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THE EFFECT OF NEIGHBOURING PLANT HEIGHT, DISTURBANCE LEVEL AND GAP SIZE ON SPONTANEOUS RECRUITMENT OF LARGE-SEEDED AND SMALL-SEEDED SPECIES IN *MOLINIETUM CAERULEAE* MEADOWS

ABSTRACT: The seed size is considered as a key feature, which effects both: colonizing and competitive abilities of species. The disturbance dependent small-seeded taxa are regarded as superior colonists, whereas the large-seeded taxa are considered as best competitors able to survive the competition from adjacent plants and negative effect of litter. The impact of character of standing vegetation, disturbance level and size of gaps on spontaneous recruitment of seedlings of selected light-seeded species (*Gentiana pneumonanthe* L., *Dianthus superbus* L.) and heavy-seeded taxa (*Serratula tinctoria* L., *Gladiolus imbricatus* L.) were conducted. The investigations were carried out in two areas (A and B). Both of them were consisted of three adjacent patches of *Molinietum caeruleae*: dominated by small meadow species (labeled MC), prevailed by large-tussocks grasses (labeled GR), and overgrown by willows (labeled SA). In the year 2007, in all patches, ten permanent experimental plots were randomly arranged. In Area A, plots were divided in four square-shaped, neighboring subplots subjected to: no treatment; the removal of litter and moss layers; the removal of litter, bryophytes, and above-ground parts of plants; the removal of litter, moss and the plants, as well as top soil stripping. In the Area B, plots were divided into four

subplots measuring from 0.16 m² to 0.01 m². In each of them the litter and above-ground part of plant biomass were clipped and removed. The recruitment of seedlings was monitored from 2007 through 2010. In both studied areas, regardless of seed mass, the greatest abundance of seedlings were found in patches MC, and decreased gradually in sites GR and SA. Irrespective of seed size, in subplots where plant cover and litter stayed intact no new genets was noted, moderate appearance of seedlings was observed in subplots without plant necromass, whereas the greatest number of generative progeny was found in subplots without necromass and aboveground parts of plants. The lower abundance of seedlings in subplots without litter, plants and topsoil might have been caused by depletion of seed bank reserves. Moreover, in all patches the number of seedlings of small- and large-seeded taxa increased significantly with augmentation of gap size.

In light of performed studies it might be concluded, that the disturbances might play very important role in active protection of studied taxa. The positive impact of disruption diminishes with increasing of plant canopy height. The removal of plant cover and litter contributes to the greatest seedling recruitment but its beneficial effect diminishes with decreasing of opening size.

KEY WORDS: abandoned meadow, active protection, *Dianthus superbus*, *Gentiana pneumonanthe*, *Gladiolus imbricatus*, natural recruitment, seed mass, *Serratula tinctoria*

1. INTRODUCTION

Seed mass is an essential trait, which occupies a pivotal position in regeneration strategy of species. The studies conducted in wide spectrum of habitats from old-fields (McConnaughay and Bazzaz 1987, Reader 1993), through pastures (Eriksson and Eriksson 1997) and grasslands (Ryser 1993, Burke and Grime 1996, Eriksson and Jakobsson 1998, Jakobsson and Eriksson 2000, Leishman 2001, Turnbull *et al.* 2004), to forests (van Ulft 2004) have documented that seed mass affects both colonizing and competitive abilities of species. The aforementioned investigations brought evidence, that the light-seeded taxa are superior colonists in result of substantial diaspore production and fast growth of seedlings. However, due to substantial requirements for the light availability, their recruitment in closed habitats strongly depends on disturbances in continuous plant canopy and necromass. In contrary, the large seeds contain high amounts of nutrient storage tissues, which enable the survivability under the several environmental hazards such as shading, defoliation, as well as nutrient deprivation. In result, the heavy-seeded taxa are considered as best competitors able to recruit offsprings in crowded habitats with high litter accumulation.

The review of voluminous international literature on the role of seed mass in recruitment processes showed, that the majority of findings is based on experiments involving seed supplementation (*i.e.* Moles and Westoby 2004, 2006), whereas the investigations of spontaneous establishment of offsprings are not numerous (Kern *et al.* 2013). Particularly needed are studies conducted in populations of the rare species, because they provide valuable data for successful protection programs. Given the unsatisfactory state of knowledge the present studies were undertaken to investigate the natural recruitment in populations of selected endangered small- and large-seeded species occurring in *Molinietum caeruleae* meadows with different

habitat conditions. The detailed goals focused on assessment of: (1) the impact of height of neighboring vegetation on seedling abundance, (2) the influence of disturbance level on appearance of seedlings, (3) the effect of gap size on emergence of seedlings.

I hypothesize that: (1) the number of seedlings of each species decreases with augmentation of height of standing vegetation, (2) the abundance of seedlings of small- and large-seeded species increases with growing disturbance level, (3) large-seeded species colonize small gaps, while small-seeded taxa appear in large openings.

2. STUDY SPECIES

Four species typical for wet meadows of the *Molinion* alliance (Matuszkiewicz 2012), occurring in Atlantic, Central and Eastern Europe, as well as northern and western Asia (Meusel *et al.* 1965) and belonging to plants threatened in entire Europe (APPENDIX I), have been chosen for investigation. All of selected taxa represent perennial herbs, which create inflorescences consisting of several insect-pollinated flowers and produce numerous self-sowing or wind-dispersed seeds. The main character differing selected taxa is seed mass. Kahmen and Poshold (2008a) divide seeds into two categories: small, with mass <0.5 mg, and large, 0.5–2.0 mg. In the present study, the small-seeded species are represented by *Gentiana pneumonanthe* L., with seeds weighing 0.05 mg (Simmonds 1946) and *Dianthus superbus* L., with seeds reaching 0.07 mg (Bu *et al.* 2007). The large-seeded taxa are represented by *Serratula tinctoria* L., with seeds attaining 1.74 mg (Cerabolini *et al.* 2003) and *Gladiolus imbricatus* L., with seeds achieving 1.8 mg (Kubikova and Zeidler 2011).

3. STUDY AREA

The studies were carried out in Kostrze district localized on western edge of Krakow, south of the Vistula River (southern Poland). The research area is located at ca 210 m a.s.l., on a low flood terrace of the Vistula, 3.0–6.0 m high. Most of it is covered by unmanaged *Molinietum caeruleae* patches characterized by presence of *Dianthus superbus*, *Gentiana*

pneumonanthe, *Gladiolus imbricatus* and *Serratula tinctoria* (Dubiel 1991, 1996 and personal observations). The studies were carried out in two areas (A and B). In both of them, three adjacent, abandoned *Molinietum caeruleae* patches with different species composition and habitat conditions were selected (APPENDIX II). The patches labeled MC were dominated by meadow species, creating delicate, creeping stems or small-tussocks. The small species intercepted very low amount of irradiance, contributing to strong insolation of the plot. The places labeled GR were prevailed by the large-tussock grasses, with tall-growing stems and closely packed leaves, strongly prevented the penetration of sunlight on the surface of the ground. The sites named SA were overgrown by willows, with wide leaves and densely distributed stems, which shaded the whole patch.

4. MATERIAL AND METHODS

In the year 2007, in each of above described patches ten permanent experimental plots were randomly arranged. They were established at least 2.0 meters from the border of patch to avoid edge effect. The experimental design is given by Kostrakiewicz-Gierałt (2012).

In Area A plots were divided into four, square-shaped, adjacent subplots measuring 0.09 m². The subplots were subjected:

- no treatment (subplot 1),
- low disturbance level *i.e.* the removal of litter and moss layers (subplot 2),
- medium disturbance level *i.e.* the removal of litter, bryophytes, and above-ground parts of plants (subplot 3),
- high disturbance level *i.e.* the removal of litter, moss and the plants, as well as top soil stripping to the depth 3–5 cm (subplot 4).

In the Area B plots were divided into four, square shaped, neighboring subplots, reaching with the area of 0.16 m² (subplot I), 0.09 m² (subplot II), 0.04 m² (subplot III), and 0.01 m² (subplot IV). In each of them the litter and the above-ground part of biomass were clipped and removed. The establishment of generative descendants was monitored once a week in May, June, July and August, and once every two weeks in April, September and October, from year 2007 to

year 2010. The effect of adjacent plant height, disturbance level and gap size on recruitment process was characterized by absolute, cumulative number of seedlings calculated by adding seedlings that appeared in particular plots (subplots) over the course of four years.

The statistical analysis were based on the nonparametric Kruskal-Wallis test, which was applied to examine whether there were significant differences in:

- the mean number of seedlings that appeared in permanent plots among patches in both areas;
- the mean number of seedlings that appeared in different treatment (size) subplots within each patch.

5. RESULTS

5.1. The impact of neighbouring plant height on seedling abundance

The greatest abundance of both small- and large-seeded species seedlings were found in patches MC, while it decreased gradually in sites GR and SA (Table 1). The number of seedlings of small-seeded species in places MC achieved from 11.1 to 21.1, in sites GR it ranged from 7.5 to 15.3, while in places SA it reached from 6.2 to 10.5. The abundance of seedlings of large-seeded taxa in sites MC achieved from 11.2 to 58.9, in patches GR it amounted from 9.2 to 21.0, whereas in places SA it ranged from 8.6 to 18.1. The statistical significance of differences in number of seedlings of each species noted in successive patches is shown in Table 2.

5.2. The influence of disturbance level on appearance of genets

In subplots, where plant cover and litter stayed intact (I) new genets were not noted, moderate appearance of generative offsprings was observed in subplots without necromass (II), whereas the greatest number of descendants was found in subplots without necromass and aboveground parts of plants (III). The lower seedling abundance was observed in subplots without litter, plants and topsoil (IV).

The detailed recruitment rates of the small-seeded species are given in Table 3. The

Table 1. The recruitment of seedlings of *Gentiana pneumonanthe*, *Dianthus superbus*, *Serratula tinctoria* and *Gladiolus imbricatus* recruited during whole study period in in experimental plots within established within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in Area A and Area B.

Species	Area	Patch	The average number of seedlings (±SD)
<i>Gentiana pneumonanthe</i>	A	MC	11.1 (4.3)
		GR	7.5 (5.0)
		SA	6.7 (3.6)
	B	MC	13.2 (4.5)
		GR	9.4 (5.7)
		SA	8.0 (5.0)
<i>Dianthus superbus</i>	A	MC	11.5 (4.9)
		GR	9.7 (8.7)
		SA	6.2 (4.8)
	B	MC	21.1 (2.6)
		GR	15.3 (2.1)
		SA	10.5 (3.3)
<i>Serratula tinctoria</i>	A	MC	25.6 (7.6)
		GR	21.0 (9.2)
		SA	18.1 (14.0)
	B	MC	58.9 (10.1)
		GR	31.8 (7.4)
		SA	18.7 (10.0)
<i>Gladiolus imbricatus</i>	A	MC	11.2 (1.8)
		GR	9.1 (3.7)
		SA	8.6 (5.6)
	B	MC	19.9 (5.3)
		GR	15.7 (6.9)
		SA	10.9 (5.0)

average number of *Gentiana pneumonanthe* seedlings ranged from 0.3 to 2.3 in subplots II, from 3.0 to 5.9 in subplots III, and from 2.4 to 4.9 in subplots IV. The mean number of descendants of *Dianthus superbus* reached from 0.2 to 2.8 in subplots II, from 3.8 to 5.4 in subplots III, and from 1.2 to 5.0 in subplots IV. The statistical significance of differences of seedling recruitment in particular subplots is given in Table 4.

The data of establishment of the large-seeded taxa are given in Table 5. The mean number of seedlings of *Serratula tinctoria* attained from 1.0 to 3.8 in subplots II, from 11.8 to 18.2 in subplots III, and from 2.5 to 6.4 in subplots IV. The mean number of seedlings of *Gladiolus imbricatus* reached from 1.0 to 1.2 in subplots II, from 4.0 to 7.4 in subplots III, and from 2.6 to 3.9 in subplots IV. The statistical significance of differences in seedling recruitment in particular subplots is given in Table 6.

5.3. The effect of gap size on emergence of generative progeny

Irrespective of patch character the number of seedlings of all taxa diminished gradually from the largest subplots (I), via intermediate openings (II and III), to the smallest ones (IV). The number of seedlings of the small-seeded species in particular subplots is shown in Table 7. The average abundance of generative offsprings of *Gentiana pneumonanthe* reached from 2.6 to 6.3 in the largest gaps, from 1.5 to 3.5 in the intermediate openings and from 1.1 to 1.4 in the smallest ones. The mean number of *Dianthus superbus* individuals achieved from 3.8 to 7.5 in the greatest gaps, from 2.3 to 5.3 in the medium openings and from 1.5 to 3.5 in the smallest ones. The statistical significance of differences in seedling recruitment in consecutive subplots is given in Table 8.

Also the abundance of descendants of the large-seeded taxa raised gradually with aug-

mentation of opening area (Table 9). The average recruitment rates of *Serratula tinctoria* attained from 7.2 to 24.5 in the largest gaps, from 3.6 to 18.7 in the medium openings and from 2.6 to 7.4 in the smallest ones. The mean number of descendants of *Gladiolus imbricatus* achieved from 4.4 to 7.4 in the greatest gaps, from 2.0 to 5.0 in the medium openings and from 1.8 to 3.5 in the smallest ones. The statistical significance of differences in abun-

dance of seedlings noted within successive subplots is given in Table 10.

6. DISCUSSION

6.1. The impact of neighbouring plant height on seedling abundance

Performed investigations document significant diminishing of recruitment rates of

Table 2. The statistical variability of number of seedlings of *Gentiana pneumonanthe*, *Dianthus superbus*, *Serratula tinctoria* and *Gladiolus imbricatus* recruited during whole study period in experimental plots within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in Area A and Area B. Asterisks mean that the mean number of seedlings differed significantly at $P \leq 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) ; ns-not significant differences (H Kruskal-Wallis test).

<i>Gentiana pneumonanthe</i>	The value of Kruskal-Wallis test		4.65 ^{ns}	
	Area A	The level of statistical significance of differences among sites:	MC and GR	ns
			MC and SA	ns
			GR and SA	ns
	The value of Kruskal-Wallis test		6.42 ^{ns}	
	Area B	The level of statistical significance of differences among sites:	MC and GR	ns
MC and SA			$P \leq 0.05$	
GR and SA			ns	
<i>Dianthus superbus</i>	The value of Kruskal-Wallis test		4.76 ^{ns}	
	Area A	The level of statistical significance of differences among sites:	MC and GR	ns
			MC and SA	ns
			GR and SA	ns
	The value of Kruskal-Wallis test		23.20***	
	Area B	The level of statistical significance of differences among sites:	MC and GR	$P \leq 0.05$
MC and SA			$P < 0.001$	
GR and SA			ns	
<i>Serratula tinctoria</i>	The value of Kruskal-Wallis test		5.43 ^{ns}	
	Area A	The level of statistical significance of differences among sites:	MC and GR	ns
			MC and SA	ns
			GR and SA	ns
	The value of Kruskal-Wallis test		22.43***	
	Area B	The level of statistical significance of differences among sites:	MC and GR	$P \leq 0.05$
MC and SA			$P < 0.001$	
GR and SA			ns	
<i>Gladiolus imbricatus</i>	The value of Kruskal-Wallis test		6.14 ^{ns}	
	Area A	The level of statistical significance of differences among sites:	MC and GR	ns
			MC and SA	$P \leq 0.05$
			GR and SA	ns
	The value of Kruskal-Wallis test		13.48*	
	Area B	The level of statistical significance of differences among sites:	MC and GR	ns
MC and SA			$P < 0.001$	
GR and SA			ns	

both, small- and large-seeded species along the gradient of growing shading. These results correspond well with previous observations (Kostrakiewicz 2010, 2011, Kostrakiewicz-Gierałt 2012, 2013), which showed greater recruitment of seedlings of various taxa in patches of *Molinietum caeruleae* dominated by small meadow species, than in plots prevailed by large-tussock grasses and willows. In contrary, the laboratory experiments (Grime and Jeffrey 1965, Leishman and Westoby 1994, Milberg *et al.* 2000, Pearson *et al.* 2002, Jankowska-Błaszczuk and Daws 2007), as well as the observations carried out under semi-field conditions (Wu *et al.* 2013) demonstrated that high light requirements for germination more likely characterize small- than in large-seeded taxa. Also, numerous field data have demonstrated an increase of large-seeded taxa recruitment along the gradient of rising shading. Lönnberg and Eriksson (2012) observed the weak positive correlation between seed size and recruitment

success from grasslands to forests. Bruun and ten Brink (2008) have showed, that the number of seedlings of large-seeded species increases, while the abundance of offsprings of small seeded taxa diminishes from open to closed habitats. Furthermore, the augmentation of positive relationship between seed size and seedling number along the gradient diminishing of light availability was documented in intra-specific studies (Lönnberg and Eriksson 2013).

6.2. The influence of disturbance level on appearance of genets

The obtained results, showing lack of 'recruits' in control subplots and the slight occurrence of 'recruits' in places without dead parts of plants suggest the negative effect of litter on seedling recruitment irrespective of seed mass. The unfavourable effect of necromass on seedling establishment, which has been observed previously in several productive

Table 3. The number of seedlings of small-seeded taxa *Gentiana pneumonanthe* and *Dianthus superbus* in subplots with no disturbance (1), low disturbance level (2), medium disturbance level (3) and high disturbance level (4) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007-2010.

Species	Patch	Subplot	The average number of seedlings ($\pm$ SD)
<i>Gentiana pneumonanthe</i>	MC	1	0
		2	0.3 (0.4)
		3	5.9 (3.3)
		4	4.9 (4.0)
	GR	1	0
		2	2.3 (1.8)
		3	3.0 (2.4)
		4	7.5 (5.0)
	SA	1	0
		2	1.2 (0.9)
		3	3.1 (1.7)
		4	2.4 (3.2)
<i>Dianthus superbus</i>	MC	1	0
		2	1.1 (2.3)
		3	5.4 (3.9)
		4	5.0 (2.9)
	GR	1	0
		2	2.8 (3.2)
		3	3.8 (3.5)
		4	3.1 (2.0)
	SA	1	0
		2	0.2 (0.4)
		3	4.8 (2.8)
		4	1.2 (1.6)

Table 4. The statistical variability of number of seedlings of *Gentiana pneumonanthe* and *Dianthus superbus* in subplots with no disturbance (1), low disturbance level (2), medium disturbance level (3) and high disturbance level (4) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007-2010. Asterisks mean the probability that mean number of seedlings among subplots differed significantly at $P \leq 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) ; ns-not significant differences (H Kruskal-Wallis test).

<i>Gentiana pneumonanthe</i>		The value of Kruskal-Wallis test	26.52***	
	MC	The level of statistical significance of differences among subplots	1 and 2	ns
			1 and 3	<i>P</i> <0.001
			1 and 4	<i>P</i> <0.01
			2 and 3	<i>P</i> ≤0.05
			2 and 4	ns
			3 and 4	ns
		The value of Kruskal-Wallis test	13.63*	
	GR	The level of statistical significance of differences among subplots	1 and 2	ns
			1 and 3	<i>P</i> <0.01
			1 and 4	ns
			2 and 3	ns
			2 and 4	ns
			3 and 4	ns
		The value of Kruskal-Wallis test	16.18*	
	SA	The level of statistical significance of differences among subplots	1 and 2	ns
			1 and 3	<i>P</i> <0.01
			1 and 4	ns
2 and 3			ns	
2 and 4			ns	
3 and 4			ns	
<i>Dianthus superbus</i>		The value of Kruskal-Wallis test	21.57**	
	MC	The level of statistical significance of differences among subplots	1 and 2	ns
			1 and 3	<i>P</i> ≤0.05
			1 and 4	<i>P</i> ≤0.05
			2 and 3	ns
			2 and 4	ns
			3 and 4	ns
		The value of Kruskal-Wallis test	12.10*	
	GR	The level of statistical significance of differences among subplots	1 and 2	ns
			1 and 3	<i>P</i> ≤0.05
			1 and 4	<i>P</i> ≤0.05
			2 and 3	ns
			2 and 4	ns
			3 and 4	ns
		The value of Kruskal-Wallis test	22.62***	
	SA	The level of statistical significance of differences among subplots	1 and 2	ns
			1 and 3	<i>P</i> <0.001
			1 and 4	ns
2 and 3			<i>P</i> <0.01	
2 and 4			ns	
3 and 4			ns	

Table 5. The number of seedlings of large-seeded *Serratula tinctoria* and *Gladiolus imbricatus* in subplots with no disturbance (1), low disturbance level (2), medium disturbance level (3) and high disturbance level (4) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007–2010.

Species	Patch	Subplot	The average number of seedlings ($\pm$ SD)
<i>Serratula tinctoria</i>	MC	1	0
		2	1.0 (0.8)
		3	18.2 (6.4)
		4	6.4 (4.7)
	GR	1	0
		2	0.8 (1.3)
		3	16.6 (10.3)
		4	3.6 (2.7)
	SA	1	0
		2	3.8 (4.6)
		3	11.8 (8.8)
		4	2.5 (1.8)
<i>Gladiolus imbricatus</i>	MC	1	0
		2	1.2 (1.3)
		3	7.4 (1.9)
		4	2.6 (1.9)
	GR	1	0
		2	1.0 (0.6)
		3	4.2 (2.7)
		4	3.9 (2.6)
	SA	1	0
		2	1.2 (2.4)
		3	4.0 (2.9)
		4	3.4 (4.6)

riparian plant communities (Xiong and Nilsson 1997, 1999, Xiong *et al.* 2003) and grasslands (Loydi *et al.* 2013) might be caused by diminishing of amplitude of temperature, reduction of the quality and amount of light reaching to the soil surface, isolation of seeds from available resources, attraction of predators, releasing allelochemicals, as well as fostering pathogens.

The sharp increase of seedling abundance of all studied species in effect of litter, moss and plant cover removal, might be linked with an enlargement of microhabitat suitable for seedling recruitment. In particular, the attention is drawn to the substantial number of offsprings of *Serratula tinctoria*, which might be connected with proximity of generative stems and dispersal of diaspores in short distances by wind or water (Bischoff 2002). Moreover, the chances for an increase of recruitment success might be caused by the dispersal of group of seeds connected by pappus.

Furthermore, it should be added that a rise of seedling abundance in effect of moderate disruption showed also other meadow taxa such as *Iris sibirica* and *Trollius europaeus* (Kostrakiewicz-Gierałt 2012).

The observed augmentation of number of offsprings of all studied taxa support the sowing experiments of Stammel *et al.* (2006). The aforementioned authors showed that regardless of seed mass, greater recruitment of seedlings occurred in mowing sites, than in gaps without plants and litter, as well as in abandoned plots. On the other hand Kahmen and Poshlod (2008b) argued, that mowing and grazing applied in semi-natural grasslands favor the germination of small seeds, whereas the patch abandonment contribute to the germination success of larger seeds.

Observed in presented studies diminishing number of offsprings of *Gentiana pneumonanthe*, *Dianthus superbus*, *Serratula tinctoria*

Table 6. The statistical variability of number of seedlings of *Serratula tinctoria* and *Gladiolus imbricatus* in subplots with no disturbance (1), low disturbance level (2), medium disturbance level (3) and high disturbance level (4) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007-2010. Asterisks mean the probability that mean number of seedlings among subplots differed significantly at $P \leq 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***); ns-not significant differences (H Kruskal-Wallis test).

<i>Serratula tinctoria</i>	MC	The level of statistical significance of differences among subplots	The value of Kruskal-Wallis test		32.15***
			1 and 2	ns	
			1 and 3	$P<0.001$	
			1 and 4	$P<0.01$	
			2 and 3	$P<0.001$	
			2 and 4	ns	
			3 and 4	ns	
	GR	The level of statistical significance of differences among subplots	The value of Kruskal-Wallis test		30.93***
			1 and 2	ns	
			1 and 3	$P<0.001$	
			1 and 4	$P\leq 0.05$	
			2 and 3	$P<0.001$	
			2 and 4	ns	
			3 and 4	ns	
	SA	The level of statistical significance of differences among subplots	The value of Kruskal-Wallis test		23.15***
			1 and 2	ns	
			1 and 3	$P<0.001$	
			1 and 4	ns	
			2 and 3	ns	
			2 and 4	ns	
3 and 4			ns		
<i>Gladiolus imbricatus</i>	MC	The level of statistical significance of differences among subplots	The value of Kruskal-Wallis test		28.21***
			1 and 2	ns	
			1 and 3	$P<0.01$	
			1 and 4	ns	
			2 and 3	$P<0.01$	
			2 and 4	ns	
			3 and 4	ns	
	GR	The level of statistical significance of differences among subplots	The value of Kruskal-Wallis test		21.44**
			1 and 2	ns	
			1 and 3	$P<0.01$	
			1 and 4	$P<0.01$	
			2 and 3	ns	
			2 and 4	ns	
			3 and 4	ns	
	SA	The level of statistical significance of differences among subplots	The value of Kruskal-Wallis test		15.85*
			1 and 2	ns	
			1 and 3	$P<0.01$	
			1 and 4	ns	
			2 and 3	ns	
			2 and 4	ns	
3 and 4			ns		

Table 7. The number of seedlings of small-seeded taxa *Gentiana pneumonanthe* and *Dianthus superbus* in subplots measured 0.16 m² (I), 0.09 m² (II), 0.04 m² (III), and 0.01 m² (IV) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007–2010.

Species	Patch	Subplot	The average number of seedlings ($\pm$ SD)
<i>Gentiana pneumonanthe</i>	MC	I	6.3 (2.2)
		II	3.5 (1.7)
		III	2.0 (2.1)
		IV	1.4 (0.9)
	GR	I	3.7 (1.6)
		II	3.1 (1.3)
		III	1.5 (1.3)
		IV	1.1 (1.3)
	SA	I	2.6 (1.1)
		II	2.8 (1.2)
		III	1.5 (1.3)
		IV	1.1 (1.3)
<i>Dianthus superbus</i>	MC	I	7.5 (2.1)
		II	5.3 (2.3)
		III	4.8 (0.9)
		IV	3.5 (1.2)
	GR	I	4.7 (1.3)
		II	4.0 (2.0)
		III	3.8 (1.3)
		IV	2.8 (1.8)
	SA	I	3.8 (3.0)
		II	2.6 (1.1)
		III	2.3 (0.8)
		IV	1.5 (0.9)

and *Gladiolus imbricatus* in gaps originated in effect of soil stripping might suggests the impoverishment of seed bank reserves. On the other hand numerous authors (Thompson *et al.* 1993, 1994, Thompson 2000, Bakker *et al.* 1996, Bekker *et al.* 1998) argued, that light-seeded taxa might reach great soil depth maintaining germination ability, while species with large diaspores are less likely to be incorporated into the soil. Additionally it should be pointed out, that artificial introducing of diaspores into places with bare soil increases the recruitment of seedlings of *Gentiana pneumonanthe* (Křenová and Lepš 1996) and *Dianthus superbus* (Poshold and Biewer 2005). The positive role of sod cutting and diaspore sowing on emergence of generative offsprings of numerous meadow taxa was documented by Špačková *et al.* (1998), Kotorová and Lepš (1999), whilst Hölzel and Otte (2003) considered this

treatment as an extremely successful method of restoring wet meadows.

6.3. The effect of gap size on emergence of generative progeny

The observations presented above, bringing evidence that irrespective of habitat conditions the germination success of small- and large-seeded species is greater in wide gaps than in smaller ones, are consistent with observations of colonization of openings by seedlings of *Iris sibirica* and *Trollius europaeus* (Kostrakiewicz-Gierałt 2012). Also studies conducted in heatlands (Miles 1974) and forests (Denslow 1987, Bullock 2000, Sapkota and Odén 2009) documented, that the majority of species tend to perform better in larger openings. Such phenomenon might be connected with an increase of the area in which taxa can successfully establish

Table 8. The statistical variability of number of seedlings of *Gentiana pneumonanthe* and *Dianthus superbus* in subplots measured 0.16 m² (I), 0.09 m² (II), 0.04 m² (III), and 0.01 m² (IV) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007-2010. Asterisks mean the probability that mean number of seedlings among subplots differed significantly at $P \leq 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) ; ns-not significant differences (H Kruskal-Wallis test).

<i>Gentiana pneumonanthe</i>	The value of Kruskal-Wallis test		15.36*	
	MC	The level of statistical significance of differences among subplots	I and II	ns
			I and III	$P<0.01$
			I and IV	$P<0.01$
			II and III	ns
			II and IV	ns
			III and IV	ns
	The value of Kruskal-Wallis test		13.58*	
	GR	The level of statistical significance of differences among subplots	I and II	ns
			I and III	ns
			I and IV	$P\leq 0.05$
			II and III	ns
			II and IV	ns
			III and IV	ns
	The value of Kruskal-Wallis test		8.42 ^{ns}	
	SA	The level of statistical significance of differences among subplots	I and II	ns
			I and III	$P<0.01$
			I and IV	ns
II and III			ns	
II and IV			ns	
III and IV			ns	
<i>Dianthus superbus</i>	The value of Kruskal-Wallis test		15.37*	
	MC	The level of statistical significance of differences among subplots	I and II	ns
			I and III	ns
			I and IV	$P<0.001$
			II and III	ns
			II and IV	ns
			III and IV	ns
	The value of Kruskal-Wallis test		3.98 ^{ns}	
	GR	The level of statistical significance of differences among subplots	I and II	ns
			I and III	ns
			I and IV	ns
			II and III	ns
			II and IV	ns
			III and IV	ns
	The value of Kruskal-Wallis test		1.32 ^{ns}	
	SA	The level of statistical significance of differences among subplots	I and II	ns
			I and III	ns
			I and IV	ns
II and III			ns	
II and IV			ns	
III and IV			ns	

and grow, as well as with an augmentation of level of resources available for exploitation. They asserted, that the surface of the soil in a large opening generally receives higher light intensities, and thus the soil temperature is generally higher than that in small openings. In contrast, observations made in habitats as diverse as old fields (McConnaughay and Bazzaz 1987), grasslands (Burke and Grime 1996), meadows (Kostrakiewicz 2011), as well as forests (Foster and Janson 1985, Alvarez-Buylla and Martinez-Ramos 1990, Seiwa and Kikuzawa 1996, Ellison *et al.* 1993, van Uft 2004) have brought evidence that the germination success of small-seeded species rises, while that of large-seeded species diminishes as the gap area increases. The above mentioned authors observed an increased risk of desiccation in wider gaps. Such phenomenon might be a far more important seed mortality agent for large than for smaller seeded species.

Table 9. The number of seedlings of large-seeded taxa *Serratula tinctoria* and *Gladiolus imbricatus* in subplots measured 0.16 m² (I), 0.09 m² (II), 0.04 m² (III), and 0.01 m² (IV) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007-2010.

Species	Patch	Subplot	The average number of seedlings (±SD)
<i>Serratula tinctoria</i>	MC	I	24.5 (14.4)
		II	18.7 (18.1)
		III	8.3 (2.6)
		IV	7.4 (3.3)
	GR	I	12.4 (5.1)
		II	9.9 (1.1)
		III	5.8 (4.1)
		IV	3.7 (3.1)
	SA	I	7.2 (2.6)
		II	5.3 (2.4)
		III	3.6 (2.9)
		IV	2.6 (2.5)
<i>Gladiolus imbricatus</i>	MC	I	7.4 (3.5)
		II	5.0 (3.1)
		III	4.0 (2.0)
		IV	3.5 (1.4)
	GR	I	6.2 (2.2)
		II	4.1 (2.7)
		III	2.8 (1.3)
		IV	2.6 (0.9)
	SA	I	4.4 (1.5)
		II	2.7 (2.0)
		III	2.0 (0.9)
		IV	1.8 (0.7)

7. CONCLUSIONS

Concluding, it should be pointed out, that the gap creating might be an effective way for active protection of both: small- and large-seeded taxa. The role of gaps as microhabitats suitable for seedling recruitment diminishes gradually: from the patch dominated by low-stature meadow species, *via* the place prevailed by tall grasses, to the site overgrown by willows. Regardless of character of standing vegetation, the best results brings removal of above-ground parts of plants and litter layer. Such treatment allows the reduction of competition from neighbors and the preservation of soil seed bank reserves, simultaneously. Moreover, an increase of opening area contributes to significant augmentation of recruitment success of all studied species.

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Table 10. The statistical variability of number of seedlings of *Serratula tinctoria* and *Gladiolus imbricatus* in subplots measured 0.16 m² (I), 0.09 m² (II), 0.04 m² (III), and 0.01 m² (IV) within patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in the years 2007-2010. Asterisks mean the probability that mean number of seedlings among subplots differed significantly at $P \leq 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***); ns-not significant differences (H Kruskal-Wallis test).

		The value of Kruskal-Wallis test	16.91**
<i>Serratula tinctoria</i>	MC	The level of statistical significance of differences among subplots	I and II ns
			I and III $P \leq 0.05$
			I and IV $P < 0.01$
			II and III ns
			II and IV $P \leq 0.05$
			III and IV ns
	GR	The level of statistical significance of differences among subplots	I and II ns
			I and III ns
			I and IV $P \leq 0.05$
			II and III ns
			II and IV $P \leq 0.05$
			III and IV ns
	SA	The level of statistical significance of differences among subplots	I and II ns
			I and III ns
			I and IV $P \leq 0.05$
			II and III ns
			II and IV ns
			III and IV ns
<i>Gladiolus imbricatus</i>	MC	The level of statistical significance of differences among subplots	I and II ns
			I and III ns
			I and IV $P \leq 0.05$
			II and III ns
			II and IV ns
			III and IV ns
	GR	The level of statistical significance of differences among subplots	I and II ns
			I and III $P \leq 0.05$
			I and IV $P \leq 0.05$
			II and III ns
			II and IV ns
			III and IV ns
	SA	The level of statistical significance of differences among subplots	I and II ns
			I and III $P \leq 0.05$
			I and IV $P \leq 0.05$
			II and III ns
			II and IV ns
			III and IV ns

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APPENDIX I

The categories of threat of studied species in Eastern and Central Europe.
Numbers refer to Red Lists/Books cited in alphabetical order:

Species	The IUCN categories					
	Extinct (EX)	Critically endangered (CE)	Endangered (EN)	Vulnerable (VU)	Near threat- ened (NT)	Least concern (LC)
<i>Gentiana pneumo- nanthe</i>	Kaliningrad Region ⁸		Czech Republik ^{5,11}	Switzerland ⁹ Poland ¹² Lithuania ⁶ Latvia ⁶ Meclenburg state ⁶ Schleswig -Holstein state ⁶	Hungary ¹³	Great Britain ¹
<i>Dianthus superbus</i>		Czech Republik ^{5,11}	Lithuania ⁶ Latvia ⁶ Schleswig- Holstein state ⁶ Austria ¹⁰	Leningrad Region ⁸ Poland ¹² Germany ⁷	Hungary ¹³	Switzerland ⁹
<i>Serratula tinctoria</i>	Ireland ⁴	Luxembourg ²	Italy ²			Great Britain ¹
<i>Gladiolus imbricatus</i>		Czech Republik ^{5,11}	Hungary ¹³ Switzerland ⁹ Poland ¹² Kaliningrad Region ⁸	Lithuania ⁶ Leningrad Region ⁶		

¹Cheffings and Farrell 2005; ²Colling 2005; ³Conti et al 1997; ⁴Curtis and McGough 1988; ⁵Holub and Procházka 2000; ⁶Ingelög *et al.* 1993; ⁷Korneck et al. 1998; ⁸Kotiranta *et al.* 1998; ⁹Moser *et al.* 2002; ¹⁰Nikl-feld and Schratt-Ehrendorfer 1999; ¹¹Procházka 2001; ¹²Zarzycki and Szeląg 2006; ¹³Zoltan *et al.* 1990.

APPENDIX II

The characteristic of the habitat conditions in patches of *Molinietum caeruleae* dominated by low meadow species (MC), large-tussock grasses (GR) and willows (SA) in Area A and Area B.

Area	Patch	Coordinates	Patch size (m ²)	Dominants	Maximal height of plant canopy (cm)	Mean height of plant canopy (cm)
A	MC	50°01'55.76"N, 19°52'03.06"E	1400	<i>Lathyrus pratensis</i> , <i>Lychnis flos-cuculi</i> , <i>Succisa pratensis</i>	159	96
	GR	50°01'55.23"N, 19°52'05.48"E	1300	<i>Molinia caerulea</i> , <i>Deschampsia caespitosa</i> ,	195	155
	SA	50°01'55.60"N, 19°52'04.45"E	1450	<i>Salix repens</i> ssp. <i>rosmarinifolia</i> , <i>S. cinerea</i>	228	185
B	MC	50°01'50.4"N, 19°52'03.2"E	1 600	<i>Lotus corniculatus</i> , <i>Briza media</i> , <i>Lychnis flos-cuculi</i>	157	120
	GR	50°01'52.5"N, 19°52'03.0"E	1 200	<i>Molinia caerulea</i> , <i>Deschampsia caespitosa</i>	210	170
	SA	50°01'50.9"N, 19°52'02.9"E	1 500	<i>Salix repens</i> ssp. <i>rosmarinifolia</i> , <i>S. cinerea</i> , <i>S. aurita</i>	247	180