

Experimental assessment of dispersal and habitat limitation in an oligotrophic wet meadow

Alena Vítová · Jan Lepš

Received: 28 June 2010 / Accepted: 27 January 2011 / Published online: 18 February 2011
© Springer Science+Business Media B.V. 2011

Abstract The ability of a non-resident species to establish a viable population when sown or transplanted into a target community is often considered as indirect evidence that its absence in the community is caused by dispersal limitation. We evaluated the importance of dispersal and habitat limitation in an oligotrophic wet meadow community by a sowing experiment where seeds of three resident and three non-resident dicotyledonous species were added to the target community, into disturbed (litter and moss removal by raking) and undisturbed plots. The sowing experiment was supplemented with a transplant experiment, to overcome possible seedling failure in early life stages. Recruitment and survival of seedlings and transplants were monitored for 5 years. Disturbance increased recruitment, but decreased survival, in both resident and non-resident species. Recruitment was slightly better in the non-resident species, while survival was slightly better in

the resident species. Three species reached the reproductive stage at the end of the experiment, one resident and two non-resident. The two non-resident species would probably be able to establish viable populations and consequently their absence can be caused by dispersal limitation. On the contrary, one of the non-resident species was not able to overcome the seedling state, and this inability can cause its absence. Two of resident species failed to establish themselves; this failure to establish is probably related to the prevailing vegetative propagation of these species. This shows that the failure to establish is not sufficient evidence that a species is habitat limited.

Keywords Habitat limitation · Seedling recruitment · Species pool · Sowing experiment · Transplant experiment

A. Vítová (✉) · J. Lepš
Department of Botany, Faculty of Science, University
of South Bohemia, Branišovská 31, 370 05 České
Budějovice, Czech Republic
e-mail: alena.vitova@prf.jcu.cz

J. Lepš
e-mail: suspa@prf.jcu.cz

J. Lepš
Institute of Entomology, Biology Center, Academy
of Sciences of the Czech Republic, Branišovská 31, 370
05 České Budějovice, Czech Republic

Introduction

Classical niche theory suggests that species composition of plant communities is determined by environmental conditions, which limit the ability of species to survive in a site, and which also determine the biotic interactions in the community; of these, competition is the most important restriction of species ability to live in a site (Tilman 1982). This

suggests only limited variation in community composition in sites with similar ecological conditions. On the contrary, several studies have shown that some species are absent from sites apparently suitable for them (Foster and Tilman 2003; Tilman 1997; Turnbull et al. 2000), so from sites where they are limited neither by abiotic conditions nor by competition.

It is generally accepted that species composition is shaped by the regional species pool, dispersal limitation and local conditions including both abiotic conditions and biotic interactions (Zobel 1997). If a species occurs in a regional species pool, its absence in a community is considered to be caused either (1) by low species potential to disperse or (2) the inability of the species to establish and survive in the community (or both). (1) Dispersal limitation means that a non-resident species can establish in a not yet occupied site, but its propagules are not able to reach the site (Dalling et al. 2002; Tilman 1994; Zobel et al. 2006). (2) Habitat or niche limitation signifies that the species cannot establish in the target community because of unsuitable abiotic conditions or negative biotic interactions (Moore and Elmendorf 2006).

At small spatial scales, community composition is influenced mainly by seed availability (Dalling et al. 2002; Kolb et al. 2007; Poschlod and Biewer 2005), gap abundance for germination and establishment (Eriksson and Ehrlén 1992; Zobel et al. 2000) and species ability to disperse to such sites (Pärtel and Zobel 2007, Primack and Miao 1992, Zobel and Kalamees 2005; Zobel et al. 2005). To weight the relative contribution of these factors for the assembly of communities, sowing experiments where seeds of resident or non-resident species of a target community are added are often used. The establishment of non-resident species is often considered to be indirect evidence of their dispersal limitation, whereas failure to establish is an evidence of negative biotic interactions or abiotic factors, i.e., habitat limitation (Dupré and Ehrlén 2002; Foster and Tilman 2003; Turnbull et al. 2000). It is expected that resident species have their regeneration niche in the community and neither abiotic nor biotic conditions restrict their establishment and survival—consequently, they should be able to establish and grow in the community. Thus, the behavior of non-resident species can be compared to the success of resident species. For

the non-resident species, the existence of a regeneration niche cannot be assumed a priori; successful establishment of their seedlings in a sowing experiment suggests that their regeneration niche exists in the community. However, the existence of regeneration niche is necessary, but not sufficient condition for the ability of species to establish as a viable population in a community: the species should also have a potential to grow. Seeds of many species can germinate, but they often cannot establish a self-perpetuating viable population. Such populations can survive in a community for several years in an immature stage until they die out (Foster and Tilman 2003; Klimeš 2005; Moles and Westoby 2002; Primack and Miao 1992), or some of them can even reach the reproductive state. If we are able to construct a matrix model for the newly establishing population (Caswell 2001), dispersal limitation can be evidenced by population ability to increase, i.e., $\lambda > 1$. With respect to interannual variability (Zobel and Kalamees 2005), several years are required to account for it, and a relatively high number of individuals to reliably estimate the transition probabilities—consequently reliable estimates of λ are usually not available.

In many cases, establishment from seed is a rare event and depends on certain types of disturbance. This way it is also interesting to evaluate whether the inability to form a viable population is only due to the inability to establish from seeds (with the possibility that once established, the population can survive for a very long period, e.g., by vegetative propagation), or whether also established plants would be competitively excluded from the community. The seedlings establishment is often limited to specific “safe sites”, often gaps in the matrix of established vegetation or variously disturbed places (e.g., in meadows the positive effect of moss and litter removal has been demonstrated by Špačková and Lepš 2004).

We aim here to evaluate the potential of dispersal and habitat limitation to influence a species composition of rich meadow community. To achieve our aims, we designed a sowing experiment, where the behavior of selected non-resident species from the local species pool was compared with selected resident species. To prevent premature conclusions, we followed the fate of sown plots for 5 years. To account for possible microsite limitation, we compared the behavior of undisturbed plots with moderately disturbed plots. And

finally, to separate the limitation on seedling establishment from the inability of grown plants to survive in the site, the sowing experiment was compared with a transplant experiment. Last but not least, by evaluation of the resident species (which are not habitat limited by definition), we wanted to test the potential for false declaration of habitat limitation on the basis of sowing experiments.

Materials and methods

Study site

The experiments were conducted at an oligotrophic wet species-rich meadow 10 km southeast of České Budějovice, Czech Republic (48°57' N, 14°36' E, 510 m a.s.l.). Mean annual temperature is 7.8°C, and mean annual precipitation is 620 mm (České Budějovice Meteorological Station, 9 km from the plots). The experimental plots were established in a homogeneous part of the meadow characterized as *Molinietum caeruleae* (Molinion) with some elements of the *Violion caninae* according to phytosociological classification. The meadow is surrounded on three sides by forest, while the fourth side borders a field. It has been mown twice a year; the experimental plots were mown once a year with a sickle at the beginning of August (at height 3–5 cm), therefore it involved seedlings and transplants of monitored species taller than this.

Study species

The locality is used for experiment for more than a decade (e.g., Špačková et al. 1998; Špačková and Lepš 2004; Pálková and Lepš 2008), so its species composition is well known. In addition, we have compiled phytosociological relevés from similar sites in the surroundings of the target community to (to 1 km) to characterize regional species pool. On the basis of these data, we chose three species resident at the site (*Lathyrus pratensis*, *Lysimachia vulgaris*, *Plantago lanceolata*) and three species occurring in surrounding communities but not at the target one (*Campanula patula*, *Lotus corniculatus*, *Solidago virgaurea*, non-resident species hereafter). Each group contains one legume species with rather large seeds (even though *Lathyrus* has ten times

larger seeds than *Lotus*). We did not manage to form analogous pairs from other species (Table 1); we selected dicots which are sufficiently common in the target community (residents) and in surrounding meadows. Resident species had frequency in 1 m² plots over 70% but rather low biomass (less than 0.1% of total community biomass), non-resident species were abundant at least in some places close to the experimental site.

Seed sowing experiment

In autumn 2004, we established permanent plots, 0.5 m × 0.5 m in size, in five blocks separated by 1 m² in a relatively homogeneous part of the meadow. Blocks were double rows consisting of 14 plots, seven undisturbed plots and seven plots where moss and litter were removed by raking (factor “disturbance” hereafter) at the beginning of the experiment. This “disturbance” did not remove the established plants, but removal of moss (having originally cover up to 30 %) and litter created small gaps in the vegetation. Moreover, our previous experience from the locality (Špačková and Lepš 2004) shows that moss and litter removal increased seedling establishment. The disturbed and undisturbed plots were distributed in a checkerboard design. To each plot, 200 seeds of one species were sown close to the center of each plot (0.3 m × 0.3 m) to avoid edge effect (i.e., in total, 2,000 seeds of each species were sown). Seed addition was not repeated in following years. Each block contained two control plots, one with and one without disturbance, where no seeds were added to monitor possible germination from natural seed rain and seed bank. Plots from different blocks were often closer to each other than plots from the same block. Consequently, we considered blocks as a way to prevent clustering of plots of the same species and disturbance levels, and were not used in the analyses. Plots were mown with a sickle at the beginning of August.

The numbers of recruited individuals of the sown species were monitored regularly during season from May 2005 to June 2009. In the first year, we considered as recruited individuals of all species but *L. pratensis* seedlings with developed primary leaves. For *L. pratensis*, the only species with hypogeal germination (i.e., the only species where primary leaves are not seen), the small individuals were

Table 1 Species used in sowing and transplant experiments

Species	Residency	Life span	Guild	Rosettes	Seed weight (mg)	Seed number per shoot	Diaspore type	Dispersal agent	Clonal growth organs
<i>Campanula patula</i>	n	Bi/poly-annuals, strict monocarpic	Forb	s	0.02	14,700	Seed	–	Epigeogenous stem/rhizome, root splitter
<i>Lathyrus pratensis</i>	r	Perennials, polycarpic	Legume	e	11.65	79	Seed	Explosive mechanism, unspecialized	Hypogeogenous stem/rhizome
<i>Lotus corniculatus</i>	n	Perennials, polycarpic	Legume	e	1.2	641	Seed	Explosive mechanism, ants, unspecialized	Root splitter
<i>Lysimachia vulgaris</i>	r	Perennials, polycarpic	Forb	e	0.25	972.5	Seed	Water	Hypogeogenous stem/rhizome
<i>Plantago lanceolata</i>	r	Perennials, polycarpic	Forb	r	1.72	1,500	Seed with mucilage	Eaten by birds, Uns specialized	Epigeogenous stem/rhizome, root splitter, roots with adventitious buds
<i>Solidago virgaurea</i>	n	Perennials, polycarpic	Forb	s	0.42	2,909	Fruit with appendage (pappus)	Wind	Epigeogenous stem/rhizome

Data on life span, rosettes, and seed number per shoot originate from the Leda traitbase (Kleyer et al. 2008), data on guild and diaspore type from BioFlor (Klotz et al. 2002), data on seed weight from Šerá and Šerý (2004), data on dispersal agent from Ecoflora (Fitter and Peat 1994), data on clonal growth organs from Clo Pla (Klimešová and Klimeš). All species are hemicryptophytes. Residency: *n* non-resident, *r* resident. Rosettes: *s* semirosette, *e* ersolate, *r* rosette

considered as seedlings, based on our field experience. In the following years, all individuals of studied species were counted. In resident species, this might include some newly arriving individuals from natural seed resources—in this way, we could overestimate survivor numbers at these species. Nevertheless, the numbers of their individuals in the control (unsown) plots were negligible, so that possible overestimation was very small.

Transplant experiment

In spring 2005, seedlings of the study species were grown to an average height of 10 cm for the primary stem or to rosette development. The numbers of transplanted individuals varied, this difference was caused by variable seed germinability. We used all seedlings that presented appropriate size and were available at the planting date (*C. patula* 26 individuals, *L. pratensis* 10, *L. corniculatus* 7, *L. vulgaris* 12, *P. lanceolata* 28, *S. virgaurea* 26). All the transplants were planted into one plot, 2 m × 2 m, to a mown part of meadow just next to the sowing experiment (without any other disturbance than mowing). Individuals of all the species were evenly dispersed over the area (mixed with other species, so that no pair of individuals was closer than 15 cm from each other). Transplant survival was monitored regularly during season from May 2005 to June 2009.

Data analysis

Prior to the analyses, seedling numbers were log-transformed [$Y' = \log(Y + 1)$] to improve homoscedasticity and normality. Monitoring of seedlings of investigated resident species in control plots, i.e., without seed addition, demonstrated that their spontaneous establishment from the seed bank and seed rain was negligible and therefore was not considered in the analyses.

Seedling establishment and survival, and their response to disturbance in the sowing experiment, were analyzed by repeated measures ANOVA with two categorical factors (species and disturbance). Mean numbers of recruited individuals were calculated per plots with and without disturbance for each species.

Finally, we amended the repeated measures ANOVA model by including the factor ‘resident/

non-resident’ species. The factor ‘species’ was then hierarchically nested in the factor ‘resident/non-resident’. We used two forms, one with factor ‘species’ being fixed, and the other with it being a random effect. Taking ‘species’ as a fixed effect, we compared just the three residents with the three non-resident species used in the experiment. Two error levels are then used as in classical repeated measures ANOVA: for the “main-plots effects” (i.e., disturbance, species, residency and their appropriate interactions), the error term is the variability among the plots with the same species under the same disturbance regime. Time and all the interactions with time are tested against the residual variability. The null hypothesis for the residency is that there are no differences between the three selected resident and the three selected non-resident species. Taking ‘species’ as random effect factor, the three selected species were considered as representatives of the whole set of possible resident/non-resident species. The null hypothesis is then that there are no differences between all the species in the pools of resident and non-resident species; the selected experimental species are then considered randomly selected representatives of these pools. In this case, we tested general hypothesis about residency effect. The effect of residency must be then tested against the variability among species within the residency group (and similarly for the interactions with time). However, because of the low number of species tested, this test has rather low power.

Transplant survival was analysed using Survival analysis. The repeated measurement ANOVA was calculated in Statistica 5.5, the Survival analysis was conducted and graphs were created in Statistica 8 (StatSoft Inc. 2007).

Results

Seed sowing experiment

The seeds of all sown species germinated and survived in the meadow for some time period (Table 2). The species *P. lanceolata* (resident) and *L. corniculatus* (non-resident) exhibited the highest germination rates, while *S. virgaurea* (non-resident) and *L. pratensis* (resident) showed the worst (Fig. 1). All the species but *L. pratensis* germinated very

quickly, and showed the typical pattern (Kotorová and Lepš 1999) with the highest number of germinated seeds in the spring of the first season, with no noticeable increase in their numbers during summer and the following years.

The sharpest decrease in recruited individuals of the sown species was recorded after the first

Table 2 The results of repeated measures ANOVA, with categorical factors disturbance and species and factor time (individual observation) which is the repeated measures (i.e., within subject) factor

Effect	df effect	df error	F	P level
Disturbance	1	48	9.249	0.0038
Species	5	48	33.084	0.0000
Time	25	1200	123.566	0.0000
Disturbance × species	5	48	0.453	0.8093
Disturbance × time	25	1200	6.276	0.0000
Species × time	125	1200	5.114	0.0000
Disturbance × species × time	125	1200	0.527	0.9999

All factors are factors with fixed effect

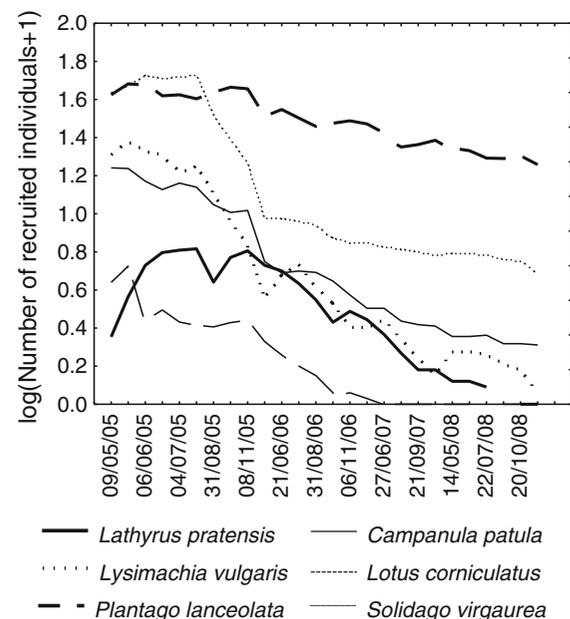


Fig. 1 Mean numbers of survivor individuals per 0.5 m × 0.5 m plot for the sown species during the 5-year monitoring period. Disturbed and undisturbed plots were pooled. Dates are in the form day/month/year. Heavy lines are used for the resident, and light line for the non-resident species

overwintering. Another significant decrease in most species was noticed after mowing in the first season (with the exception of *P. lanceolata* and *S. virgaurea*). Neither mowing nor winter season caused abrupt decrease in survivors in the following 4 years.

Individuals of all sown species but *L. pratensis* and *S. virgaurea* were recorded in the plots at the end of the five-year period (Table 4). In the course of the last 3 years, individuals of *C. patula* (3 individuals in 2008, 6 in 2009), *L. corniculatus* (2 individuals in 2007, 5 in 2008, 11 in 2009) and *P. lanceolata* (7 individuals in 2008, 6 in 2009) were flowering; no other species reached the reproductive stage.

Disturbance increased seedling germination at the beginning, and the numbers of individuals remained higher in the disturbed plots during the whole period, although the decrease of the recruited individuals was steeper there (both the main effect of disturbance and its interaction with time are significant, Table 2). Noting the logarithmic scale of the number of recruited individuals in Fig. 1, the steeper decrease in disturbed plots implies that not only a higher absolute numbers, but also higher proportions of individuals died there. The effect was very similar for all species (non-significant species × disturbance interaction). At the end of the experiment, 103 individuals of all of the species survived in all undisturbed plots and 168 in all disturbed plots out of 12,000 seeds sown (i.e., 2.25%). However, the numbers of flowering individuals in disturbed and undisturbed plots were roughly the same for the three species that have reached maturity in the course of experiment (*L. corniculatus* 10 flowering individuals in undisturbed plots and 8 in disturbed ones, *P. lanceolata* 6 in undisturbed and 7 in disturbed, *C. patula* 4 in undisturbed and 5 in disturbed).

The effect of residency and its interaction with time are only significant when species is considered as a fixed effect factor, i.e., only when we compare the six experimental species (Table 3). If the selected species were considered as representative sample of resident and non-resident species (i.e., factor species with random effect), effect of residency was inconclusive (Table 3).

Surprisingly, non-resident species seeds germinated slightly better and faster than seeds of resident species. On the other hand, survival of residents was slightly higher. Overall, 191 individuals of resident species and 80 individuals of non-resident species were alive at the end of monitoring (Figs. 2, 3).

Table 3 The results of repeated measures ANOVA, with categorical factors disturbance, species, residency and factor time (individual observation) which is the *repeated measures* (i.e., *within subject*) factor

Effect	df effect	Species fixed effect			Species random effect		
		df error	F	P level	df error	F	P level
Disturbance	1	48	9.249	0.0038	4	16.431	0.0154
Species	4	48	39.265	0.0000	48	39.266	0.0000
Residency	1	48	8.359	0.0058	4	0.213	0.6685
Time	25	1200	123.566	0.0000	100	22.483	0.0000
Disturbance × species	4	48	0.563	0.6907	48	0.563	0.6907
Disturbance × residency	1	48	0.011	0.9153	4	0.020	0.8936
Disturbance × time	25	1200	6.276	0.0000	100	11.599	0.0000
Species × time	100	1200	5.496	0.0000	1200	5.496	0.0000
Residency × time	25	1200	3.588	0.0000	100	0.653	0.8890
Disturbance × species × time	100	1200	0.541	0.9999	1200	0.541	0.9999
Disturbance × residency × time	25	1200	0.470	0.9882	100	0.868	0.6468

Factor species is hierarchically nested in factor residency, and once is considered as factor with fixed, and once with random effect

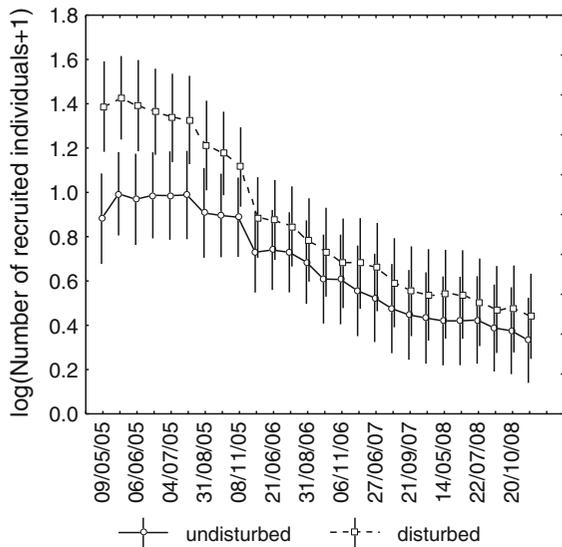


Fig. 2 Mean numbers of survivor individuals in disturbed and undisturbed plots during the 5-year monitoring period (pooled over all sown species). Dates are in the form day/month/year. Error bars are 95% confidence intervals

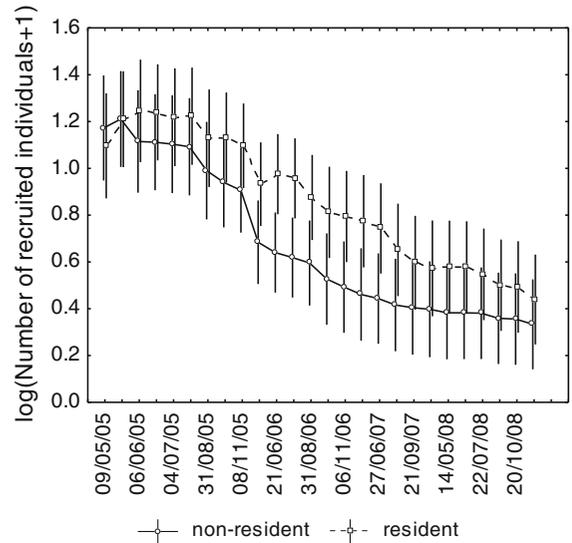


Fig. 3 Mean numbers of survivor individuals of resident and non-resident species over the 5-year monitoring period. Values are pooled over all plots. Dates are in the form day/month/year. Error bars denote 95% confidence intervals

In both undisturbed and disturbed plots, seeds of non-resident species germinated well but their seedlings started dying out earlier than resident ones. However, more individuals of non-resident species reached the reproductive stage (27 non-resident against 6 resident). It means that 0.275% of all the sown seeds produces reproductive individual in the 5 years after sowing.

Transplant experiment

Most transplanted plants established successfully in the meadow community (Table 4) and several individuals of each species were recorded over the entire 5-year monitoring period. Transplant survival differed significantly among species (survival analysis, $\chi^2 = 29.4601$, $df = 5$, $P = 0.00002$, Fig. 4). *S. virgaurea* had the highest survival rate. *L. pratensis* and

Table 4 The summarizing table of seedling and transplant survival

Species	Residency	Seedling survival		Transplant survival	
		Disturbed	Undisturbed	No. transplants	No. survivors
<i>Campanula patula</i>	n	7	19	26	12
<i>Lathyrus pratensis</i>	r	0	0	10	0
<i>Lotus corniculatus</i>	n	23	38	7	2
<i>Lysimachia vulgaris</i>	r	0	2	12	0
<i>Plantago lanceolata</i>	r	73	116	28	15
<i>Solidago virgaurea</i>	n	0	0	26	23

Residency: *n* non-resident, *r* resident

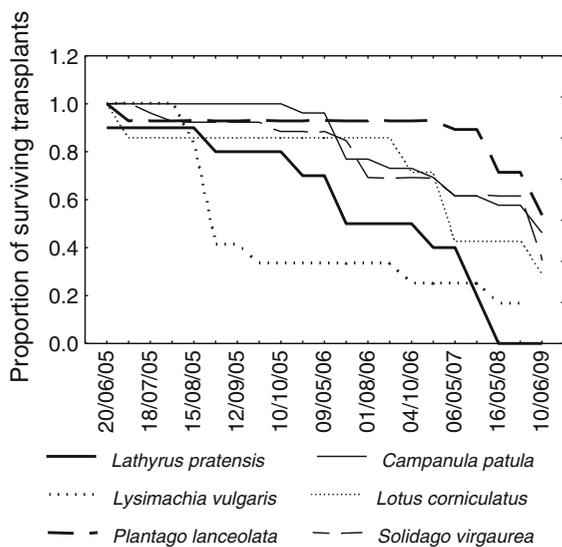


Fig. 4 Transplant survival over the five-year monitoring period. Values are the proportion of surviving individuals from the total numbers planted. Dates are in the form day/month/year

L. vulgaris survival decreased after mowing in the first season. In the course of the last three years, several individuals of *C. patula* were recorded flowering (3 individuals in 2006, 2 in 2009).

Discussion

Most sown seeds germinated in the spring of the first season and their numbers did not obviously increase since that time. The only delayed seed germination was observed in species with large seeds (*L. corniculatus* and *L. pratensis*), as suggested by Leishman (2001). The fact that seeds of non-resident species germinated better than resident species suggests that

seed germination and initial recruitment itself are not limiting for establishment of non-resident species (Ehrlén et al. 2006).

Isselstein et al. (2002) distinguished two specific stages in species recruitment—seed germination and seedling establishment, each of them needing different conditions and having species specific demands. In our case, the species response to disturbance, i.e., litter and moss removal, was similar for all of the species—germination was highest in the disturbed and survival in undisturbed plots. Small gaps created by raking provide more light, nutrients and lower competition from the surrounding vegetation (Morgan 1997), whereas individuals are more exposed to harsh conditions (more desiccation, herbivory; Foster 2001, Kolb et al. 2007, Kotorová and Lepš 1999, Morgan 1997). Germination and initial establishment are, therefore, positively affected, while survival is reduced by disturbance (Foster and Gross 1997, Foster et al. 2004, Jakobsson and Eriksson 2000, Janeček and Lepš 2005, Křenová and Lepš 1996, Marsico and Hellmann 2009). At the end of the experiment, ca. 1.5 times more surviving individuals were still found in disturbed plots; of those, 16 individuals reached reproductive stage in undisturbed plots against 17 in disturbed ones.

We suppose that the recruit mortality was caused mainly by abiotic factors or competition from the surrounding vegetation (Jakobsson and Eriksson 2000, Janeček and Lepš 2005, Münzbergová 2005) but herbivory and pathogens were also reported as important (Moles and Westoby 2004). In our case, the highest mortality was observed after the first year mowing and during the first winter. We recorded higher mortality in the disturbed plots as well; this suggests that mortality is caused mainly by harsh

environmental factors. Nevertheless, we can not exclude also the effect of herbivory: an invasive slug *Arion lusitanicus* is common at our locality and is efficient seedling grazer (Pálková and Lepš 2008). In their 11-year experiment, Ehrlén et al. (2006) found no obvious difference between establishment in unoccupied and occupied patches for most species; consequently, they concluded that habitat suitability is very probably crucial factor affecting establishment only for species with narrow niches.

In our study, the number of surviving individuals was far too low to enable a reliable estimation of transition matrix probabilities to estimate λ (the population growth rate). Nevertheless, we can ask whether the individuals established from the sown seeds are able to produce at least as many seeds as were sown (during their whole lifespan). Although it is difficult to estimate this, at least for *C. patula*, *L. corniculatus* and *P. lanceolata*, we have some individuals that are still surviving and flowering. The chance is particularly high for *C. patula*, in which there were flowering individuals in the last 2 years of the sowing experiment. Based on the database data about seed production (Table 1), we estimated that the flowering individual produced more seeds than were originally sown. Moreover, in the transplant experiment, we recorded several flowering individuals in 2006 and 2009 and many seedlings in the understorey (from flowering individuals in 2006). There are no records about it being clonal; only occasionally there may be an epigeogenous rhizome (Klimešová and Klimeš 2010). Together with the fact that the species is strictly monocarpic but without any obvious dispersal mechanism except low seed weight, it suggests that the absence of *C. patula* at the locality is probably due to dispersal limitation.

Similarly, several individuals of *L. corniculatus* in the sowing experiment achieved the reproductive stage. Plants of this species produce fairly lower numbers of seeds per individual than *C. patula*. Nevertheless, *Lotus* is a polycarpic perennial and, very likely, the individuals are able to survive at the locality for a longer time (and so, produce more seeds). Because of its heavy seeds, dispersed probably only by some explosive mechanism or myrmecochory, we can expect also dispersal limitation for this species.

Nevertheless, even for those two species, reaching the reproductive stage is not unequivocal proof of

dispersal limitation. Local extinctions can be caused by extreme events, appearing once in several years, which were not captured in our 5 year experiment. For example, extremely wet years causing the water level reaching the soil surface for several months can cause simultaneous death of many individuals, or complete germination failure, leading to local extinction (in our locality, water level was extremely high in the summer of the extremely wet year 2002). *Lotus*, a species with heavy seeds might also be limited by its ability to spread within the meadow. Our field experience (Krahulec and Lepš 1994) demonstrated that many species were able to reach a locality, establish some population there, but became extinct after several years, sometimes for unclear reasons.

No individuals of *S. virgaurea* survived till the end of the sowing experiment. On the other hand, the species showed the best survival rates in the transplant experiment, although, none of them reached the reproductive stage. Consequently, the absence in the locality might probably be caused by an inability of this species to establish at the locality from seeds. As the seeds of this species are easily dispersed and there are seeds sources close to the site, dispersal limitation can probably be excluded for this species to reach the meadow environment.

From the resident species, only the behavior of *P. lanceolata* corresponds to a viable population. The species survived best of all the sown species, and several individuals reached a reproductive state. The clear increase of this species after sowing suggests that the population size of the species is at the locality partially limited by seed availability. The remaining two resident species, *L. vulgaris* and *L. pratensis* performed very poorly. Their survival was very low, in both the sowing and transplant experiments, and none of the survivors was able to reach the reproductive state. If considered as non-resident species, such a performance would very probably be (erroneously) considered as evidence for niche limitation. However, both species are frequent in the local community (their biomass is rather low, less than 0.1% of total community biomass, but their frequency in 1 m² plots is over 70%). *L. vulgaris* is able to spread efficiently by long hypogeogenous rhizomes, which is considered to be its dominant mean of propagation; for this species, establishment from seeds is probably very rare (Fitter and Peat

1994). In our locality, seedlings of this species are rarely found, but the species has particularly strong individuals in unmown plots and from them, it sends hypogeogenous rhizomes all around. Similarly, *L. pratensis* can spread by hypogeogenous rhizomes which may spread far too, but generative and vegetative propagation is considered to be equal in this species (Klotz et al. 2002), even though vegetative regeneration is more successful (personal observation).

Also, the homogeneity of experimental plots can be one of the disadvantages for establishment and survival of some species. We have noticed that in similar habitats, *L. vulgaris* is doing best in small remnants of vegetation non-mown in individual year (which are usually not rare in similar wet meadows). By its long hypogeogenous stolons, it can support new ramets even in mown parts; this effect of clonality advantage in heterogeneous environment was experimentally demonstrated by Stuefer et al. (1996). As we have recorded high seedling mortality after the first year mowing, these remnants can be also important for seedling establishment.

Spontaneous seedling establishment of the resident species was very limited and so seed addition caused a considerable increase in seedling recruitment; this suggests seed limitation. This agrees with the results of most seed addition experiments, which found either insufficient seed availability in a target community (e.g., Ehrlén et al. 2006; Münzbergová 2005; Poschlod and Biewer 2005) or poor species dispersal (Cadotte 2006; Ehrlén et al. 2006; Foster 2001; Münzbergová 2005; Takahashi and Kamitani 2004; Vandvik and Goldberg 2005; Zobel et al. 2000, 2005). However, to say whether seed limitation is really an ecologically important factor limiting population size, we need information on the relative importance of vegetative spread and generative reproduction.

Two of the three resident species failed to establish themselves. This suggests that the inability to establish a population is not sufficient evidence of habitat limitation; very probably, many species in the community are able to survive by vegetative propagation for a long period. It seems the ability to establish is not an unequivocal evidence of dispersal limitation, and failure to establish is not unequivocal proof of habitat limitation—these are just useful leads. The species-specific factors, including ability

to cope with disturbance, interannual variability in seedling recruitment, seed size, and importance of vegetative reproduction should all be taken into account. Our experiment also demonstrated that only very small proportion of sown seeds produced a reproductive individual. The seedling success is always a probabilistic event. This suggests that the two factors, habitat and dispersal limitation are not independent of each other: the species where the “community filter” is rather strong (i.e., with low probability of establishment) will need much more seeds to arrive to the site to have chance to establish. In many cases, the species absence is caused by co-limitation of the two factors: the seed input is not sufficient to provide sufficient number of individual able to overcome the community filter.

Although not our primary aim, our results might be also used in restoration ecology where artificial seed additions of target species are often used. In accordance with Wallin et al. (2009), our results demonstrate that transplanting is much more successful and effective than seed sowing in long-term species (re)introduction to meadows.

Acknowledgments The study was financially supported by grants GACR 206/09/1471, GAJU 138/2010/P, and MSMT 6007995801. The authors are grateful for English corrections made by Keith Edwards.

References

- Cadotte MW (2006) Dispersal and species diversity: a meta-analysis. *Am Nat* 167:913–924
- Caswell H (2001) Matrix population models: construction, analysis, and interpretation. Second edition. Sinauer Associates, Sunderland
- Dalling JW, Muller-Landau HC, Wright SJ, Hubbel SP (2002) Role of dispersal in the recruitment limitation of neotropical pioneer species. *J Ecol* 90:714–727
- Dupré C, Ehrlén J (2002) Habitat configuration, species traits and plant distribution. *J Ecol* 90:796–805
- Ehrlén J, Münzbergová Z, Diekmann M, Eriksson O (2006) Long-term assesment of seed limitation in plants: results from an 11-year experiment. *J Ecol* 94:1224–1232
- Eriksson O, Ehrlén J (1992) Seed and microsite limitation of recruitment in plant populations. *Oecologia* 91:360–364
- Fitter AH, Peat HJ (1994) The ecological flora database. *J Ecol* 82:415–425. <http://www.ecoflora.co.uk>. Accessed 8 March 2010
- Foster BL (2001) Constraints on colonization and species richness along a grassland productivity gradient: the role of propagule availability. *Ecol Lett* 4:530–535

- Foster BL, Gross KL (1997) Partitioning the effects of plant biomass and litter on *Andropogon gerardi* in old-field vegetation. *Ecology* 78:2091–2104
- Foster BL, Tilman D (2003) Seed limitation and the regulation of community structure in oak savanna grassland. *J Ecol* 91:999–1007
- Foster BL, Dickson TL, Murphy CA, Karel IS, Smith VH (2004) Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. *J Ecol* 92:435–449
- Isselstein J, Tallowin JRB, Smith REN (2002) Factors affecting seed germination and seedling establishment of fen-meadow species. *Restor Ecol* 10:173–184
- Jakobsson A, Eriksson O (2000) A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos* 88:494–502
- Janeček Š, Lepš J (2005) Effect of litter, leaf cover and cover of basal internode of dominant species *Molinia caerulea* on seedling recruitment and established vegetation. *Acta Oecol* 28:141–147
- Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, Van Groenendael JM, Klimeš L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel A-K, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stauder M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E, Peco B (2008) The LEDA Traitbase: a database of life-history traits of Northwest European flora. *J Ecol* 96:1266–1274. <http://www.leda-traitbase.org/LEDAportal/>. Accessed 8 March 2010
- Klimeš L (2005) A transient expansion of sown plants and diaspore limitation. *Folia Geobot* 40:69–75
- Klimešová J, Klimeš L (2010) Clo-Pla3—database of clonal growth of plants from Central Europe. <http://clopla.butbn.cas.cz/>. Accessed 8 March 2010
- Klotz S, Kühn I, Durka W [Hrsg.] (2002) BIOLFLOR—Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. Schriftenreihe für Vegetationskunde 38. Bundesamt für Naturschutz, Bonn. <http://www.ufz.de/biolflor/index.jsp>. Accessed 8 March 2010
- Kolb A, Leimu R, Ehrlén J (2007) Environmental context influences the outcome of a plant-seed predator interaction. *Oikos* 116:864–872
- Kotorová I, Lepš J (1999) Comparative ecology of seedling recruitment in an oligotrophic wet meadow. *J Veg Sci* 10:175–186
- Krahulec F, Lepš J (1994) Establishment success of plant immigrants in a new water reservoir. *Folia Geobot Phytotax* 29:3–14
- Křenová Z, Lepš J (1996) Regeneration of *Gentiana pneumonanthe* population in an oligotrophic wet meadow. *J Veg Sci* 7:107–112
- Leishman MR (2001) Does the seed size/number trade-off model determine plant community structure? An assessment of the model mechanisms and their generality. *Oikos* 93:294–302
- Marsico TD, Hellmann JJ (2009) Dispersal limitation inferred from an experimental translocation of *Lomatium* (Apiaceae) species outside their geographic ranges. *Oikos* 118:1783–1792
- Moles AT, Westoby M (2002) Seed addition experiments are more likely to increase recruitment in larger-seeded species. *Oikos* 99:241–248
- Moles AT, Westoby M (2004) What do seedlings die from and what are the implications for evolution of seed size? *Oikos* 106:193–199
- Moore KA, Elmendorf SC (2006) Propagule vs. niche limitation: untangling the mechanisms behind plant species distributions. *Ecol Lett* 9:797–804
- Morgan JW (1997) The effect of gap size on establishment, growth and flowering of the endangered *Rutidosia leptorrhynchoidea* (Asteraceae). *J Appl Ecol* 34:566–576
- Münzbergová Z (2005) Factors limiting distribution of dry grassland species at different spatial scales. *Oikos* 113–150
- Pálková K, Lepš J (2008) Positive relationship between plant palatability and litter decomposition in meadow plants. *Comm Ecol* 9:16–26
- Pärtel M, Zobel M (2007) Dispersal limitation may result in the unimodal productivity-diversity relationship: a new explanation for a general pattern. *J Ecol* 95:90–94
- Poschlod P, Biewer H (2005) Diaspore and gap availability are limiting species richness in wet meadows. *Folia Geobot* 40:13–34
- Primack RB, Miao SL (1992) Dispersal can limit local plant distribution. *Cons Biol* 6:513–519
- Šerá B, Šerý M (2004) Number and weight of seeds and reproductive strategies of herbaceous plants. *Folia Geobot* 39:27–40
- Špačková I, Lepš J (2004) Variability of seedling recruitment under dominant, moss, and litter removal over four years. *Folia Geobot* 29:41–55
- Špačková I, Kotorová I, Lepš J (1998) Sensitivity of seedling recruitment to moss, litter and dominant removal in an oligotrophic wet meadow. *Folia Geobot* 33:17–30
- StatSoft, Inc. (2007) STATISTICA (data analysis software system), version 8.0
- Stuefer JF, de Kroon H, During HJ (1996) Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct Ecol* 10:328–334
- Takahashi K, Kamitani T (2004) Effect of dispersal capacity on forest plant migration at a landscape scale. *J Ecol* 92:778–785
- Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton
- Tilman D (1994) Competition and biodiversity in spatially structured habitats. *Ecology* 75:2–16
- Tilman D (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* 78:81–92
- Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88:225–238
- Vandvik V, Goldberg DE (2005) Distinguishing the roles of dispersal in diversity maintenance and in diversity limitation. *Folia Geobot* 40:45–52
- Wallin L, Svensson BM, Lönn M (2009) Artificial dispersal as a restoration tool in meadows: sowing or planting? *Restor Ecol* 17:270–279

- Zobel M (1997) The relative role of species pools in determining plant species richness: an alternative explanation of species coexistence? *Tr Ecol Evol* 12:266–269
- Zobel M, Kalamees R (2005) Diversity and dispersal—can the link be approached experimentally? *Folia Geobot* 40:3–11
- Zobel M, Otsus M, Liira J, Moora M, Möls T (2000) Is small-scale species richness limited by seed availability or microsite availability? *Ecology* 81:3274–3282
- Zobel M, Otsus M, Rünk K, Liira J (2005) Can long-distance dispersal shape the local and regional species pool? *Folia Geobot* 40:35–44
- Zobel M, Öpik M, Moora M, Pärtel M (2006) Biodiversity and ecosystem functioning: it is time for dispersal experiments. *J Veg Sci* 17:543–547