EFFECT OF PLANT COMMUNITY ON RECRUITMENT OF *PULSATILLA PRATENSIS* IN DRY GRASSLAND*

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The most critical life phase of many plant species is seedlings recruitment. We investigated emergence and survival of seedlings of endangered *Pulsatila pratensis* subsp. *bohemica* on dry grassland close to Prague (Czech Republic). We asked: (1) How are the field emergence and survival of seedlings affected by different plant communities? (2) Is the *in situ* seeding of local seeds a perspective method for the reinforcement of the declining population? In May 2010, we selected plots with dominant *Festuca pallens* (*Festuca* treatment), *Geranium sanguineum* accompanied by *Festuca rupicola* (*Geranium* treatment), and plots without any vegetation (Control treatment) and sewed 1350 seeds of *P. pratensis*. In October 2010, field emergence was 1.7, 2.6, and 1.3% in *Festuca*, *Geranium,* and Control treatments. In March 2011, only 4 and 3 seedlings were recorded in *Festuca* and Control treatments and no seedlings survived in *Geranium* treatment. By the end of 2011, only one seedling was alive in Control treatment and this seedling survived till October 2012. We concluded that *in situ* seeding was not an effective method for reinforcement of *P. pratensis* populations because of very low emergence rate and following survival of seedlings.

field emergence; germination; pasqueflower; steppe plant communities

INTRODUCTION

The endemic taxon *Pulsatilla pratensis* (L.) subsp. *bohemica* Skalický (Ranunculaceae, pasqueflower, thereafter referred as *P. pratensis*) occurs only in Central Europe – in Germany, Czech Republic, Slovakia, Poland, Austria, and Hungary (Skalický, 1988). In the Czech Republic, this taxon grows on low productive steppe grasslands or occurs within forest-steppe vegetation in North and Central Bohemia and in South Moravia and it belongs to endangered species (Grulich, 2012). It is a perennial herb flowering from March till May with bell-like pendulous flowers and with leafs which develop after flowering (Skalický, 1988; Jonsson et al., 1991; Widen, Lindell, 1996). The species is predominantly cross pollinated by bees and bumbles and is not completely selfcompatible (Torvik et al., 1998). New plants establish from seeds or clonally from the underground rhizome. *Pulsatilla* seeds have feathery pappus and are dispersed by wind to a distance of up to 100 m (Tackenberg, 2001). As reported by Wells, Barling (1971) successful natural recruitment of *P. pratensis* seedlings is very infrequent. Survival of *P. pratensis* seedlings can be improved by the mycorrhizal colonization (Moora et al., 2004).

In the past, low productive steppe grasslands with *P. pratensis* were used as pastures and therefore they were regularly defoliated and disturbed (Háková et al., 2004; Karlík, Řezáč, 2005; Mayerová et al., 2010). The retreat of *P. pratensis* and many other endangered species was recorded after the interruption of agricultural management followed by encroachment of dry grasslands by trees, shrubs, and tall grasses making recruitment of endangered species difficult (Matus et al., 2003; Dostálek, Frantík, 2012).

As the most critical life phase of many endangered species is recruitment of seedlings, and poor recruitment is believed to be responsible for their recent decline (Münzbergová, 2004; Poorter, 2007; Eriksson, Eriksson, 2008), it is necessary to look for methods how to reinforce populations of remaining endangered species. One method can be the *in situ* seeding of seeds collected from remaining plants directly at their localities under controlled conditions.

Under natural conditions, seeds of *Pulsatilla* species lose germination ability within two years and thus they are present only in the transient soil seed bank (Wells,

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Barling, 1971; Lhotská, Moravcová, 1989; Bakker et al., 1996; Kaligarič et al., 2006). The soil seed bank cannot be used for reestablishment of *P. pratensis* populations if fertile plants have not occurred at the locality for several years. Seeds of *Pulsatilla* species can survive viable for decades deeply frozen and they can be effectively stored in gene banks and seeded with long-term delay (Godefroid et al., 2010). Wells, Barling (1971) studied *in situ* germination of *P. vulgaris* which yielded 16% for seeds on the soil surface and 29% for seeds covered with a 3 mm layer of soil.

To test effectiveness of *in situ* seeding, we focused on a dry grassland locality in Prague (Czech Republic), where the last four living and flowering plants of *P. pratensis* were recorded in 2010. Using the seeding experiment, we studied recruitment of seedlings defined as an emergence and following survival of seedlings up to the first flowering. We asked (1) how are the field emergence and survival of seedlings affected by different plant communities and (2) whether *in situ* seeding of local seeds is a perspective method for the reinforcement of the declining population of *P. pratensis*.

MATERIAL AND METHODS

Study site

The experiment was established in April 2010, approximately 300 m NW of the Baba nature reserve (50°7´15.767˝N, 14°23´20.722˝E) on the NW edge of Prague. Altitude of the study site is 180–250 m a.s.l., mean annual temperature is 9°C, and mean annual precipitation is 500 mm. The study site is a NW slope with inclination of 20–30° with poorly developed ranker soils on Proterozoic shales (*Festuca* and Control treatments) and volcanic rock with base-rich vein (*Geranium* treatment). The steppe grassland was managed by goats grazing some 50 years ago and since that time it has been left ungrazed (Ložek, 1992; Karlík, Řezáč, 2005).

Pulsatila pratensis was a common species at the study site in the past, but in the spring of 2010 only four plants with 56 flowers were recorded. In 2010, the average number of seeds (achenes) per one fruiting flower of *P. pratensis* from the Prague's surroundings was 114 and seed germination under laboratory conditions was 35% (Jiras, 2011).

Design of the experiment

In May 2010, three plant communities for the seeding experiment were selected. We selected plots with dominant *Festuca pallens* (*Festuca* treatment), with dominant *Geranium sanguineum* accompanied by *Festuca rupicola* (*Geranium* treatment), and plots where vegetation was manually cleared (Control treatment).

Festuca treatment was slightly shaded by shrubs, *Geranium* treatment was medium-shaded by trees, and in Control treatment there was low shading from shrubs. Each treatment was nine times replicated (3 treatments × 9 replicates = 27 individual plots in total), each plot had 0.5 × 1 m in size. Local seeds of *P. pratensis* were collected in May 2010 and stored in a paper bag at room temperature before seeding. Seeds were sown into 1 cm deep hollows and covered with a 0.5 cm layer of soil to improve their germination (July 15th, 2010). Fifty seeds were sown into each individual plot (450 seeds per treatment and 1350 per the experiment). Position of each individual seed was marked by a stick (Fig. 1). Vegetation and

Table 1. Plant species composition and soil chemical properties in investigated *Festuca*, *Geranium,* and Control treatments. Phytosociological classification of investigated treatments follows Chytrý (2007).

		Festuca	*Geranium*	Control
Vegetation classification	union	*Alysso-Festucion pallentis* Moravec in Holub et al., 1967	*Festucion valesiacae* Klika 1931	early stage of succession
	association	*Seselio ossei-Festuc,um pallentis* Klika 1933 corr. Zólyomi 1966	*Festuco rupicolae-Caric,um humilis* Klika 1939	early stage of succession
Soil chemical properties	C org (g/kg)	184	48	130
	N tot (g/kg)	14.1	4.2	8.9
	C/N ratio	13.1	11.59	14.6
	P (mg/kg)	171 (very high)	7 (extremely low)	118 (high)
	K (mg/kg)	205 (good)	278 (high)	220 (good)
	Ca (mg/kg)	1115 (low to medium)	5332 (high)	1164 (low to medium)
	Mg (mg/kg)	132 (good)	289 (high)	120 (satisfactory)
	pH/H_2O	5.1 (moderate acid)	7.4 (slightly alkaline)	4.9 (moderate acid)

C org = content of organic carbon in the upper 3–8 cm soil layer, N tot = content of total nitrogen; plant available P, K, Ca, and Mg concentrations were extracted by Mehlich III reagent and obtained values evaluated according to Sáňka, Materna (2004) for productive grasslands

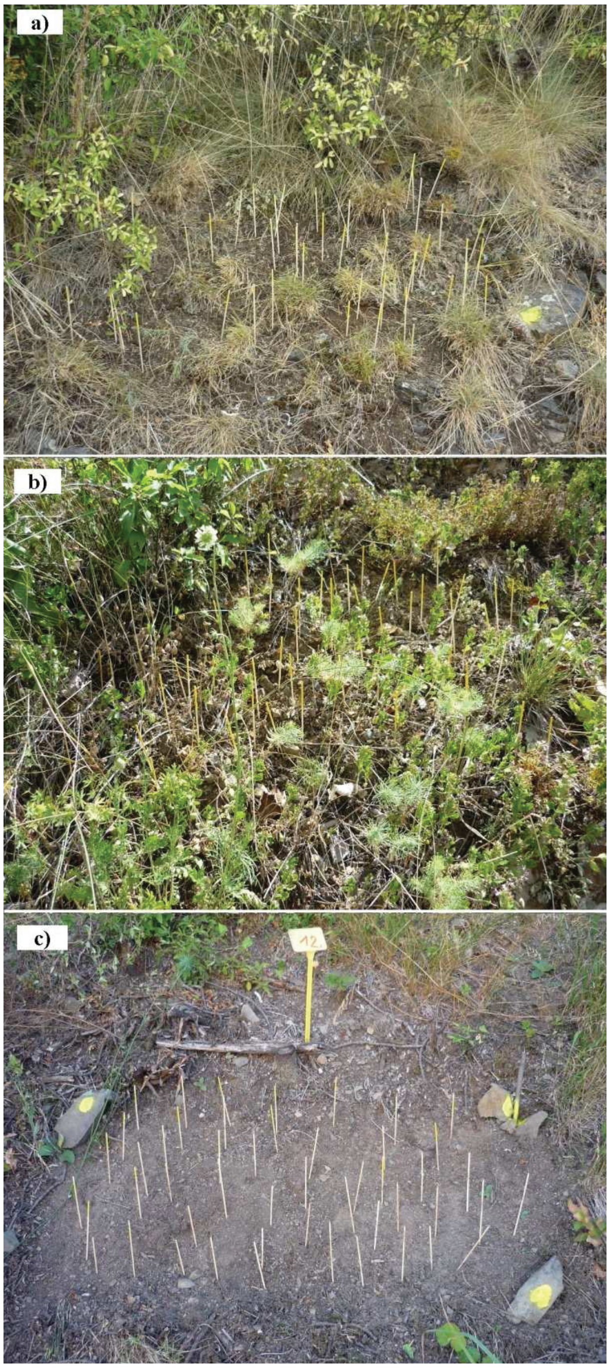

Fig. 1. Treatments: (a) *Festuca*, (b) *Geranium*, and (c) Control (photos by M. Bochenková, August 23rd, 2010). Position of each seed was marked by a plastic stick

soil chemical properties in the investigated treatments are thoroughly described in Table 1. Soil samples from nine individual plots were collected from the upper 10 cm layer and mixed to make one composite sample per each treatment.

Data collection and analysis

The number of seedlings per each plot was counted in October and November of 2010, in March and November of 2011, and in April and October of 2012. Because of the low number of the recorded seedlings any statistical tests were unfeasible, so the results were presented without statistical evaluation.

RESULTS

On October 15th, 2010 at least one seedling was recorded in three, six, and two plots from nine plots per each treatment in *Festuca*, *Geranium,* and Control treatments, respectively. In October 2010, field emergence was 1.7, 2.6, and 1.3% out of 450 sown seeds in each treatment in *Festuca*, *Geranium,* and Control. Seedling survival was high in all treatments between October and November 2010 (Fig. 2). In *Festuca* treatment, 2 seedlings out of 8 died and mortality between October and November 2010 was 25%. In *Geranium* treatment, 3 seedlings out of 12 died and mortality between October and November 2010 was 25%. In Control treatment, no mortality was recorded and all six seedlings survived.

In March 2011, only 4 and 3 seedlings were recorded in *Festuca* and Control treatments, respectively. No seedlings survived the winter in *Geranium* treatment. At the end of 2011, only one seedling was alive in Control treatment and this seedling survived also the second winter and was recorded again in April as well as in October 2012. The seedling was 3 cm high in April 2012 and it had three leaves and no flowers. The height of the seedling in October 2012 was 5 cm.

DISCUSSION

Our study showed that emergence of seedlings and their following survival were very low under the field conditions and the effect of grassland community on their survival was negligible. The field emergence lower than 3% of the used seeds in all the communities indicates that *in situ* seeding is not an effective method of production of viable seedlings particularly under limited number of available seeds. Emergence was very low although all seeds were covered with a 0.5 cm soil layer to increase their germination rate as recommended by Wells, Barling (1971). To successfully germinate, *P. pratensis* probably requires gaps without vegetation cover – typical localities of *P. pratensis* occurrence are light and disturbed sites (Uotila, 1996; Pilt, Kukk, 2002; Kalamees et al., 2012). Very low field emergence (below 3%) contrasted with 35 and 58% germination rate for *P. pratensis* recorded in laboratory conditions by Jiras (2011) and by Naumovski et al. (2009). This was because germination in the laboratory was performed in ideal conditions and also partly because the germination rate of different *Pulsatilla* species depends on the locality from which the seeds come (Wells, Barling 1971; Šedivá, 2002).

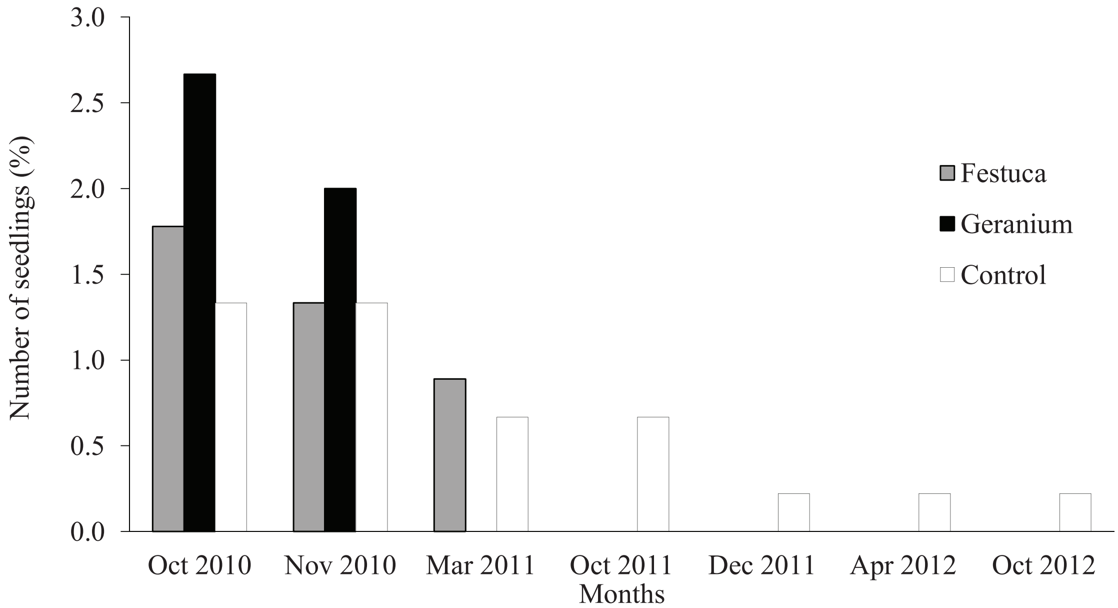

Fig. 2. Percentage of survived *Pulsatilla pratensis* seedlings from total number (450) of seeds sown into each treatment in *Festuca*, *Geranium*, and Control treatments from October 2010 till October 2012. Only one living seedling was recorded in December 2011 and again in October 2012

The higher emergence recorded in *Geranium* than in the other two treatments was probably given by better water supply under partial canopy shading and by deep soils rich in minerals. This emergence contrasted with extinction of seedlings in this community in winter, probably at least partly given by very low P availability in the soil and therefore low viability of seedlings. It seems that low vitality of *P. pratensis* seedlings and therefore their high mortality is a typical feature of this species; we have recorded substantially worse vitality and therefore survival of *P. pratensis* than of *P. grandis* seedlings in laboratory as well as in garden conditions (unpublished data of the present authors). High mortality of *P. pratensis* seedlings in our experiment during the first winter is consistent with the experience of Granér et al. (2011) for *P. patens*, but in contrast to our experiment, young seedlings of *P. patens*, which survived the winter, build up a stable population without any effect of light conditions in open, semi-open or shaded areas.

In our experiment, we recorded a certain effect of random factors on the survival of the last several seedlings which were still alive during the growing season of 2011. Seedlings exhibiting several cm in size suffered from mechanical disturbances caused by wildlife. Although wildlife activity can increase probability of emergence by creation of vegetation-free gaps, it can substantially decrease survival of seedlings. In our experiment several seedlings died because of trampling caused by wild animals. We observed also cut flowers on adult *P. pratensis* plants probably because of grazing by pheasants. We therefore recommend using exclosure cages or contemporary fencing of experimental plots to prevent the seedlings from negative effects of wildlife. In England, cages are used to protect *P. vulgaris* during the flowering (Walker, 2011).

The last seedling, which survived the second winter, was only 3 cm high in April 2012. This indicates that the transition from the seedling to the flowering plant can take a long time, more than three years in real natural conditions. This highly contrasts with ideal garden conditions where plants of *P. pratensis* flowered during the second year of their life (Bochenková, 2011). Although no exact data are available, adult plants with many flowers are probably decades-old. Very slow growth of seedlings of various plant species in steppe grasslands was recorded also by Ryser (1993). The locality was repeatedly inspected in 2012, however no new seedlings were recorded.

CONCLUSION

Finally we may conclude that *in situ* seeding was not an effective method for reinforcement of *P. pratensis* populations because of very low emergence rate and following survival of seedlings. We demonstrated that recent decline of *P. pratensis* populations in Central Europe could be caused by insufficient recruitment of seedlings. If the number of seeds is limited, producing viable juveniles in garden conditions and their following transplantation at the target locality may be more effective than *in situ* seeding. As juvenile plants can be highly damaged by wildlife, contemporary fencing or using exclosure cages seems to be an alternative measure to increase the success of *in situ* seeding.

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Vliv rostlinného společenstva na vzcházení koniklece lučního v suchém travním porostu

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Nejkritičtější fází pro přežití ohrožených druhů rostlin je vzcházení a přežívání semenáčků. V našem výzkumu jsme se zabývali vzcházením a přežíváním druhu koniklece lučního českého (*Pulsatila pratensis* subsp. *bohemica*), který se dříve hojně vyskytoval na stepních trávnících v Praze a okolí. Naše otázky byly: (1) jaká je polní vzcházivost a přežívání semenáčků v závislosti na rostlinném společenstvu? (2) je možné pomocí *in situ* výsevů místních semen posílit stávající populace koniklece? V květnu 2010 bylo založeno devět pokusných ploch s dominantním druhem *Festuca pallens* (*Festuca* varianta), devět s dominantními druhy *Geranium sanguineum* a *Festuca rupicola* (*Geranium* varianta) a devět ploch, kde byla odstraněna veškerá vegetace (kontrolní varianta). Do každé plochy bylo vyseto padesát semen (celkem 1350 semen na celý experiment). V říjnu 2010 dosáhla polní vzcházivost ve variantách *Festuca*, *Geranium* a kontrola hodnot 1.7, 2.6 a 1.3%. V březnu 2011 byly nalezeny pouze 4 semenáčky ve variantě *Festuca* a 3 ve variantě kontrola. Konce roku 2011 se dočkal pouze jediný semenáček v kontrolní variantě, který přežil i druhou zimu a byl opět zaznamenán v říjnu 2012. Metoda *in situ* výsevů je málo účinná pro posílení mizejících populací koniklece lučního českého, a to díky velmi nízké vzcházivosti a následně nízkému přežívání semenáčků.

polní vzcházivost; klíčení; koniklec luční český; stepní společenstva

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