The effect of succession stage on seed rain and seedling recruitment in overgrown *Molinia caeruleae* meadows

Kinga Kostrakiewicz-Gierałt

Department of Plant Ecology, Institute of Botany, Jagiellonian University, Lubicz 46, 31-512 Kraków, Poland

E-mail: kinga.kostrakiewicz@uj.edu.pl

Abstract: Investigations into the effect of succession stage on seed rain and seedling recruitment were conducted in Kraków-Kostrze (southern Poland) in 2014 and 2015. Observations were carried out in *Molinia caeruleae* meadows representing early (Patch I), temporary (Patch II) and advanced (Patch III) stage of secondary succession. Patch I was dominated by meadow species creating delicate stems, Patch II abounded in large tussock grasses and tall-growing rhizomatous grasses and Patch III was overgrown by willow shrubs and bordered by trees. The number of seeds and taxa in the seed rain decreased gradually in the successive study sites. Species resident in the standing vegetation were the most abundantly represented in the seed rain and their share augmented remarkably in Patch III, most likely due to its spatial isolation by the surrounding shrubs and trees. The share of seedlings and taxa in artificially made gaps in the plant cover and litter decreases along the successional gradient. The majority of taxa appearing in the seedling pool of all the patches occurred in the seed rain, while the taxa in the soil seed-bank reserves were modestly represented only in Patches I and II. Combining the obtained results and published data, it can be stated that, being abundant in the seed rain, the best colonizers show the greatest germination capability. Although the best colonizers are meadow taxa, fast-growing weeds, trees as well as alien, invasive species also appeared in the seedling pool.

Key words: secondary succession; seed dispersal; seedling pool; species composition

INTRODUCTION

At the beginning of 20th century the semi-natural rich-in-species humid meadows (order *Molinietalia*) were common across the whole of Europe [1]. During the 1970s, the traditional agricultural management was either heavily intensified or abandoned for socio-economic reasons. The lack of land-use contributing to secondary succession promoted the development of swamps and brushwoods, which finally brought about the disappearance of numerous plant species and a decrease in biodiversity. The encroachment of tall-growing native and alien perennials, as well as shrubs and trees, leading to habitat fragmentation and diminishing of the aforementioned communities' area, was also noted across the whole of Europe during this period [2-7]. Consequently, the most endangered types of humid meadows were listed among other protected habitats in the Annex I Habitat Directive and are included in the net NATURA 2000 [8].

Although the creation of gaps in the continuous plant cover and litter seems to be a very effective way of active conservation of humid meadows, studies of natural colonization of openings are rather scarce. So far, the studies of natural seedling recruitment of artificially made gaps during meadow overgrowing were conducted in relation to gap size [9], shape [10], the mode of gap creation [11-17] and time of origination [18]. Moreover, Fibich et al. [19] investigated the establishment of seedlings and vegetative sprouts in openings.

Considering the insufficient state of knowledge, the present studies were undertaken. Their main aim was to assess the relations between the plant canopy, seed rain and seedling pool in *Molinia caeruleae* patches with different habitat conditions. Specific goals focused on answering the following questions: i) What is the number of taxa and seeds in the seed rain? ii) How abundantly are the taxa present in the

Table 1. Site conditions in observed patches of *Molinietum caeruleae* in the year 2014. Names of Patches: Patch I – dominated by small meadow species, Patch II – dominated by large tussocks grasses and macroforbs), Patch III – overgrown by willow shrubs and bordered by trees.

Patch	I	II	III
Patch area (m²)	1700	1900	1600
Successional stage	Initial	Temporary	Advanced
Number of species in patch	56	47	39
Dominants (species, with cover >20%)	*Lathyrus pratensis Lotus corniculatus*	*Molinia caerulea, Deschampsia caespitosa, Lysimachia vulgaris,*	*Salix repens ssp. rosmarinifolia, S. caprea, S. cinerea*
Subdominants (species, with cover 5-20%)	*Lychnis flos-cuculi, Succisa pratensis*	*Serratula tinctoria, Polygonum bistorta*	*Betula pendula, Populus tremula*

The value of statistical significance of differences (Kruskal-Wallis H test, *df=2*) among patches in vascular plant height achieved 39.39 (*p<0.001*)

established vegetation represented in the seed rain? iii) Which species occur in the seed rain? iv) What is the number of taxa and seedlings in the seedling pool? v) How abundantly are taxa present in the seed rain represented in the seedling pool? and vi) Which species occur in the seedling pool?

MATERIALS AND METHODS

Study area

The investigations were carried out in the Kostrze district on the western border of Krakow, south of the Vistula River (southern Poland). The research area is situated at ca. 210 m a.s.l, on the low flood terrace of the Vistula. Peaty or clay- and silt-laden soils with fluctuating water tables are covered by *Molinietum caeruleae* patches [20]. The abandonment of mowing for at least the past dozen years has favored the encroachment of vegetation from the alliances *Phragmition* and *Magnocaricion*, leading to the fragmentation of meadows [21,22]. The meadows are surrounded by diverse vegetation types, including deciduous forests covering the slopes of the Vistula valley, calcareous grasslands occurring on the limestone and chalk hill slopes, and ruderal communities appearing near buildings and along the edges of roads.

The studies were carried out in an area comprising three adjacent abandoned patches of *Molinietum caeruleae* sensu Matuszkiewicz [23], characterized by the presence of the following taxa: *Betonica officinalis, Dianthus superbus, Gentiana pneumonanthe, Gladiolus imbricatus, Iris sibirica, Molinia caerulea, Potentilla erecta, Sanguisorba officinalis, Selinum carvifolia,*

and *Succisa pratensis*. The studied patches differed as to successional stage and dominant species. Patch I (50° 01′ 55.7″ N; 19° 52′ 03.1″ E), abandoned for ca. 20 years, was dominated by meadow species creating delicate erect (up to 30 cm) or procumbent stems (i.e. *Lathyrus pratensis Lotus corniculatus*); Patch II (50° 01′ 55.2″ N; 19° 52′ 05.5″ E), not used for ca. 35 years, by large tussock grasses and tall-growing rhizomatous grasses (e.g. *Molinia caerulea, Phragmites australis*); and Patch III (50° 01′ 55.6″ N; 19° 52′ 04.4″ E), unmanaged for at least 45 years, was overgrown by willow shrubs and bordered by trees (i.e. *S. caprea, S. cinerea*). The floristic similarity among the study sites was checked by the Jacquard index. In July 2014, an evaluation of the plant height from soil surface to the top flower (n=30, randomly chosen stems) was performed using a folding tape measure. The habitat conditions in the experimental patches are described in Table 1.

The characteristic of seed traps and seed identification

Twenty traps for the seed rain were placed in each study site in both study seasons during the first week of April. The traps were separated from each other by belts of untouched vegetation at least 3 m wide and were located ca. 2 m from the borders of the patches. The plastic-made traps consisted of a transparent, cylindrical container half-buried in the soil. The containers were covered on top by a fine mesh (pore size 0.4 cm) to reduce insect capture. The volume of containers was 2260.8 cm³ (the height of the containers was 20 cm, while their radius was 6 cm). The traps were emptied twice a month in the laboratory. Each time the containers and mesh were washed and then reused. Seeds deposited in the traps were identified directly

under a binocular microscope (at 10-60x magnification) or a dissecting microscope using the author's comparative collection, as well as the keys and manuals for seed determination [24-26] and monographs [27-29]. Altogether, in the seed rain of the successive patches, 145 seeds – most likely from the *Poaceae* and *Cyperaceae* families, indeterminable at the species-level – were excluded from the analyses. Then, for each patch the following parameters were counted: i) the cumulative number of species/seeds in all the traps; ii) the average number of species/seeds per trap; iii) the percentage of seeds representing the species present and absent in the plant cover; iv) the Jacquard index between the species composition of the plant canopy and the seed rain. At each study site the number of diaspores representing particular species appearing in the seed rain were counted, and the five most abundantly represented in the traps were identified.

Gap creation and seedling determination

Next to each trap a gap with an area 20 cm x 20 cm was artificially made. The gaps were created by clipping all the aboveground parts of the plants at soil level and removing them along with the litter layer. The underground organs of the plants were not dug up. This treatment is considered optimal for seedling recruitment in wet meadows according to Kotorová and Lepš [30], as well as Kostrakiewicz [9]. The ramets of the adjacent plants encroaching on the gap area during the growing season were not removed.

Habitat conditions in the openings were monitored in a central part of each gap once a week from April until November, between 10:00 and 12:00 in the years 2014 and 2015. Altogether, measurements were taken during 30 weeks each year. The light intensity at the soil level was examined in full sunshine with a Voltcraft MS-1300 digital light meter (accuracy ±5%; measuring range 0.01-50 000 lx). The humidity at ground level was measured using an OMEGA HSM50 handheld digital soil moisture sensor (accuracy ±5%; measuring range 0-50% moisture content of soil). Soil temperature at a depth of 5 cm was measured using a Hanna electronic temperature sensor (accuracy ±0.1°C; measuring range -10-60°C). The weekly average values of the abovementioned parameters were calculated for each Patch separately and are presented

together with the dates of measurements in Suppl. Table S1.

The process of gap colonization by generative offspring was monitored once a week from April until November in the studied years. All the recruits were removed and examined according to the guides for seedling determination [31,32] with the support of the author's own comparative collection. The genets of rare and protected plants were identified *in situ* without digging them up and marked with plastic rings and sticks. The nomenclature of the taxa follows Mirek et al. [33]. Altogether in the seedling pool of successive patches, 87 seedlings (most likely from *Poaceae* family, indeterminable at the species level) were excluded from the analyses. Then, for each patch the following parameters were counted: i) the average light availability, soil moisture and soil temperature within gaps; ii) the cumulative number of species/seedlings found in all the gaps; iii) the average number of species/seedlings per gap; iv) the percentage of seedlings representing the species present in the plant cover and the seed rain, present only in the plant cover, present exclusively in the seed rain, as well as absent in the plant canopy and seed rain of the particular patches.

In each study site the number of seedlings of each taxa appearing in the seedling pool was counted and the five most abundantly represented species in the gaps were distinguished.

Statistical analyses

The normal distribution of the untransformed data (number of taxa/seeds in the traps) was tested using the Kolmogorov-Smirnov one-sample test at the significance level of $p < 0.05$. Subsequently, the variance homogeneity was tested using the Brown-Forsythe test at the significance level of $p < 0.05$. As the distribution of characteristics in some groups of data was not consistent with the normal distribution and the variance was not homogeneous, the statistical analysis was based on the nonparametric Kruskal-Wallis H test. It was applied to examine if there were significant differences in: i) light availability, soil moisture and soil temperature within gaps between particular patches; ii) the number of taxa/seeds in the seed rain between

particular patches. After a significant value of the test was achieved, post-hoc comparisons were made.

Chi-square statistics were used to check whether there were significant differences among the study sites in i) species composition of the seed rain regarding the share of species present/absent in the established vegetation and ii) the species composition of the seedling pool regarding the presence/absence of species in the plant canopy and seed rain.

RESULTS

Habitat conditions

The floristic composition of plant canopy did not differ substantially between study sites. The Jacquard index values, reaching 0.40 between Patches I and II, 0.33 between Patches I and III and 0.36 between Patches II and III, indicate the greatest floristic similarity between Patches I and II. The average height of standing vegetation rose in consecutive sites and reached from 44.9 cm in Patch I, via 88.6 cm in Patch II, to 114.2 cm in Patch III. Performed observations showed considerable differences in site conditions within gaps in subsequent study sites. The statistical analysis showed that within openings made in successive patches, light availability decreased, soil humidity increased, whereas the soil temperature did not differ significantly in the openings made in the consecutive patches (Table 2).

Seed rain

The total number of species recorded in the seed rain in the consecutive patches was 46, 34 and 33, respectively. The average number of all taxa per trap diminished. The total number of seeds in the seed rain in the consecutive patches amounted to 1868, 1185 and 442, respectively. Similarly, the mean number of seeds per trap decreased remarkably in successive patches (Fig. 1).

Species present and absent in the plant canopy were observed in the seed rain of all the patches; however their contribution was different. In Patches I and II, the share of seeds representing species occurring and not occurring in the standing vegetation was rather similar, whereas the share of seeds representing species present in the plant cover dominated in Patch III (Fig. 3). The Jacquard index values between species composition in the seed rain and plant canopy in particular patches came to 0.35, 0.34 and 0.47, respectively. The full list of names of the species with their occurrence in the plant canopy and seed rain is given in Suppl. Table S2.

Different species dominated in the seed rain in particular study sites. These were: *Betonica officinalis*, *Cirsium arvense*, *Filipendula ulmaria*, *Sanguisorba officinalis* and *Solidago canadensis* in Patch I, *Betula pendula*, *Inula salicina*, *Iris sibirica*, *Sanguisorba officinalis*, and *Succisa pratensis* in Patch II, and *Betula pendula*, *Filipendula ulmaria* , *Selinum carvifolia*, *Iris sibirica*, and *Sanguisorba officinalis* in Patch III (Suppl. Table S3).

Table 2. The average (±SD) light availability (Lx), soil humidity (% of water content) and soil temperature (ºC) in artificially created gaps in the years 2014 and 2015. Names of Patches as in Table 1

Year	Parameter	Patch			Statistical significance level (the value of Kruskal-Wallis H test, df=2; P)
		I	II	III	
2014	Light intensity	44 560[a] (±2 680)	40 812[b] (±2 159)	33 667[c] (±5 889)	63.7 P≤0.001
	Soil humidity	21.7[a] (±7.0)	26.2[b] (±5.6)	28.4[b] (±5.7)	18.2 P≤0.001
	Soil temperature	13.9 (±5.2)	13.2 (±5.1)	12.9 (±4.3)	1.01 P>0.05
2015	Light intensity	38 927[a] (±4 088)	35 649[b] (±3 774)	32 172[c] (±4 081)	27.9 P≤0.001
	Soil humidity	25. 8[a] (±5.2)	32.5[b] (±6.8)	37.9[c] (±4.3)	45.4 P≤0.001
	Soil temperature	16.1 (±6.0)	15.1 (±5.6)	14.2 (±5.3)	2.2 P>0.05

The same letters denote the absence of statistical differences among study Patches.

Seedling pool

The total number of species recorded in the seedling pool amounted to 40 in Patch I and 30 in Patches II and III. The average number of taxa per gap in the consecutive study sites diminished gradually. The total number of seedlings in the consecutive patches was 2428, 1454 and 944, respectively (Fig. 2).

In all the patches the seedlings representing species occurring in the plant canopy and seed rain recruited most abundantly. The contribution of seedlings representing taxa occurring exclusively in the seed rain decreased sharply in Patch III, while taxa absent in the plant cover and seed rain were slightly represented in the seedling pool only in Patches I and II (Fig. 4). The names of the species with their occurrence in the seed rain and seedling pool are presented in Suppl. Table S2.

Different species prevailed in the seedling pool in particular study sites. These were: *Betonica officinalis*, *Filipendula ulmaria*, *Sanguisorba officinalis*, *Serratula tinctoria*, *Succisa pratensis*, *Serratula tinctoria* and *Valeriana officinalis* in Patch I, *Filipendula ulmaria*, *Sanguisorba officinalis*, *Selinum carvifolia*, *Serratula tinctoria* and *Succisa pratensis* in Patch II, and *Betonica officinalis*, *Filipendula ulmaria*, *Geum rivale*, *Sanguisorba officinalis* and *Selinum carvifolia* in Patch III (Suppl. Table S3).

DISCUSSION

Seed rain

The obtained results proving that the abundance of species and seeds in traps diminishes gradually in the consecutive patches correspond with those of Jensen [34], who reported that the number of taxa and diaspores in the seed rain decreases along a successional gradient. Such a scenario might be due to the diminishing species richness in *Molinia caeruleae*, as confirmed by numerous authors [6,35-37]. This phenomenon might also be caused by a gradual decrease in seed production by plants resident in the standing vegetation. Such a scenario was found in abandoned xeric grassland by Soukupová [38].

Contrary to the findings of Jensen [34], the analysis of species composition in the seed rain produced evidence that diaspores representing species resident in the standing vegetation dominate in all the study patches, and their share increases remarkably along the successional gradient. The observed sharp decrease in Patch III of species representing species absent in the plant cover might be due to its spatial isolation by the surrounding shrubs and trees. Other studies [39,40] found that spatial isolation of meadow patches might successfully prevent the transfer of diaspores by water [41], animals [42], as well as wind [43,44].

Surprisingly, the substantial number of *Cirsium arvense* and *Solidago canadensis* seeds in traps placed in Patch I, the considerable number of *Betula pendula* seeds in Patch II, as well as the large number of *Filipendula ulmaria* seeds in Patches I and II, despite the absence of mature individuals in the plant canopy, might suggest the effective transfer of seeds from the other sites. A large body of literature has confirmed the very effective anemochorous transport of *Cirsium arvense* [45-47], *Solidago canadensis* [48,49] and *Betula pendula* [50,51]. The hydrochorous transport of *Filipendula ulmaria* seeds during prolonged spring inundations or heavy rains in the summer or autumn might occur due to the ability of the seeds to survive long-lasting flotation on the water surface [52]. Additionally, Soomers et al. [53] found that the typical wind- and water-dispersers in overgrown, abandoned meadows might be transferred by water. On the other hand, the substantial appearance of *Betonica officinalis* and *Sanguisorba officinalis* in the seed rain might be result of the occurrence of numerous mature individuals in the plant canopy, which seems to compensate for the rather moderate seed production noted in both taxa [54,55].

Seedling pool

The results of the presented studies are also in accordance with previous observations that the number of species and offspring in the seedling pool along the successional gradient decreases in overgrown meadows from *Molinia* ordo [9,15,17]. Such a phenomenon could be due to the increased period of water stagnation that might accelerate the development and
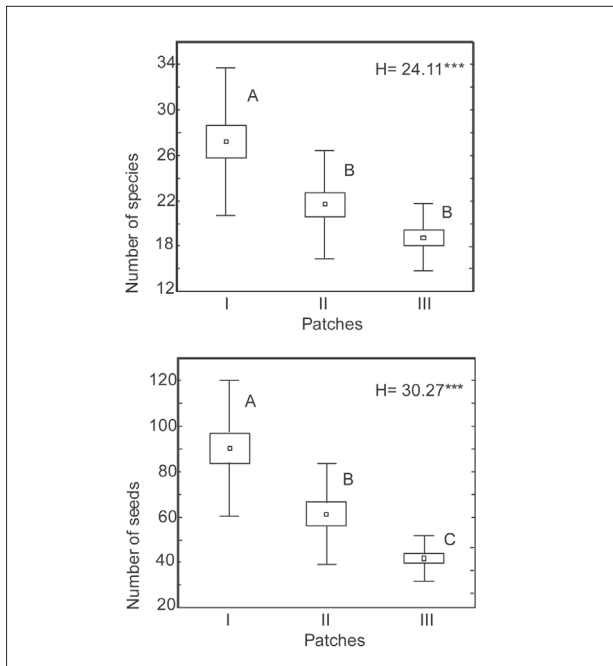
Fig. 1. Number of species and seeds in traps in study patches in the years 2014 and 2015. Names of Patches as in Table 1. Box and whisker plots give the mean (square), SE (box) and SD (whiskers). Asterisks mean that there are significant differences among Patches at the 0.001 level (Kruskal-Wallis H test).
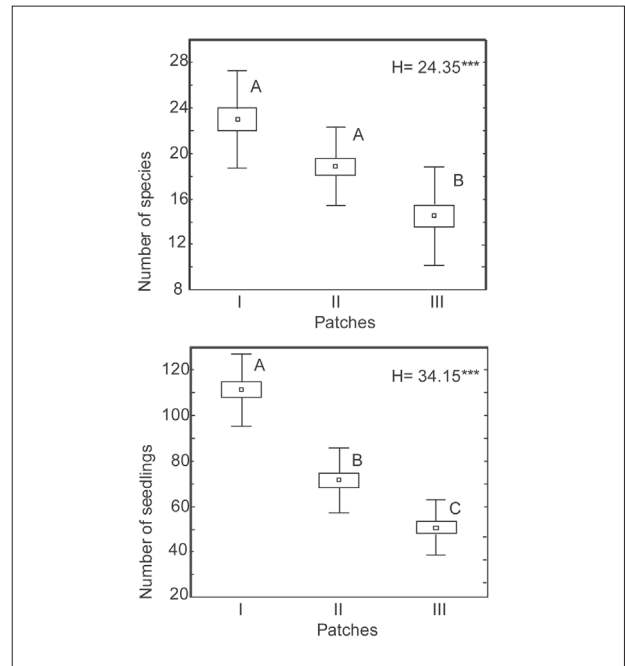

Fig. 2. Number of species and seedlings in gaps in study patches in the years 2014 and 2015. Names of Patches as in Table 1, description of graphs and the statistical significance level as in Fig. 1.

infectivity of fungal pathogens, a major cause of mortality of diaspores deposited in the soil [56]. On the other hand, Bekker et al. [57] argued that the seeds of most wetland plants survive better in waterlogged conditions. The successive diminishment of species

and seedling number might also be caused by growing shading of the soil surface in openings, particularly by the neighboring large-tussock grasses and shrub willows frequent in Patches II and III. The diminishing of seedling recruitment caused by insufficient
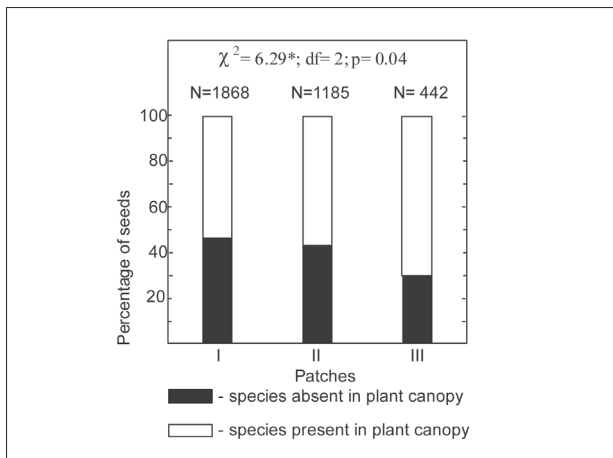

Fig. 3. Share of species absent and present in plant canopy appearing in traps set in study patches in the years 2014 and 2015. Names of Patches as in Table 1 Asterisk – significant differences among Patches at the 0.01 level (χ^2 test); "N" – total number of seeds.
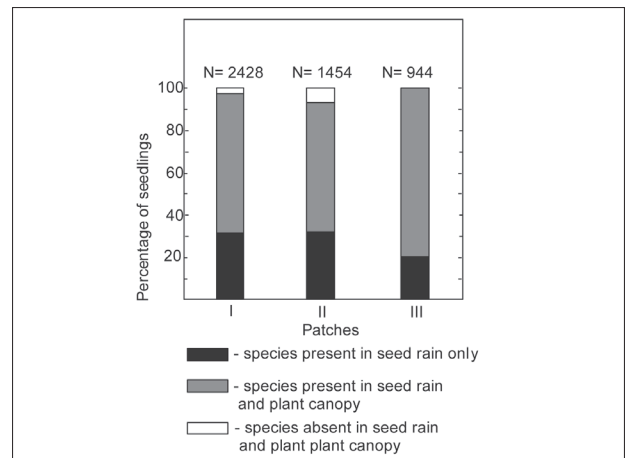

Fig. 4. Share of species present and absent in the seed rain appearing in gaps artificially created in study patches in the years 2014 and 2015. Names of Patches as in Table 1. Symbol "ns" – no significant differences among Patches (χ^2 test); "N" – total number of seedlings.

light availability was observed in many meadow species [58-61], while Hautier et al. [62] added that in grasslands, lack of light is the main cause of seedling mortality and species richness loss. Moreover, the lowest recruitment of seedlings in Patch III might be due of the allelopathic activities of *Salix caprea* inhibiting the appearance of meadow species [63].

The observed sharp increase in Patch III of seedlings representing species found in the plant cover also corresponds with previous observations showing the gradual augmentation of the share of offspring representing taxa resident in standing vegetation along the successional gradient in *Molinia caeruleae* meadows [9]. Furthermore, the occurrence of species absent in the plant canopy and seed rain in Patches I and II might suggest seedling recruitment from the reserves of the soil seed bank, appearing as an effect of diaspore accumulation combined with suppressed germination by thick litter and shading. The lack of seedlings representing species not occurring in the plant canopy and seed rain in Patch III contrasts with other studies conducted along the successional gradient in wet meadows [11,34,64-66], marshlands [67] and oxbow wetlands [68], proving that even in the advanced stages of overgrowth, the soil seed bank contains viable diaspores of taxa deposited in earlier successional phases.

The observed abundant recruitment of *Filipendula ulmaria* L., *Betonica officinalis* L. (Trevis.), *Sanguisorba officinalis* L., *Selinum carvifolia* (L.), *Serratula tinctoria* L., *Succisa pratensis* Moench., *Geum rivale* L. and *Valeriana officinalis* L. confirms previous observations [9,10,17,18]. The colonization success of the aforementioned taxa producing non persistent diaspores [69] might be a consequence of their substantial presence in the seed rain (Suppl. Table S3) and great germination capability. The studies of Grime et al. [70] provided evidence that the seeds of *Betonica officinalis, Filipendula ulmaria, Geum rivale, Serratula tinctoria* and *Succisa pratensis* could germinate in Petri dishes immediately after collection, as well as after storage at room temperatures for up to a year. Also, further laboratory tests proved that the seeds of *Betonica officinalis* [30,55], *Selinum carvifolia* [30,71], *Succisa pratensis* [30,72] and *Sanguisorba officinalis* [30,73] present substantial germination ca-

pacity under controlled conditions. The experimental seed sowing in gaps left after the removal of the plant canopy and litter layer in meadows showed considerable seedling recruitment of *Betonica officinalis* [30], *Geum rivale* [74], *Serratula tinctoria* [30,72,75,76], *Selinum carvifolia* [30,77,78], *Sanguisorba officinalis* [30] and *Succisa pratensis* [30,72,78].

Additionally, it should be pointed out that although the majority of species appearing in the seedling pool are meadow taxa, the recruitment of native weeds (i.e. *Chenopodium album, Papaver rhoeas)* and pioneer trees (i.e. *Betula pendula*), as well as alien, invasive perennials (i.e. *Solidago canadensis*) was also noted. Moreover, the successful, abundant seedling recruitment of the aforementioned taxa was repeatedly observed in the laboratory [79-81], as well as in natural habitat conditions [10]. Similarly, other authors emphasized the significant survival of seedlings and juveniles of *Betula pendula* and *Solidago canadensis* even in competitive environmental conditions [82,83]. Additionally, the investigations of Mei et al. [84], as well as Sun et al. [85], proved the allelopathic activity of *Solidago canadensis*, contributing to inhibition of the recruitment of seedlings of other species. Taking all this into account, it might be stated that the recruitment of pioneer trees and invasive perennials in *Molinia caeruleae* meadows should be carefully monitored to avoid promotion of the secondary succession process.

CONCLUSIONS

The number of seeds and taxa appearing in the traps decreased gradually along the successional gradient. Species resident in the standing vegetation dominated in the seed rain in all the study patches, and their share augmented remarkably in the successive sites. To the more successful dispersers (after the species present in plant canopy) belong taxa producing wind- or water-assisted diaspores that might be transported from other locales.

The abundance of seedlings and taxa appearing in openings in the plant cover and litter decreases along the successional gradient. The majority of taxa appearing in the seedling pool of all the patches occurred in the seed rain, while the contribution of taxa

recruiting from the soil seed bank reserves (only in the patches in the early and temporary successional stages) was significantly lower. Creating gaps in the plant cover and litter might be a very effective way of active protection of *Molinia caeruleae* meadows, enabling the recruitment of meadow taxa. On the other hand, it might also contribute to the recruitment of undesired native and alien taxa and should be closely monitored to avoid promotion of the secondary succession process.

REFERENCES

1. Ellenberg H. Vegetation Ecology of Central Europe. New York: Cambridge University Press; 1988. 732 p.
2. Fuller RM. The changing extent and conservation interest of lowland grasslands in England and Wales: a review of grasslands surveys 1930-84. Biol Conserv. 1987;40:281300.
3. Green BH. Agricultural intensification and loss of habitat, species and amenity in British grasslands: a review of historical change and assessment of future prospects. Grass Forage Sci. 1990;45:365-72.
4. Joyce CB, Wade PM. European wet grasslands: biodiversity, management and restoration. Chichester: Wiley; 1998. 385 p.
5. Muller S. Diversity of management practices required to ensure conservation of rare and locally threatened plant species in grasslands: a case study at regional scale (Lorraine, France). Biodivers Conserv. 2000;11:1173-84.
6. Kącki Z. Variability and long-term changes in the species composition of *Molinion* meadows in Poland: a case study using a large data set from the Polish Vegetation Database. Acta Bot Siles Monogr. 2012;7:1-131.
7. Joyce Ch. Ecological consequences and restoration potential of abandoned wet grasslands. Ecol Eng. 2014;66:91-102.
8. Interpretation Manual of European Union Habitats – EUR 25 – NATURA 2000. [Brussels]: European Commission – DG Environment; 2003. 127 p.
9. Kostrakiewicz K. The effect of gap size on colonization process in *Molinietum caeruleae* meadows with different habitat conditions. Pol J Ecol. 2011;59:677-86.
10. Kostrakiewicz-Gierałt K. The effect of the shape of gaps on microenvironmental conditions and seedling recruitment in *Molinietum caeruleae* meadows. Acta Agrobot. 2015;68(2):143-51.
11. Falińska K. Alternative pathways of succession: species turnover patterns in meadows abandoned for 30 years. Phytocenosis 15 (N.S.) Archiv Geobot. 2003;9:1-104.
12. Špačková I, Lepš L. Variability of seedling recruitment under dominant, moss, and litter removal over four years. Folia Geobot. 2004;39(1):41-55.
13. Janeček S, Lepš J. Effect of litter, leaf cover and cover of basal internodes of the dominant species *Molinia caerulea* on seedling recruitment and established vegetation. Acta Oecol. 2005;28(2):141-7.
14. Borkowska L. Patterns of seedling recruitment in experimental gaps on mosaic vegetation of abandoned meadows. Acta Soc Bot Pol. 2004;73:343-50.
15. Borkowska L. Wzorce rekrutacji siewek gatunków klonalnych w zbiorowisku niekoszonej łąki *Cirsietum rivularis* Ralski 1931. Phytocenosis 16 (N.S) Archiv Geobot. 2004;10:1-71.
16. Borkowska L. The effect of disturbances on the floristic composition of vegetation in abandoned meadows. Pol Bot Stud. 2006;22:63-70.
17. Kostrakiewicz-Gierałt K. The impact of disturbance gradient on recruitment of clonal plant species in *Molinietum caeruleae* meadows. Pol J Ecol. 2013;61(3):519-33.
18. Kostrakiewicz-Gierałt K. The impact of time of gap origin on microsite conditions and seedling recruitment in *Molinietum caeruleae* meadows. Inter J Conserv Sci. 2015;6(1):111-24.
19. Fibich P, Vítová A, Macek P, Lepš J. Establishment and spatial associations of recruits in meadow gaps. J Veg Sci. 2012;24(3):496-505.
20. Dubiel E, carthographer. Plant communities of the 3rd Campus of the Jagiellonian University and its surroundings [map]. Kraków: Institute of Botany Jagiellonian University; 2005. 1 sheet : 1; 96 x 56 cm. color.
21. Dubiel E. Map of actual vegetation of the city of Cracow. Zesz Nauk UJ, Prace Bot. 1991;22:121-33.
22. Dubiel E. Meadows in Cracow. I. *Molinio-Arrhenatheretea* class. Studia Ośr Dok Fizjogr. PAN. 1996;24:145-71.
23. Matuszkiewicz W. A guide to identification of Polish plant communities. Warszawa: Wydawnictwo Naukowe PWN; 2001. 536 p.
24. Aulak W. Klucze do oznaczania nasion, siewek, pędów drzew i krzewów leśnych. Warszawa: Fundacja Rozwój SGGW; 1994. 118 p.
25. Kulpa W. Fruits and seeds. The key for determination of weed seeds. Warszawa: Państwowe Wydawnictwo Naukowe; 1958. 419 p.
26. Cappers RTJ, Bekker RM. A Manual for the Identification of plant seeds and fruits. Groningen Archaeological Studies 2013;23:1-273.
27. Kostrakiewicz K. Taxonomic studies on Polish species of the genus Vicia L. Springfield: U.S. Department of Agriculture and National Science Foundation by the Foreign Scientific Publications Department of the National Center for Scientific. 1956. 54 p.
28. Szafer W, Pawłowski B, editors. Flora of Poland. The Vascular Plants of Poland and neighboring lands. Vol. 7. Warsaw: Państwowe Wydawnictwo Naukowe; 1955; 302 p.
29. Jasiewicz A., editor. Flora of Poland. The Vascular Plants of Poland and neighboring lands. Vol. 4. Warsaw: Państwowe Wydawnictwo Naukowe; 1985; 306 p.
30. Kotorová I, Lepš J. Comparative ecology of seedling recruitment in an oligotrophic wet meadow. J Veg Sci. 1999;10(2):175-86.

31. Csapodý V. Keimlingsbestimmungsbuch der Dikotyledonen. Budapeszt: Akademiai Kiado;1968. 286 p. German.

32. Muller FM. Seedlings of the North-Western European lowland. A flora of seedlings. 1st ed. The Hague, Boston: Dr W. Junk B.V. Publishers; 1978. 653 p.

33. Mirek Z, Piękoś-Mirkowa H, Zając A, Zając M. Flowering plants and pteridophytes of Poland. A checklist. 1st ed. Kraków: W. Szafer Institute of Botany PAS; 2002. 442 p.

34. Jensen K. Species composition of soil seed bank and seed rain of abandoned wet meadows and their relation to aboveground vegetation. Flora. 1998;193(4):345-59.

35. Michalska-Hejduk D. Changes in species composition in purple moor-grass meadows *Molinietum caeruleae* W. Koch 1926 in the Kampinos National Park in the period 1994-2004. Studia Naturae. 2006;54(1):159-72.

36. Michalska-Hejduk D. Changes of semi-natural vegetation with a focus on *Molinion* meadows after 50 years of strict protection. Pol J Environ Stud. 2012;6:1731-41.

37. Kulik M. Changes in biodiversity and species composition of *Molinia* meadow depending to use method. Pol J Environ Stud. 2014;23(3):773-82.

38. Soukupová L. Life histories in principal plant populations, including their allelopathic interferences. In: Osbornová J, Kovárová M, Lepš J, Prach K, editors. Succession in Abandoned Fields: Studies in Central Bohemia, Czechoslovakia. Dordrecht, Boston, London: Kluver Academic Publishers. 1990. p. 32-8.

39. Middleton B, van Diggelen R, Jensen K. Seed dispersal in fens. Appl Veg Sci. 2006;9(2):279-84.

40. Ozinga WA, Romermann C, Bekker RM, Prinzing A, Tamis WLM, Schaminée JHJ, Hennekens SM, Thompson K, Poschlod P, Kleyer M, Bakker JP, van Groenendeael JM. Dispersal failure contributes to plant losses in NW Europe. Ecol Lett. 2009;12(1): 66-74.

41. Galatowitsch SM, van der Valk AG. The vegetation of restored and natural prairie wetlands. Ecol Appl. 1996;6:102-12.

42. Debinski DM, Holt RD. A survey and overview of habitat fragmentation experiments. Conserv Biol. 2000;14(2):342-55.

43. Soons MB, Messelink JH, Jongejans E, Heil GW. Habitat fragmentation reduces grassland connectivity for both short-distance and long-distance wind-dispersed forbs. J Ecol. 2005;93:1214-25.

44. Pounden E, Greene DF, Quesadaand M, Contreras Sánchez JM. The effect of collisions with vegetation elements on the dispersal of winged and plumed seeds. J Ecol. 2008;96:591-8.

45. Sheldon JC, Burrows FM. The dispersal effectiveness of the achene-pappus

46. units of selected compositae in steady winds with convection. New Phytol. 1973;72:665-75.

47. Moore RJ. The biology of Canadian weeds. 13. *Cirsium arvense* (L.) Scop. Can J Plant Sci. 1975;55:1033-48.

48. Becker RL, Haar MJ, Kinkaid BD, Klossner LD, Forcella F. Production and wind dispersal of Canada Thistle (*Cirsium arvense* L.) achenes. Minnesota: Minnesota Department of Transportation. 2008. 42 p.

49. Werner PA, Bradbury IK, Gross RS. The biology of Canadian weeds. 45. *Solidago canadensis* L. Can J Plant Sci. 1980;60:1393-409.

50. Tackenberg O, Peter P, Bonn S. Assessment of wind dispersal potential in plant species. Ecol Monogr. 2003;73(2):191-205.

51. Atkinson MD. *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. J. Ecol. 1992;80:837-70.

52. Wagner S, Wälder K, Ribbens E, Zeibig A. Directionality in fruit dispersal models for anemochorous forest trees. Ecol Model. 2004;179:487-98.

53. Merritt DM, Nilsson C, Jansson R. Consequences of propagule dispersal and river fragmentation for riparian plant community diversity and turnover. Ecol Monogr. 2010;80(4):609-26.

54. Soomers H, Karssenberg D, Soons,MB, Verweij PA, Verhoeven JTA, Wassen MJ. Wind and Water Dispersal of Wetland Plants Across Fragmented Landscapes. Ecosystems. 2013;16(3): 434-51.

55. Nordborg G. Studies on *Sanguisorba officinalis* L. Bot Not. 1963;116:267-88.

56. Dušek K, Dušková E, Smékalová K. *Betonica officinalis* L. in the Czech Republic. II. Seed production and quality and variability of total polyphenols content. Herba Polonica. 2010;56(3):15-23.

57. Blaney SC, Kotanen PM. Effects of fungal pathogens on seeds of native and exotic plants: a test using the congeneric pairs. J Appl Ecol. 2001;38:1104-13.

58. Bekker RM, Oomes MJM, Bakker JP. The impact of groundwater level on soil seed bank survival. Seed Sci Res. 1998;8(3):399-404.

59. Křenova A, Lepš J. Regeneration of a *Gentiana pneumonanthe* population in an oligotrophic wet meadow. J Veg Sci. 1996;7(1):107-12.

60. Jensen K, Meyer C. Effects of light competition and litter on the performance of *Viola palustris* and on species composition and diversity of an abandoned fen meadow. Plant Ecol. 2001;155(2):169-81.

61. Fu-Ping T, You L, Gao-Lin W, Shang-Li S. Seedling recruitment of forb species under experimental habitats in alpine grassland. Pak J Bot. 2015;47(6):2127-34.

62. Li W, Cheng J, Yu K, Epstein HE, Du G. Short-term responses of an alpine meadow community to removal of a dominant species along a fertilization gradient. J Plant Ecol. 2015;8(5):1-10.

63. Hautier Y, Niklaus PA, Hector A. Competition for light causes plant biodiversity loss after eutrophication. Science. 2009;324:636-8.

64. Mudrák O, Frouz J. Allelopathic effect of *Salix caprea* litter on late successional plants at different substrates of post-mining sites: pot experiment studies. Botany. 2012;90(4):311-18.

65. Falińska K. Long-term changes in size and composition of seed bank during succession: from meadow to forest. Acta Soc Bot Pol. 1998;67(3-4):301-11.

66. Falińska K. Seed bank dynamics in abandoned meadows during a 20-year period in the Białowieża National Park. J Ecol. 1999;87(3):461-75.

67. Ma M., Du G., Zhou X. Role of the soil seed bank during succession in a subalpine meadow on the Tibetan Plateau. Arc Antarc Alp Res. 2009;41(4):469-77.

68. Amiaud B, Touzard B. The relationships between soil seed bank, aboveground vegetation and disturbances in old embanked marshlands of Western France. Flora. 2004;199:25-35.

69. Yang D, Li W. Soil seed bank and aboveground vegetation along a successional gradient on the shores of an oxbow. Aquat Bot. 2013;110:67-77.

70. Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnerschein 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, Stadler M, Schlegelmilch J, Steenddam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eroksson O, Garnier E, Peco B. The LEDA Traitbase: A database of life-history traits of Northwest European flora. J Ecol. 2008;96:1266-74.

71. Grime JP, Mason G, Curtis AV, Rodman J, Band SR, Mowfort MAG, Neal AM, Shaw S. A comparative study of germination characteristics in a local flora. J Ecol. 1981;69(3):1017-59.

72. Vandelook F, Bolle N, Van Assche JA. Multiple environmental signals required for embryo growth and germination of seeds of *Selinum carvifolia* (L.) L. and *Angelica sylvestris* L. (Apiaceae). Seed Sci Res. 2007;17:283-91.

73. Stammel B, Kiehl K, Pfadenhauer J. Effects of experimental and real land use on seedling recruitment of six fen species. Basic Appl Ecol. 2006;7:334-46.

74. Holloway PS, Matheke GEM. Seed Germination of Burnet, *Sanguisorba* spp. Native Plants Journal 2003;4(2):95-9.

75. Kiviniemi K. Population dynamics of *Agrimonia eupatoria* and *Geum rivale*, two perennial grassland species. Plant Ecol. 2002;159(2):153-69.

76. Bissels S, Donath TW, Hölzel N, Otte A. Effects of different mowing regimes on seedling recruitment in alluvial grasslands. Basic Appl Ecol. 2006;7:433-42.

77. Donath TW, Hölzel N, Otte A. Influence of competition by sown grass, disturbance and litter on recruitment of rare flood-meadow species. Biol. Conserv. 2006;130(3): 315-23.

78. Špačková I, Kotorová I, Lepš J. Sensitivity of seedling recruitment to moss, litter and dominant removal in an oligotrophic wet meadow. Folia Geobot. 1998;33(1):17-30.

79. Poschlod P, Biewer H. Diaspore and gap availability are limiting species richness in wet meadows. Folia Geobot. 2005;40(1):13-34.

80. Henson IE. The effect of light, potassium nitrate and temperature on the germination of *Chenopodium album* L. Weed Res. 1970;10:27-39.

81. Thompson LJ, Naeem S. The effects of soil warming on plant recruitment. Plant Soil. 1996;182(2):339-43.

82. Yao S, Lan H, Zhang F. Variation of seed heteromorphism in *Chenopodium album* and the effect of salinity stress on the descendants. Ann Bot. 2010;105(6):1015-25.

83. Hartnett DC, Bazzaz FA. The genet and ramet population dynamics of *Solidago canadensis* in an abandoned field. J Ecol. 1985;73(2):407-13.

84. Kuznetsova T, Rosenvald K, Ostonen I, Helmisaaric H-S, Mandre M, Lõhmus K. Survival of black alder (*Alnus glutinosa* L.), silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) seedlings in a reclaimed oil shale mining area. Ecol Eng. 2010;36(4):495-502.

85. Mei L, Chen X, Tang J. Allelopathic effects of invasive weed *Solidago canadensis* on native plants. J Appl Ecol. 2005;16(12):2379-82.

86. Sun B, Tan JZ, Wan ZG, Gu FG, Zhu MD. Allelopathic effects of extracts from *Solidago canadensis* L. against seed germination and seedling growth of some plants. J Environ Stud. 2006;18(2):304-9.

Supplementary Data

Supplementary Table S1. The average values (± SD) of light availability (Lx), soil humidity (water content in %) and soil temperature (ºC) within artificially created gaps in the years 2014 and 2015. Names of Patches as in Table 1.

Table S1 can be accessed via the link: http://serbiosoc.org.rs/sup/3/SupplementaryTableS1.docx

Supplementary Table S2. The list of taxa occurring in plant canopy, seed rain or seedling pool in study Patches in the years 2014-2015. Names of Patches as in Table 1.; symbol "+" means presence, symbol "-" means absence.

Table S2 can be accessed via the link:
http://serbiosoc.org.rs/sup/3/SupplementaryTableS2.docx

Supplementary Table S3. Total number of seeds found in traps and total number of seedlings occurred in artificially created gaps in study Patches in the years 2014-2015. Names of Patches as in Table 1.

Table S3 can be accessed via the link:
http://serbiosoc.org.rs/sup/3/SupplementaryTableS3.docx