals	329	

***, $P < 0.001$

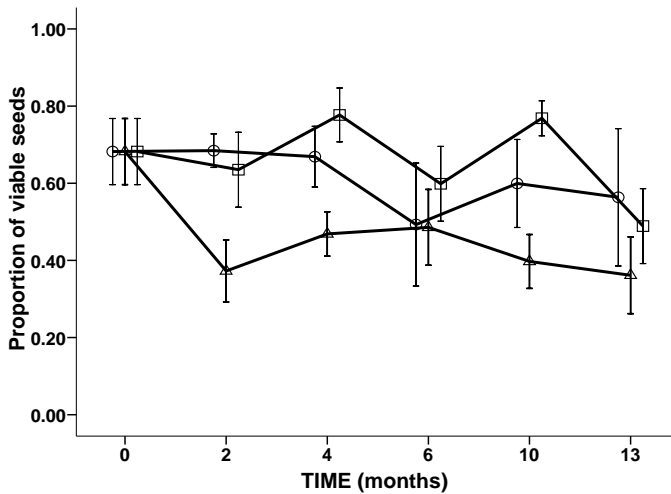
¹ Subscript number denotes the line used as denominator for the test statistics.

Fig. 2. Seed survival of *C. epigejos* in three habitats (A, sedimentation basin with vegetation; B, sedimentation basin without vegetation; C, nearby meadow) during a period of 13 months. Seeds were buried to three depths – 0 cm (circles), 5 cm (triangles) and 10 cm (squares). Means \pm SDs are shown.

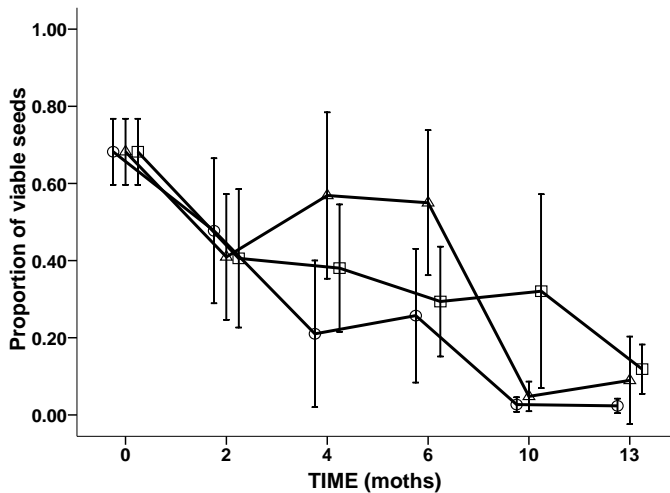
A) Abandoned sedimentation basin with vegetation



B) Abandoned sedimentation basin without vegetation



C) Meadow



Seed burial experiment

After 13 months of exposure in the field, the initial proportion of seeds ($68.21 \pm 10.76\%$ (mean \pm SD)) changed very little in the part of basin with vegetation ($65.84 \pm 14.11\%$ (mean \pm SD)), but declined to $46.73 \pm 18.61\%$ (mean \pm SD) in the bare part. The lowest seed survival was recorded in the adjacent meadow as only $7.72 \pm 11.05\%$ (mean \pm SD) viable seeds were left (Fig. 2, Table 4). Proportion of seeds recovered after 13 months was $39.83 \pm 32.77\%$, $34.42 \pm 24.81\%$ and $44.66 \pm 28.16\%$ (mean \pm SD) % at the corresponding burial depth of 0 cm, 5 cm and 10 cm respectively (Fig. 2). In pair-wise

comparisons (after sequential deletion of the third habitat type) it was shown that all three habitats differed significantly from each other in their effect on seed survival. In a similar procedure with the soil depth it was shown that seeds survived similarly in 0 cm and 5 cm layers, but significantly more seeds survived in the 10 cm layer. In a description of seed decay over time we therefore treated habitats and depths within habitats separately (0 cm and 5 cm vs. 10 cm).

Negative exponential model described seed decay better than log-logistic model in all cases. However, the fit was low (8–43 % of explained variation) and in the case of both habitat types within the sedimentation basin at 0–5 cm depth, no model could be applied ($p > 0.10$; Table 5). We further calculated how long it takes to reduce the mean seed density recorded in traps (576 seeds m^{-2}) to a half amount: it takes only 86 days at the meadow habitat (0–5 cm), but at the basin with vegetation at depth of 10 cm it takes almost two years (Table 5).

Table 5: Description of seed decay over time (t , in days) by negative exponential models at respective habitats and depths. Half-life indicates the time needed to reduce density of landing seeds (576 seeds m^{-2}) to a half amount. R^2 refers to proportion of variation explained by the model and P indicates the significance. NA, model explained too little variation and thus is not displayed

Habitat	Depth	Regression equation	Half-life (in days)	R^2	df	P
ABANDONED BASIN WITH VEGETATION	0-5 cm	NA	NA	NA	NA	NA
	10 cm	$N_t = N_0 e^{-0.0010t}$	693	0.08	1,38	0.080
ABANDONED BASIN WITHOUT VEGETATION	0-5 cm	NA	NA	NA	NA	NA
	10 cm	$N_t = N_0 e^{-0.0016t}$	433	0.08	1,38	0.080
MEADOW	0-5 cm	$N_t = N_0 e^{-0.0081t}$	86	0.43	1,78	<0.001
	10 cm	$N_t = N_0 e^{-0.0051t}$	136	0.13	1,34	0.032

DISCUSSION

We aimed to find out whether the process of colonisation of vacated patches in the industrial deposit after disturbance by seeds may rely on either seed rain or soil seed bank, or both of these sources. It was shown that mainly seed rain can serve as the reliable source of revegetation after a disturbance. Although the density of seeds found in traps located in the bare area was lower than that of traps placed in the part with vegetation, the difference was not statistically significant. Seeds may thus be dispersed far beyond the distribution of adult plants which was also shown by poor correspondence between trap seed density (and soil seed density from September 1996) and cover of *C. epigejos* (in the part of the basin with vegetation). This finding accords with the fact that *C. epigejos* was one of 35 species (the most abundant one) that successfully colonised the abandoned sedimentation basin, out

of approximately 255 species present in the closest surroundings (up to 200 m from basin's margins; Dostál unpubl.), during the first 15 years of primary succession.

Concerning the soil seed bank, the sedimentation basin's substrate was almost free of any persistent seeds as demonstrated by the analysis of samples from March 1997. This pattern was found even though the soil was supplied with numerous propagules in seed rain (found in mean density of 617 seeds m⁻² in traps) that contributed to the formation of autumn (September 1996) soil seed bank of 220 seeds m⁻². We unfortunately lack similar data on soil seed bank reserves from the part of the basin without vegetation, however there is no reason to assume a higher build-up of persistent soil seed bank there. What are, therefore, the reasons for almost complete depletion of seeds from the soil? As formulated by Murdoch and Ellis (1992), the major cause may be due to low capacity, here of *C. epigejos*, to form dormant seeds and the soil seed pool is then depleted by germination. We should consider seed quality at the same time as one reason for low germination rates, and fungal pathogens (genus *Puccinia*, *Eudarlucis caricis*) or decompositors, too (Marková 2004, Spěváková 2004, Požárová 2004). The other reason may be due to the effect of seed predation and seed pathogens (Blaney & Kotanen 2001, Dostál 2005). Among possible causes could also be the effect of site environment: we hypothesised that sedimentation substrate might have direct effect due its harsh properties on seed viability, or that it could alter dormancy dynamics (Bekker *et al.* 1998). Study of spatial correlations and/or autocorrelations in fixed plots on this toxic deposit supported this idea, when *Calamagrostis epigejos* with higher values of autocorrelation form compact structures in aboveground biomass (Kovář & Herben 2004).

The fact that some seeds were viable until the 13th month during the burial experiment indicated a capacity of the surveyed species to form at least a short-term persistent seed bank (*sensu* Thompson *et al.* 1997). We must also reject the hypothesis that the environment of sedimentation basin had a negative effect on seed persistence as the least number of viable seeds was recovered in the meadow (control) environment. It may take almost two years under the established vegetation at the sedimentation basin (at a depth of 10 cm) to reduce the density of landing seeds to half as described by the exponential model.

We assume that the poor persistent soil seed bank at the abandoned basin results from a combination of several factors. The major source of depletion of freshly-produced seeds landing to the ground is probably germination, as indicated by seedling occurrence at the basin during winter and spring periods or by seedling remnants in the buried bags (Dostál & Kovář unpubl.). At the same time formation of persistent seed pool at the basin was probably constrained by various types of biotic interactions. Firstly, as shown by Kovář *et al.* (2013) seeds of multiple species are being removed at the site by ants (*Tetramorium caespitum*, *Lasius niger*, *Formica rufibarbis*) that use seeds as a source of food and building material. Ants were shown to remove not only seeds with elaiosomes, but also propagules without such adaptation. At the same time, the substrate of the basin was shown to be very poor of soil macrofauna such as earthworms (in contrast to surface-dwelling ants and beetles; Davidová-Vilimová 2004) that would assist with seed incorporation to the deeper soil layer (e. g. Willems & Huijsmans 1994). As earthworms were missing, more seeds were lost due to the aforementioned effect of seed predators as well as due to germination. If earthworms were present, they could transport the seeds to lower soil layers where darkness and more stable temperature regimes would keep them in a state of physiological dormancy (Baskin & Baskin 1998). This was also indicated by the higher number of viable seeds recovered from the lowest layer in the seed burial experiment. In contrast, the presumable low diversity and abundance of soil pathogens at the basin did not reduce seed survival as sharply as observed at the meadow habitat. However, we did not

perform experimental manipulation of the effect of any of the above-mentioned biotic groups on seeds, and thus we cannot make a robust conclusion about their relative roles in the formation of persistent soil seed bank within the surveyed habitat.

To conclude, the importance of locally produced seeds, i. e. seed rain, in recovery after a possible disturbance would depend on the size and severity of such a disturbance (Pickett & White 1985). Re-colonisation after a small-scale disturbance by fire as simulated by Štefánek *et al.* (2012) would probably be of clonal origin due to strong vegetative propagation of the studied species. The re-colonisation by local seeds would only play a role in case of mid-scale (hundreds square metres) and severe disturbance. If some patches survive such disturbance, they may provide numerous and well-dispersed seeds enabling recovery. In case of large-scale disturbance with high severity the possible small remnants of vegetation and locally produced seeds would be of minor importance, as most seeds would arrive from the surroundings at the beginning of the succession.

ACKNOWLEDGEMENTS

This study was financially supported by grant GAČR No. 206/93/2256. It was also partly supported by grant GA AVČR no. KJB600050713 and by grant AVČR no. AV0Z60050516.

We acknowledge technical and scientific help of Anna Svárovská, Ivan Suchara, Zdena Hroudová, Petr Zákavský, Lenka Moravcová, Andrej Ivan, Zdeněk Palice, Blanka Shaw, Jiří Hadinec, Pavel Dostál and Pavel Dostál jr.

REFERENCES

- Baskin, C.C. & Baskin, J.M., (1998). *Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press.
- Bekker, R.M., Knevel, I.C., Tallowin, J.B.R., Troost, E.M.L. & Bakker, J.P., (1998). Soil nutrient input effects on seed longevity: a burial experiment with fen-meadow species. *Functional Ecology*, 12: pp. 673–682.
- Blaney, C.S. & Kotanen, P.M., (2001). Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology*, 38: pp. 1104–1113.
- Bradshaw, A.D., (1993). Introduction: Understanding the fundamentals of succession. — In: Miles, J. & Walton, D.W.H. (eds.), *Primary succession on land* (Special publication number 12 of the British ecological society), (pp. 1–4). Blackwell Scientific Publications.
- Brown, J.F., (1997). Effects of experimental burial on survival, growth, and resource allocation of three species of dune plants. *Journal of Ecology*, 85: pp. 151–158.
- Bryndová, I. & Kovář, P., (2004). Dynamics of the demographic parameters of the clonal plant *Calamagrostis epigejos* (L.) Roth in two kinds of industrial deposits (Abandoned sedimentation basins in Bukovina and Chvaletice). In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (p. 267–276). Academia, Prague.
- Burschel, P., (1958). Die Bewurzelung einiger forstlicher Bodenpflanzen. *Forst- und Jagdzeitung*, 129: pp. 89–94.

- Cavieres, L.A. & Arroyo, M.T.K., (2001). Persistent soil seed banks in *Phacelia secunda* (*Hydrophyllaceae*): experimental detection of variation along an altitudinal gradient in the Andes of central Chile (33°S). *Journal of Ecology*, 89: pp. 31–39.
- Chapin, F.S., (1993). Physiological controls over plant establishment of tropical forest assemblages, Krakatoa, Indonesia. In: Miles, J. & Walton, D.W.H, (eds.), *Primary succession on land (Special publication number 12 of the British ecological society)*, (pp. 161–178). Blackwell Scientific Publications.
- Chapin, F.S., Walker, L.R., Fastie, C.L. & Sharman, L.C., (1994). Mechanisms of primary succession following deglaciation at glacier bay, Alaska. *Ecological Monographs*, 64: pp. 149–175.
- Davidová-Vilímová J., (2004). Comparison of insect biodiversity after colonization of two different types of industrial deposits. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*. pp. 324–336 Academia, Prague.
- Del Moral, R. & Bliss, C., (1993). Mechanisms of primary succession: insights resulting from the eruption of Mount St. Helens. *Advances in Ecological Research*, 24: pp. 1–66.
- Dolečková, H. & Osbornová, J., (1990). Competitive ability and plasticity of *Calamagrostis epigejos*. *Zprávy České botanické společnosti*, 25: pp. 35–38.
- Dostál, P., (2005). Effect of three mound-building ant species on the formation of soil seed bank in mountain grassland. *Flora*, 200: pp. 148–158.
- Eriksson, O., (1989). Seedling dynamics and life histories in clonal plants. *Oikos*, 55: pp. 231–238.
- Eriksson, O., (1992). Evolution of seed dispersal and recruitment in clonal plants. *Oikos*, 63: pp. 439–448.
- Fagan, W.F., Lewis, M., Neubert, M.G., Aumann, C., Apple, J.L. & Bishop, J.G., (2005). When can herbivores slow or reverse the spread of an invading plant? A test case from Mount St. Helens. *The American Naturalist*, 166: pp. 669–685.
- Franks, S.J., (2003). Facilitation in multiple life-history stages: evidence for nucleated succession in coastal dunes. *Plant Ecology*, 168: pp. 1–11.
- Grüttner, A. & Heinze, U., (2003). Welche Bedeutung hat die sexuelle Reproduktion für den Erfolg der Art *Calamagrostis epigejos* (L.) Roth (Is sexual reproduction important to the success of *Calamagrostis epigejos* (L.) Roth). *Feddes Repertorium*, 114: pp. 240–256.
- Kovář, P. & Herben, T., (2004). Small-scale spatiotemporal dynamics of plant cover during the initial phase of primary succession in an abandoned ore-washery sedimentation basin. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 277–284). Academia, Prague.
- Hesse, E., Rees, M. & Müller-Schärer, H., (2007). Seed bank persistence of clonal weeds in contrasting habitats: implications for control. *Plant Ecology*, 190: pp. 233–243.
- Hroudová, Z. & Zákavský, P., (2004). The influence of the moss layer on soil surface microclimate in an abandoned ore-washery sedimentation basin. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 235–247). Academia, Prague.
- Jakrlová, J. & Sedláková, I., (1998). Effect of cutting on biomass production and growing parameters of the population *Calamagrostis epigejos*. In: Eliáš P. (ed.), *Plant Population Biology*, V, (pp. 78–82), SEKOS, Bratislava-Nitra, [in Czech].

- Janczyk-Weglarska, J., (1996). The strategy of *Calamagrostis epigejos* (L.) Roth individual development under ecological conditions of the valley ravine of the River Warta near Poznań. In: *Ser. Biologia*, 56, (104 pp.), Wydawnictwo Naukowe Univ. A. Mickiewicza, Poznań. [in Polish]
- Jiráčková, M. & Dostál, P., (2004). Microsite versus dispersal limitation in primary succession: a case study from an abandoned ore-washery sedimentation basin. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 59-76). Academia, Prague.
- Karssen, C.M. & Hilhorst, H.W.M., (1992). Effect of chemical environment on seed germination. In: Fenner, M. (ed.), *Seeds. The Ecology of Regeneration in Plant Communities*, (pp. 327–348). C.A.B. International, Wallingford.
- Kirmer, A., Tischew, S., Ozinga, W.A., von Lampe, M., Baasch, A. & van Groenendael, J.M., (2008). Importance of regional species pools and functional traits in colonization processes: predicting re-colonization after large-scale destruction of ecosystems. *Journal of Applied Ecology*, 45: pp. 1523–1530.
- Klebingat, G., (1968): Ein Beitrag zu Biologie forstlicher Unkräuter. *SYS-Reporter* 2: pp. 3–6.
- Kovář, P., (2004). Industrial deposits of abandoned sedimentation basins – technology of the origin and vegetation. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 15-29). Academia, Prague.
- Kovář, P., Štefánek, M. & Mrázek, J., (2011). Responses of vegetation stages with woody dominants to stress and disturbance during succession of abandoned tailings in cultural landscape. *Journal of Landscape Ecology*, 4(2): pp. 35-48.
- Kovář, P., Vojtíšek, P. & Zentsová, I., (2013). Ants as ecosystem engineers in natural restoration of human made habitats. *Journal of Landscape Ecology*, 6(1), pp. 18-31.
- Kovář, P., Štěpánek, J. & Kirschner, J., (2004). Clonal diversity of *Calamagrostis epigejos* (L.) Roth in relation to type of industrial substrate and successional stage. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 285-293). Academia, Prague.
- Lehmann, C., (1997). Clonal diversity of populations of *Calamagrostis epigejos* in relation to environmental stress and habitat heterogeneity. *Ecography*, 20: pp. 483–490.
- Lehmann, C. & Rebele, F., (1994). Zum Potential sexueller Fortpflanzung bei *Calamagrostis epigejos* (L.) Roth. *Verh. Ges. f. Ökologie Bd.*, 23: pp. 445-450.
- Leishman, M.R. & Westoby, M., (1994). The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. *Journal of Ecology*, 82: pp. 249–258.
- Lonsdale, W.M., (1988). Interpreting seed survivorship curves. *Oikos*, 52: pp. 361–364.
- Lowett Doust, L. & Lowett Doust, J., (1982). The battle strategies of plants. *New Scientist*, 95: pp. 81-84.
- Marková, K., (2004). Species diversity of parasitic fungi on plants colonizing toxic substrates. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 176-182). Academia, Prague.

- McClanahan, T.R. & Wolfe, R.W., (1993). Accelerating forest succession in a fragmented landscape – the role of birds and perches. *Conservation Biology*, 7: pp. 279–288.
- Murdoch, A.J. & Ellis, R.H., (1992). Longevity, viability and dormancy. In: Fenner, M. (ed.), *Seeds. The Ecology of Regeneration in Plant Communities*, (pp. 193–229). C.A.B. International, Wallingford.
- Pennings, S.C. & Callaway, R.M., (2000). The advantages of clonal integration under different ecological conditions: A community-wide test. *Ecology*, 81: pp. 709–716.
- Pickett, S.T.A. & White, P.S., (1985). *The Ecology of Natural Disturbance and Patch Dynamics*. Academic Press, Orlando.
- Požárová, E., (2004). Soil microfungi associated with the roots of *Calamagrostis epigejos*, an expansive plant abundant in abandoned sedimentation basin in Chvaletice. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 132–146). Academia, Prague.
- Prach, K., (1988). Životní cykly rostlin ve vztahu k časovým změnám populací a společenstev. *Preslia*, 60: pp. 23–40.
- Prach, K. & Pyšek, P., (1994). Clonal plants – what is their role in succession. *Folia Geobotanica et Phytotaxonomica*, 29: pp. 307–320.
- Prach, K. & Pyšek, P., (1999). How do species dominating in succession differ from others? *Journal of Vegetation Science*, 10: pp. 383–392.
- Rauch, O., (2004). Genesis and characteristics of orewaste sulphate soils at Chvaletice. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 45–58). Academia, Prague.
- Rebele, F. & Lehmann, C., (2001). Biological flora of Central Europe: *Calamagrostis epigejos* (L.). Roth. *Flora*, 196: pp. 325–344.
- Rees, M. & Long, M.J., (1993). The analysis and interpretation of seedling recruitment curves. *The American Naturalist*, 141: pp. 233–262.
- Rydin, H. & Borgegård, S.O., (1991). Plant characteristics over a century of primary succession on islands: Lake Hjälmaren. *Ecology*, 72: pp. 1089–1101.
- Spěváková, K., (2004). Degradation of plant litter and cellulose by microscopic fungi in an abandoned sedimentation basin. In: Kovář P. (ed.): *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*, (pp. 147–152). Academia, Prague.
- Štefánek, M., Kovář, P. & Dlouhá, V., (2012). Role of fire episode, leaf litter decomposition and mulching effects in restoration of the surface soil crust microecosystem on abandoned tailings containment. *Journal of Landscape Ecology*, 5(3): pp. 57–69.
- Stöcklin, J. & Fischer, M., (1999). Plants with longer-lived seeds have in grassland remnants 1950–1985. *Oecologia*, 120: pp. 539–543.
- Thompson, K., Bakker, J.P. & Bekker, R.M., (1997). *The soil seed banks of north west Europe: Methodology, density and longevity*. Cambridge, Cambridge University Press.
- Tsuyuzaki, S., (1987). Origin of plants recovering on the volcano Usu, northern Japan, since the eruption of 1977 and 1978. *Vegetatio*, 73: pp. 53–58.
- Willems, H.H. & Huijsmans, K.G.A., (1994). Vertical seed dispersal by earthworms – a quantitative approach. *Ecography*, 17: pp. 124–130.
- Wood, D.M. & del Moral, R., (1987). Mechanisms of early primary succession in subalpine

habitats on Mount St. Helens. *Ecology*, 68: pp. 780–790.

Yarranton, G.A. & Morrison, R.G., (1974). Spatial dynamics of primary succession: nucleation. *Journal of Ecology*, 62: pp. 417–428.