Biodiv. Res. Conserv. 28: 37-44, 2012

DOI 10.2478/v10119-012-0026-1

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The impact of neighbourhood and gap character on seedling recruitment of *Trollius europaeus* L. and *Iris sibirica* L. in *Molinietum 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: The observations were carried out in the years 2007-2010, in the *Molinietum caeruleae* meadows with different habitat conditions located in Kraków-Kostrze (southern Poland). The greatest number of seedlings of *Trollius europaeus* and *Iris sibirica* was recorded in patches dominated by low-statured species with delicate, procumbent stems or small-tussocks. The diminishing of offspring emergence in places prevailed by large-tussocks grasses, as well as in sites overgrown by willows could be a consequence of poor harvesting practices, as well as the stagnation of water in local depressions. Irrespective of patch charracter, the seedling recruitment did not occur in a fully compact plant canopy, the highest number of offspring was observed in gaps without moss and necromass layers, while the greatest abundance of genets was found in openings left after the removal of bryophytes, litter and above ground parts of plants. The decrease in offspring number noted in gaps resulted from the removal of living and died plants combined with top soil raking, which might caused the partial depletion of soil seed bank reserves. Regardless of the patch type, a significantly higher appearance of seedlings of *Trollius europaeus* and *Iris sibirica* was found in the largest gaps than in the smallest ones. In light of the performed studies, it might be concluded that gap creating seems to be a very effective way of active protection of *Iris sibirica* and *Trollius europaeus* populations.

Key words: active protection, endangered plants, gaps, *Iris sibirica, Molinietum caeruleae,* recruitment, seedlings, *Trollius europaeus*

1. Introduction

The seedling recruitment results in an increase of population abundance, facilitates its persistence in the occupied site, and maintains genetic diversity. Many theoretical and empirical studies also suggests that it can have major consequences for the structure and composition of plant communities (Wright 2002; Levine & Murrell 2003). On the other hand, germination is one of the most hazardous stages in the life cycle and offspring emergence in populations of several taxa occurs rarely in temporary openings in closed vegetation (Eriksson 1989, 1995; Forbis 2003; Forbis & Doak 2004). The seedling recruitment – a critical bottleneck in the population dynamics of numerous rare taxa –becomes one of the most important parameters to be considered in species conservation biology. Although, the phenomenon of offspring emergence was studied in a variety of threatened taxa (e.g Křenová & Lepš 1996; Dinsdale *et al.* 2000; Gulias *et al.* 2004; Chien *et al.* 2008; Chesser & Brewer 2011), further investigations are still strongly desired.

Nowadays, the globeflower *Trollius europaeus* and siberian iris *Iris sibirica* belong to rare taxa in entire Europe. In Poland, they represent strictly protected (Regulation… 2012), endangered species (Zarzycki & Szeląg 2006), included in the regional Red Lists of threatened plants in Pomerania (Markowski & Buliński 2004; Olszewski & Markowski 2006), Nizina Południowopodlaska (Głowacki *et al.* 2003), Wielkopolska region (Jackowiak *et al.* 2007), Opole province (Nowak 2002; Kącki 2002), as well as Lower Silesia (Kącki *et. al.* 2003). Moreover, *Iris sibirica* is enlisted in the Carpathian List of Endangered Species (Witkowski *et al.* 2003). In other European countries, the aforementioned taxa have various conservation statuses. *Trollius europaeus* is considered:

- endangered (EN) in Meklenburg (Ingelög *et al.* 1993), Brandenburg (Benkert & Klemm 1993), Ireland (Curtis & McGough 1988) and Hungary (Zoltan *et. al* 1990),

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- vulnerable (VU) in Schleswig-Holstein (Ingelög *et al.* 1993), and Belarus (Sačanka 1993),
- near threatened (NT) in Croatia (State Institute for Nature Protection 2004),
- least concern (LC) taxon in Great Britain (Cheffings & Farrell 2005).

Iris sibirica is classified as:

- extinct (EX) in Schleswig-Holstein (Ingelög *et al.* 1993),
- endangered (EN) in Kaliningrad and Mecklenburg (Ingelög *et al.* 1993),
- vulnerable (VU) in Lithuania (Ingelög *et al.* 1993), Croatia (State Institute for Nature Protection 2004), Switzerland (Moser *et al.* 2002).

Trollius europaeus is a perennial forb consisting of numerous dark green, deeply cut basal leaves and generative shoots linked with short caudice. The self-incompatible flowers are composed of numerous multi-ovulate carpels. The fruit consists of numerous follicles and contains several angular seeds. The long-lived individuals of *Iris sibirica* create numerous leaf rosettes and flowering shoots linked with permanent rhizomes with short internodes. The bumblebee-pollinated flowers are self-incompatible. The fruit is a capsule which opens up in three parts, revealing numerous seeds within. Although the populations of *Trollius europaeus* and *Iris sibirica* are found in woodlands, brushwoods and macroforbs, most frequently they are recorded in blue moor-grass meadows *Molinietum caeruleae* (Matuszkiewicz 2001).

Given the insufficient state of knowledge, the present studies were undertaken to investigate the recruitment processes in *Trollius europaeus* and *Iris sibirica* populations occurring in *Molinietum caeruleae* meadows. The detailed goals focused on the assessment of: (*i*) the impact of adjacent vegetation on seedling abundance, (*ii*) the influence of opening character on the appearance of genets, (*iii*) the effect of gap size on the emergence of generative offsprings.

2. Materials and methods

The studies were carried out in Kostrze district located on the western edge of Krakow, south of the Vistula River (southern Poland). The research area is at *ca* 210 m a.s.l. on a low flood terrace of the Vistula, 3.0-6.0 m high. The greatest area is taken up by unmanaged *Molinietum caeruleae* patches (Dubiel 1991, 1996). The studies were carried out in two study areas (A and B). In both of them, three adjacent, abandoned patches of *Molinietum caeruleae* with different species composition and habitat conditions were selected. The patches labeled MEADOW were dominated by meadow species creating delicate, procumbent stems or small-tussocks. The small-statured meadow species intercepted very low amount of irradiance, contributing to strong insolation and a fast decrease of groundwater level in early spring. The places called GRASSES were dominated by large-tussocks of *Molinia caerulea* and *Deschampsia caespitosa* grasses. The high, closely packed shoots shaded whole patches, whereas the hummock and hollows microtopography triggered stagnation of water in local depressions, particularly in spring and autumn. The sites designated as WILLOWS were dominated by willows: *Salix repens* ssp. *rosmarinifolia, S. cinerea,* as well as *S. aurita*. Wide and spreading willow leaves

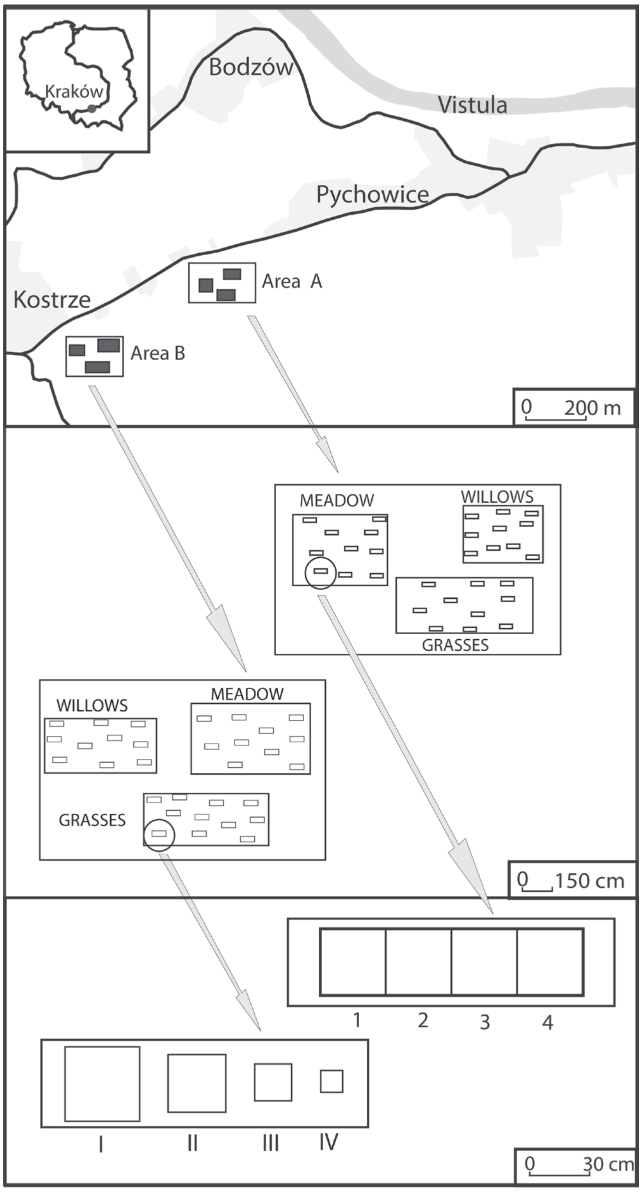

Fig. 1. The locality of the study areas and experimental design

Explanations: the patches of *Molinietum caeruleae*, MEADOW – dominated by small meadow species, GRASSES – dominated by large tussock grasses, WILLOWS – overgrown with willows. The experimental treatements, 1 – control subplots, 2 – subplots without litter and moss layer, 3 – subplots without litter, moss layer, and above-ground parts of plants, 4 – subplots without litter, moss layer, above-ground parts of plants and top soil. The area of experimental subplots, I – 0.16 m^2, II – 0.09 m^2, III – 0.04 m^2, IV – 0.01 m^2

strongly prevented the solar radiation and reduced water evaporation from soil surface.

In each of the above-described patches, ten permanent experimental plots were randomly established. They were established at least 2.0 meters from the border of patches to avoid edge effect. In area A, plots were divided into four square-shaped, neighboring subplots of 0.09 m^2. The subplots were subjected to no treatment (subplot 1), the removal of litter and moss layers (subplot 2), the removal of litter, bryophytes, and above-ground parts of plants (subplot 3) and removal of litter, moss and plants, as well as top soil raking to the depth 3-5 cm (subplot 4). In area B, plots were divided into four square-shaped adjacent subplots measuring 0.16 m^2 (subplot I), 0.09 m^2 (subplot II), 0.04 m^2 (subplot III), and 0.01 m^2 (subplot IV). In each of them, the litter and above-ground part of biomass were clipped and removed. The design of the experiment is shown in Fig. 1. The generative offspring establishment was monitored once a week in May, June, July and August, and once every two weeks in April, September and October, from 2007 through to 2010. Seedlings were counted in each subplot, using aluminium frame.

The effect of site conditions and gap character on the recruitment process was characterized by mean cumulative number of seedlings calculated by adding seedlings that appeared in particular plots (subplots) over the course of four years. The normal distribution of collected data was tested using the Shapiro-Wilk test, whereas the homogeneity of variances was checked using the Brown-Forshyte test. The data were not transformed to meet the normal distribution and improve homogeneity of variances. Because of inequality of variances, the statistical analysis was based on the nonparametric Kruskal-Wallis test which was applied to examine whether there were significant differences: (*i*) in mean number of seedlings that appeared in permanent plots among patches in both areas; (*ii*) in mean number of seedlings that appeared in different treatment (size) subplots within each patch.

3. Results

The recruitment of *Iris sibirica* and *Trollius europaeus* seedlings in patches with different habitat conditions varied significantly. The greatest abundance of *Trollius europaeus* genets was found in patches of MEADOW and they decreased gradually in sites of GRASSES and WILLOWS (Table 1). The highest number of *Iris sibirica* seedlings observed in places of MEADOW, dropped substantially in patches of GRASSES and WILLOWS (Table 2).

In all patches situated in both areas, the recruitment seedling of *Trollius europaeus* (Table 3) and *Iris*

Table 1. The recruitment of *Trollius europaeus* L. seedlings in patches of *Molinietum caeruleae* dominated by small meadow species (MEADOW), prevailed by large tussock grasses (GRASSES) and overgrown by willows (WILLOWS) in the years 2007-2010

The study area	Patch	The mean cumulative number of seedlings per experimental plot (±SD)	The statistical significance
Area A	MEADOW	137.70±50.31	a*, b***
	GRASSES	82.10±10.59	
	WILLOWS	60.50±20.79	
Area B	MEADOW	271.60±50.05	a*, b***
	GRASSES	178.10±30.60	
	WILLOWS	110.60±30.62	

Explanations: asterisks mean probability that average values of seedling number appeared in patches MEADOW and GRASSES (a), MEADOW and WILLOWS (b), WILLOWS and GRASSES (c) do not differ, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Table 2. The recruitment of *Iris sibirica* L. seedlings patches of *Molinietum caeruleae* dominated by small meadow species (MEADOW), prevailed by large tussocks grasses (GRASSES) and overgrown by willows (WILLOWS) in the years 2007-2010

The study area	Patch	The mean cumulative number of seedlings per experimental plot (±SD)	The statistical significance
Area A	MEADOW	163.90±61.36	a*, b***
	GRASSES	88.50±12.90	
	WILLOWS	61.30±24.58	
Area B	MEADOW	286.90±50.98	b***, c*
	GRASSES	207.70±20.66	
	WILLOWS	119.10±30.57	

Explanations: asterisks mean probability that average values of seedling number appeared in patches MEADOW and GRASSES (a), MEADOW and WILLOWS (b), WILLOWS and GRASSES (c) do not differ, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Table 3. The recruitment of *Trollius europaeus* L. seedlings in control subplots (1), subplots without moss and litter (2), subplots without moss, litter and above ground part of plants (3) and subplots without moss, litter, above ground part of plants and top soil (4) in patches of *Molinietum caeruleae* dominated by small meadow species (MEADOW), prevailed by large tussocks grasses (GRASSES) and overgrown by willows (WILLOWS) in the years 2007-2010

Patch	Subplot	Treatment	The mean cumulative number of seedlings per experimental subplot±SD	The statistical significance
MEADOW	1	No treatment	0±0	a*, b***, d***, e*
	2	The removal of moss and litter	21.80±11.98	
	3	The removal of moss, litter and above ground part of plants	73.30±55.16	
	4	The removal of moss, litter, above ground part of plants and top soil	42.60±23.17	
GRASSES	1	No treatment	0±0	b***
	2	The removal of moss and litter	12.50±10.17	
	3	The removal of moss, litter and above ground part of plants	49.80±12.75	
	4	The removal of moss, litter, above ground part of plants and top soil	19.80±10.93	
WILLOWS	1	No treatment	0±0	b***, d*
	2	The removal of moss and litter	13.00±10.05	
	3	The removal of moss, litter and above ground part of plants	28.90±21.07	
	4	The removal of moss, litter, above ground part of plants and top soil	18.60±10.57	

Explanations: asterisks mean probability that average values of seedling number appeared in subplots 1 and 2 (a), 1 and 3 (b), 1 and 4 (c), 2 and 3 (d), 2 and 4 (e), 3 and 4 (f) do not differ, $*P\leq0.05$, $**P\leq0.01$, $***P\leq0.001$

Table 4. The recruitment of *Iris sibirica* L. seedlings in control subplots (1), subplots without litter (2), subplots without litter and above ground part of plants (3) and subplots without litter, above ground part of plants and top soil (4) in patches of *Molinietum caeruleae* dominated by small meadow species (MEADOW), prevailed by large tussocks grasses (GRASSES) and overgrown by willows (WILLOWS) in the years 2007-2010

Patch	Subplot	Treatment	The mean cumulative number of seedlings per experimental subplot±SD	The statistical significance
MEADOW	1	No treatment	0±0	b***, c*
	2	The removal of moss and litter	33.40±21.59	
	3	The removal of moss, litter and above ground part of plants	89.50±43.40	
	4	The removal of moss, litter, above ground part of plants and top soil	41.00±31.16	
GRASSES	1	No treatment	0±0	b***, c*, d*
	2	The removal of moss and litter	11.60±10.07	
	3	The removal of moss, litter and above ground part of plants	57.00±11.56	
	4	The removal of moss, litter, above ground part of plants and top soil	19.90±12.28	
WILLOWS	1	No treatment	0±0	b*
	2	The removal of moss and litter	12.40±8.34	
	3	The removal of moss, litter and above ground part of plants	29.60±20.06	
	4	The removal of moss, litter, above ground part of plants and top soil	19.30±10.25	

Explanations: asterisks mean probability that average values of seedling number appeared in subplots 1 and 2 (a), 1 and 3 (b), 1 and 4 (c), 2 and 3 (d), 2 and 4 (e), 3 and 4 (f) do not differ, $*P\leq0.05$, $**P\leq0.01$, $***P\leq0.001$

Table 5. The recruitment of *Trollius europaeus* L. seedlings in subplots measured 0.16 m^2 (I), 0.09 m^2 (II), 0.04 m^2 (III) and 0.01 m^2 (IV) in patches of *Molinietum caeruleae* dominated by small meadow species (MEADOW), prevailed by large tussocks grasses (GRASSES) and overgrown by willows (WILLOWS) in the years 2007-2010

Patch	Subplot	Area [m^2]	The mean cumulative number of seedlings per experimental subplot±SD	The statistical significance
MEADOW	I	0.16	99.60±51.52	c*
	II	0.09	73.50±30.74	
	III	0.04	56.30±21.75	
	IV	0.01	42.20±10.61	
GRASSES	I	0.16	69.00±21.40	c*
	II	0.09	49.80±11.68	
	III	0.04	33.80±22.34	
	IV	0.01	25.50±9.97	
WILLOWS	I	0.16	49.20±10.93	c**
	II	0.09	27.90±11.10	
	III	0.04	21.00±8.49	
	IV	0.01	12.50±5.08	

Explanations: asterisks mean probability that average values of seedling number appeared in subplots I and II (a), I and III (b), I and IV (c), II and III (d), II and IV (e), III and IV (f) do not differ, *$P\leq0.05$, **$P\leq0.01$, ***$P\leq0.001$

sibirica (Table 4) did not occur in a fully compact plant canopy. The greatest abundance of seedlings was found in openings left after the removal of litter and aboveground parts of plants. Much lower offspring numbers were noted in gaps caused by the uncovering of plants and top soil, while the lowest recruitment rates were observed in subplots without necromass.

Significantly higher appearance of *Trolius europaeus* seedlings was found in the largest gaps than in the smallest ones. Intermediate recruitment values were observed in openings with medium dimensions (Table 5). The highest abundance of *Iris sibirica* seedlings was noted in the openings measuring 0.16 m^2. A considerable emergence of offsprings was recorded in gaps measuring 0.09 m^2 as well. Additionally, it should be pointed out, that recruitment values in the aforementioned subplots were significantly greater than in openings with smaller sizes (Table 6).

Table 6. The recruitment of *Iris sibirica* L. seedlings in subplots measured 0.16 m^2 (I), 0.09 m^2 (II), 0.04 m^2 (III) and 0.01 m^2 (IV) in patches of *Molinietum caeruleae* dominated by small meadow species (MEADOW), prevailed by large tussocks grasses (GRASSES) and overgrown by willows (WILLOWS) in the years 2007-2010

Patch	Subplot	Area [m^2]	The mean cumulative number of seedlings per experimental subplot±SD	The statistical significance
MEADOW	I	0.16	108.50±19.70	b*, c**, e*
	II	0.09	83.30±22.40	
	III	0.04	52.00±21.42	
	IV	0.01	43.10±25.98	
GRASSES	I	0.16	78.80±31.19	c***, e**
	II	0.09	62.80±13.54	
	III	0.04	43.40±11.50	
	IV	0.01	22.70±12.80	
WILLOWS	I	0.16	49.80±26.48	b*, c*
	II	0.09	36.10±18.85	
	III	0.04	22.00±12.00	
	IV	0.01	11.20±6.78	

Explanations: asterisks mean probability that average values of seedling number appeared in subplots I and II (a), I and III (b), I and IV (c), II and III (d), II and IV (e), III and IV (f) do not differ, *$P\leq0.05$, **$P\leq0.01$, ***$P\leq0.001$

4. Discussion

The process of seedling recruitment in fen meadows is affected by the character of adjacent vegetation. The neighbouring plant regulates the level of soil irradiance (Kotowski *et al.* 2001; Kotowski & van Diggelen 2004). In the light of these findings, the greatest number of seedlings recorded in site MC might be caused by low interception of solar radiation by small-statured meadow species. The decrease of offspring emergence in GR and SA patches could be the consequence of light harvesting by tall grasses or willows with wide and spreading leaves. A similar effect of plant growing in the vicinity on recruitment of *Iris sibirica* and *Trollius europaeus* seedlings was observed previously by Kostrakiewicz (2007, 2010) and Muncaciu *et al*. (2010). Also, Hitchmough (2003) noted greater emergence of *Trollius europaeus* seedlings in mown, than in unmanaged wet grasslands.

Moreover, it is widely accepted that the habitat conditions in fen meadows are controlled by water table depth (Okruszko 1995). The greatest number of seedlings recorded in site MC might be caused by the decrease of groundwater level in early spring which accelerated seed germination. The suppression of offspring emergence in patches GR and SA could be due to stagnation of water in hollows occurring between grass tussocks or in local depressions appearing between creeping stems of *Salix rosmarinifolia*. A similar impact of water table depth on recruitment process was found in populations of *Iris pseudacorus,* where the greatest germination rates were observed in moist but not waterlogged conditions (Falińska 1986; Lenssen *et al.* 1988; Coops & van der Velde 1995).

The performed studies demonstrated that regardless of patch character, the removal of moss and litter layers contributed to a slight appearance of *Trollius europaeus* and *Iris sibirica* seedlings, while lack of litter and aboveground parts of plants provided the best conditions for genet appearance. These findings are contrary to observations of recruitment process of *Iris spuria* (Hölzel 2005). The aforementioned author reported that lack of necromass and plant cover strongly inhibited seed germination, while the addition of decayed matter facilitated successful establishment of generative offsprings. The decrease of recruitment of *Trollius europaeus* and *Iris sibirica* seedlings in openings left after the removal of dead and living plants, as well as bare ground raking might be caused by partial depletion of seed bank. Also, the top soil and vegetation removal caused abiotic stress due to ammonium accumulation which might hamper the seedling establishment (Dorland *et al.* 2003). The diminishing of recruitment of globeflower seedlings might be caused by non viability of seeds buried in the soil for several months (Milberg 1994).

It is commonly accepted that the area of gaps may affect the recruitment rates in plant populations. The presented studies provide evidence that the higher appearance of *Trollius europaeus* and *Iris sibirica* seedlings which occurred in larger gaps than in smaller ones was in accordance with the investigations carried out in populations of *Bouteloua gracilis* (Auguilera & Laurenroth 1993), *Rutidosis leptorrhynchoides* (Morgan 1997), *Themeda triandra* (Morgan 1998) and *Bromus inermis* (Liu *et al.* 2008). On the other hand, the obtained results did not confirm sowing experiments of Hitchmough (2003), who asserted that the performance of generative offsprings of *Trollius europaeus* was not affected by gap size.

In the light of the performed investigations, it should be concluded that creating gaps which facilitate the germination of seeds and seedling development is an effective way of active protection of *Iris sibirica* and *Trollius europaeus* populations. Although the highest recruitment rates were noted in meadows dominated by small-statured species, making gaps in patches overgrown by large tussock grasses and willows seems to be promising as well. Irrespective of the character of standing vegetation, the best results were observed in the case of removal of plant canopy and litter, allowing the reduction of below-ground competition and not destroying the reserves of soil seed bank. Additionally, it should be pointed out that the recruitment success of both species increased together with the enlargement of opening area.

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