

Original Research

Effects of Different Introduction Methods on Plant Species Establishment Success in Wet Grassland Restoration

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Abstract

The assessment of plant species' introduction efficiencies for wet meadows was the aim of our study. The studies were carried out as part of a project focusing on flooded meadow restoration in the Middle Vistula Valley. Twenty-six plant species of flooded meadow were in the seedling material used for renovation. The seedbed preparation covered the cutting of vegetation, ploughing, and rototilling, removing the topsoil. The diaspores after seeding were rolled and, on half of the plots, mulched with straw. The number of species, their occurrence frequency, and population abundance as well as the soil coverage by plants in the first year and the third year after diaspore seeding were determined. The seedbed preparation has restricted the occurrence and growth of *Solidago* sp., and increased soil coverage by meadow species. The share of introduced species in the sward ranged from 5% to 27% of coverage. The removal of the sod was significantly more efficient than other methods of seedbed preparation for plant species introduction to the renovated meadows, especially the species characteristic of the *Cnidion dubii* meadows. Mulching with straw generally has not improved the renovation results. Generally, *Sanguisorba officinalis*, *Achillea millefolium*, and *Hypericum perforatum* were the species of most frequent occurrence.

Keywords: wet meadows, restoration, seedbed preparation, meadows species, species occurrence

Introduction

River valleys are the sites of high biological diversity and high potential of ecosystem services. The significant parts of those valleys are covered by

meadow communities of *Arrhenatherion elatioris*, *Molinion caeruleae*, and *Cnidion dubii* meadows. The *Cnidion dubii* communities occur from southern Russia and through Ukraine, Poland, and eastern Germany into the Panonian basin and Rhine valley of southwestern Germany, France, and alpine regions [1]. In Central Europe the highest area of their presence is presumed to be located in Hungary (about 42000 ha). In Poland the area covered by these meadows is 1640 ha

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and is of a decreasing trend. *Cnidion dubii* meadows are located in fertile alluvial regions alongside the central and lower valley sections of the great rivers, which are components of the Large Valleys Belt [2]. Due to their regular flooding one or two times a year and dryings between floods they play an important role in the protection of water resources, soils, biodiversity, and landscapes.

In phytosociology the *Cnidion dubii* Bal.-Tul. 1966 alliance is classified to the order *Molinietalia*, which belongs to the class *Molinio-Arrhenatheretea*. The only representative of the *Cnidion dubii* alliance is the *Violo-Cnidietum dubii* association. The typical aspect of that association occurs in western Poland [3]. The characteristic species of those meadows, outwith *Cnidium dubium*, are *Allium angulosum*, *Sanguisorba officinalis*, *Gratiola officinalis*, *Scutellaria hastifolia*, and *Viola stagnina*. *Cnidion dubii* meadows usually occur in mosaic with other types of meadows and rarely occupy vast areas. They are often adjacent to *Alopecurion* meadows. In the area of lower-lying meadows they border *Phalaridetum arundinaceae* or with various *Magnocaricion* communities. *Cnidion dubii* meadows are protected due to a habitat directive (code 6440). The protection of *Cnidion dubii* meadows results from the fact that the regulation the majority of large rivers as well as their scarcity and diminishment of those sites [4]. Their existence is also conditioned by extensive management, which consists of cutting one or two times annually.

Due to the deterioration of those natural sites in the EU, many activities are being undertaken on their restitution, mostly in Great Britain, Germany, Holland, the Czech Republic, and Estonia [5-11]. The threat for Polish flora, although less significant than in Western European countries, is progressing rapidly [12-14]. The most serious factors comprising threats to the biodiversity of *Cnidion dubii* meadows is, besides the change of water regime, the abandonment of cutting management. The scale of these threats is reflected by the shrinking surface of the typical associations that

are being replaced by incomplete communities and by the disappearance of the species characteristic of associations and alliances [15]. The status of 51% of *Cnidion dubii* meadows is estimated as poor and of 27% as bad [16]. It is absolutely necessary to establish new reserves dedicated to the conservation of the best *Cnidion dubii* meadows. Protection throughout the agro-environmental schemes and within the framework of Natura 2000 is also a considerable conservation achievement. The necessity of restraining biodiversity loss in the UE is underlined in the the EU Biodiversity Strategy to 2020 [17]. Environmental Liability Directive 2004/35/EC [18] strengthens the preservation of natural resources and ecosystem services, being bound with the Habitat Directive and the Birds Directive [19-20] and creating a system of responsibilities for preventing and harms' repairing regarding biodiversity. Accordingly with the regulations of these directives, the persons and institutions whose activities directly threaten the environment are also responsible for undertaking preventative actions. In Poland, most often the studies of actions are being implemented in the scope of the restoration of xerothermic meadows [21] and fen habitats [22-23], or removing invasive plants – primarily *Solidago* sp. [24-26].

Property management of the city of Warsaw is stepping toward the needs of restitution of grasslands in the Middle Vistula Valley (PLB 140004) and the restitution of half-open, partially wooded landscapes with a significant share of species-rich meadows of flooded areas of the Vistula River interbank in the area of 67 ha. The restitution for improving the structure and functions of meadow plant communities is connecting, in the case of the lack of soil seedbank, with the introduction of seeds of the most important species and, in following steps, working out the rules of management (type and frequency cutting or grazing) adjusted to the growth rhythm of those species [27-28].

Assessing the possibilities of the introduction of the target species of wet grasslands is the aim of our study.

Table 1. Weather conditions in growing seasons 2015-2016 (Meteorological Station Ursynów-Warsaw).

Year	Month						Growing season
	IV	V	VI	VII	VIII	IX	IV-IX
Mean temperature (°C)							
2015	9.1	13.7	18.1	20.5	23.0	15.6	16.7
2016	10.0	16.3	19.8	20.3	19.3	16.5	17.0
Sum of precipitation (mm)							
2015	31.4	57.7	37.5	66.2	11.3	73.1	277.2
2016	34.2	23.3	56.9	116.5	71.7	9.8	312.4
Characteristics of growing seasons				Hydrothermal index of Vinczeffý ($\sum \text{mm} \cdot \sum ^\circ \text{C}^{-1}$)			
2015	Extremely dry			0.091			
2016	Very dry			0.100			

Table 2. Characteristics of introduced plant species.

No.	Species	Family	Life form	Durability	Diaspore	Type of seed bank
1	<i>Achillea millefolium</i> L.	<i>Asteraceae</i>	H	B	fruit	T, LP
2	<i>Allium angulosum</i> L.	<i>Amaryllidaceae</i>	G	B	seed	SP
3	<i>Armeria maritima</i> (Mill.) Willd.	<i>Plumbaginaceae</i>	H	B	fruit	T
4	<i>Artemisia campestris</i> L.	<i>Asteraceae</i>	C, H	B, S	fruit	T, SP, LP
5	<i>Centaurea stoebe</i> L.	<i>Asteraceae</i>	H	B, D	fruit	T
6	<i>Cnidium dubium</i> (Schkuhr) Thell.	<i>Apiaceae</i>	H	B	fruit	T
7	<i>Dianthus deltoides</i> L.	<i>Caryophyllaceae</i>	C	B	seed	T
8	<i>Eryngium planum</i> L.	<i>Apiaceae</i>	H	B	fruit	T
9	<i>Galium boreale</i> L.	<i>Rubiaceae</i>	H	B	fruit	T
10	<i>Hypericum perforatum</i> L.	<i>Hypericaceae</i>	H	B	fruit	LP
11	<i>Inula britannica</i> L.	<i>Asteraceae</i>	H	B	fruit	T, SP
12	<i>Leucanthemum vulgare</i> Lam.	<i>Asteraceae</i>	H	B	fruit	SP, LP
13	<i>Linaria vulgaris</i> Mill.	<i>Plantaginaceae</i>	G, H	B	seed	T, SP
14	<i>Lysimachia vulgaris</i> L.	<i>Primulaceae</i>	H	B	seed	T, SP, LP
15	<i>Lythrum salicaria</i> L.	<i>Lythraceae</i>	H	B	seed	T, SP, LP
16	<i>Plantago lanceolata</i> L.	<i>Plantaginaceae</i>	H	B	seed	LP
17	<i>Potentilla erecta</i> (L.) Raeusch.	<i>Rosaceae</i>	H	B	fruit segments	T, SP
18	<i>Rumex acetosa</i> L.	<i>Polygonaceae</i>	H	B	fruit	SP
19	<i>Sanguisorba officinalis</i> L.	<i>Rosaceae</i>	H	B	fruit	T
20	<i>Scabiosa ochroleuca</i> L.	<i>Dipsacaceae</i>	H	B, D	fruit	LP
21	<i>Thalictrum flavum</i> L.	<i>Ranunculaceae</i>	H	B	fruit	T
22	<i>Tragopogon pratensis</i> L.	<i>Asteraceae</i>	H	D	fruit	T
23	<i>Trifolium arvense</i> L.	<i>Fabaceae</i>	T, H	A, D	seed	LP, T
24	<i>Verbascum thapsus</i> L.	<i>Scrophulariaceae</i>	H	D	seed	LP, T
25	<i>Veronica longifolia</i> L.	<i>Plantaginaceae</i>	H	B	seed	T, SP
26	<i>Veronica spicata</i> L.	<i>Plantaginaceae</i>	C, H	B	seed	T, LP

Life form: H – hemicryptophyte, G – geophyte, C – herbaceous chamaephyte, T – therophyte. Durability: B – perennial, D – biennial plant, S – subshrub. Type of seed bank: LP = long-term persistent, SP = short-term persistent, T = transient

Material and Methods

The studies were carried out in the Vistula River interbank on Białoleka object (geographical coordinates: 52°29' N; 20°96' E) in 2014-2017. The studies were carried out as part of the "Restitution of the flooded meadows on Warsaw cross-section of Natura 2000 site Middle Vistula Valley (PLB 140004)" project.

The soil is averagely coherent with grain size distribution of the silt sand originating from the loam. In the 0-20 cm layer of the soil rubbish occurs, because storage of construction materials began in 1979. That area is being flooded at high water levels.

Weather conditions in the study period were not convenient for plant development. The value of

Vinczeffy [29] hydrothermal index, calculated as the quotient of total precipitation to the sum of temperature, was very low for growing season 2015 and amounted to 0.091 mm °C⁻¹, which means an extremely dry season (Table 1). The growing season of 2016 was not as dry as in 2015, but the rain distribution was very unfavorable. The value of the Vinczeffy hydrothermal index for growing season 2016 amounted 0.100 mm°C⁻¹, which points to the period being very dry. With the exception of a wet July, in which the precipitation was equal to 0.185 mm per 1°C, remaining months of the growing season were very dry. May and September were extremely dry, with hydrothermal indexes amounting to, respectively, 0.046 and 0.020 mm°C⁻¹.

The area was used as meadow and pasture (cattle and horse grazing). The construction of Tarchomin house settlement and the abandonment of mowing and grazing resulted in a succession of trees and shrubs and massive development of *Solidago* sp. (alien invasive) and *Erigeron annuus* (anthropophyte, domesticated, invasive). Preparation of the terrain for experiment covered the cuttings of a few trees and two clumps of *A. negundo* of less than 10 years old, and vegetation mowing.

We introduced 26 plant species of *Cnidion dubii* meadows (6440), taken from well preserved natural sites from the neighborhood of Warsaw (lower Pilica Valley: PLH 140016, 51°51' N; 21°15' E and 51°50' N; 21°17' E). The characteristics of introduced plant species and type of seed bank persistence according to Thompson [30] are presented in Table 2. The diaspores were harvested by hand on 23.09.2014. The seed rate of seedling material (diaspores with the inflorescences) was equal to 10 g m⁻², which corresponds to the recommendations of 2-5,000 of the diaspores per 1 m² [31].

In the experiment three ways of seedbed preparation were applied: 1) low cutting of vegetation, 2) ploughing to a depth about 20 cm and rototilling without surface leveling, and 3) removal the sward and topsoil (30 cm layer). In each variant two methods of diaspore sowing were applied: 1) sowing without straw mulching and 2) sowing with a thin layer of rye straw coverage and control variant without sowing for assessment of a soil's seed bank. The diaspores were sown by hand onto the prepared areas and rolled on with a light roller on 25 October 2014. The experiment was carried out on the plots (10 m x 12 m) with six replications. The plots in the sequence of the belts with various seedbed preparation were drawn, and the methods of sowing were arranged in split plot. The total number of plots was 54 (Fig. 1).

In mid-July 2015, before the flowering of *Solidago* sp., the cultivation cut was made at a height of 25 cm. In subsequent years the mowings were done once a year, in autumn.

Within the framework of the study, the effectiveness of restricting the invasive species share in the sward and the abundance of the sown species population were analyzed. The assessment of plants during vegetation period 2015 was carried out every month on 1 m² area (1 m x 1 m, micro plot), with 6 replications of each

investigated variant. The total number of sown species was registered and the quantity of species population was expressed in scale 1-6 (where 6 is the number of occurrences of the species on all plots, and 1 is the occurrence on one plot). The abundance of population was determined on points scale 0-5 on the basis of their occurrence in the sward (0 is lack of plants, 1 is single, 2 is group, 3 is pretty numerous, 4 is numerous, and 5 is very numerous).

The assessment of groundcover with plants was determined. The botanical composition of the sward was determined by noting the occurring species and the percentage of invasive and dominant ones. The state of the sward in spring 2017 was assessed not on micro plots, but on the entire area of particular tested treatment.

The obtained results of measurement and values of scale grades were analyzed statistically using the variance analysis (ANOVA). The verification of the difference significance was based on Tukey's test on the level of confidence 0.05.

Results and Discussion

The survey of the floristic composition of the sward, carried out in 2015, showed the presence of 17 species from 26 sown ones. The similar effectiveness of the introduction (total number of target species in the range 51.5–57.6) was obtained for the *Molinion* meadows in Wielkopolska Lowland [1]. The species number was affected by the method of seedbed preparation (Table 3). The lowest number of species was noted on plough and plots, independently from the method of diaspore coverage (12 in total), but the lowest one on the variant with removed topsoil (16-17 in total). The mean number was 2-3 times lower and amounted from 4 to 8 (on average 6) species.

The best conditions for the development of most species and frequency of their occurrence were noted in the variant with removed topsoil without straw mulching, where eight species have developed (Table 4). Among them were the four characteristic of *Cnidion dubii* meadows (*A. angulosum*, *S. officinalis*, *G. boreale* and *C. dubium*), two of *Arrhenatherion* meadows (*A. millefolium*, *P. lanceolata*), and two of dry meadows (*A. campestris*, *H. perforatum*). A particularly high

Removed topsoil without sowing	Ploughing and rototilling without sowing	Cutting of sward without sowing	4m
Removed topsoil without straw mulching	Ploughing and rototilling without straw mulching	Cutting of sward without straw mulching	4m
Removed topsoil with straw mulching	Ploughing and rototilling with straw mulching	Cutting of sward with straw mulching	4m
2 m	4 m	4 m	

Fig 1. Scheme of the experiment.

Table 3. Number of introduced species depending on seedbed preparation and the sowing methods in 2015 and 2017.

Number species	Cutting of sward		Ploughing and rototilling		Removed topsoil	
	Straw mulching					
	yes	no	yes	no	yes	no
Year 2015						
Total	14	14	12	12	17	16
Mean	6ab	6ab	4a	6ab	7ab	8bc
Year 2017						
Total	12	13	10	10	14	14
Mean	6ab	6ab	5a	6ab	8ab	9bc

occurrence was shown by *A. angulosum* and *S. officinalis* (6 and 5,7 points on a 1-6 scale) in that the variant of seedbed preparation before sowing. Topsoil removal, which leads to lowering of the soil surface and reduces the distance from groundwater, may be used to provide hydrological conditions, which are adequate for target species [31-32]. Ploughing without mulching favored the growth of the same species as the removal of the topsoil layer, but with the frequency lower by about 30% on average. In the case of that method of soil preparation the biggest frequency of the occurrence (5 points in 1-6 scale) was shown by 2 species characteristic of *Cnidium dubii* meadows: *S. officinalis* and *C. dubium*. Lower frequency of occurrence was characteristic for the species on mown plots, uncovered by straw (averaging 3.7 pts. on a 1-6 scale).

Mulch coverage limited the number and the occurrence frequency of the species. In the variants with mowing and ploughing only 3 species (*S. officinalis*, *A. millefolium*, *C. dubium*) were stated, with the occurrence equal to at least 3 points (on a 1-6 scale). The mulching in the variant with removed soil created better conditions for initial growth of the sown species. In such conditions the occurrence of the 7 species, with frequency from 3,7 to 5 pts. on a 1-6 scale. The highest values of this parameter were typical for *A. angulosum*,

A. millefolium, and *A. campestris* – species characterized by various demands for water conditions (periodically wet, fresh, or dry sites). It is in accordance with other studies reported that the dead plant material (litter) had an overall negative effect on seed germination and seedling establishment [33], which creates a mechanical barrier to seedling emergence and causes a change in physical conditions (light, moisture, and temperature regimes), and can also release allelochemicals, which may be toxic. In flooded grasslands, the effects of the litter were neutral or negative, because high soil moisture levels in litter for longer periods of time might also promote the development of pathogens, which may increase seedling mortality [34]. The results obtained by Eckstein and Donath [35] suggest that the transfer of seed-containing plant litter may support the restoration projects if applied at 0.2-0.4 kg m⁻². Below that range, the establishment of most species may be inhibited by drought, while higher amounts would increasingly suppress the emergence of the seedlings, especially of small-seeded species.

Statistical analysis of the occurrence frequency of four characteristic species of *Cnidion dubii* meadows in dependence on the method of restitution has not shown the significant differences only in the variant with vegetation cutting (Table 5). In the variant with

Table 4. Frequency of occurrence of introduced species depending on seedbed preparation in 2015; the table contains only species with the highest frequency of occurrence (≥ 3.0 according to 6° scale).

Introduction method	Straw mulching	
	Yes	No
Cutting of sward	<i>S. officinalis</i> (4.5), <i>A. millefolium</i> (3.3)	<i>S. officinalis</i> (4.0), <i>A. millefolium</i> (4.0), <i>C. dubium</i> (3.7), <i>G. boreale</i> (3.7), <i>H. perforatum</i> (3.0) <i>A. angulosum</i> (3.0)
Ploughing and rototilling	<i>C. dubium</i> (3.0), <i>S. officinalis</i> (3.0)	<i>S. officinalis</i> (5.3), <i>C. dubium</i> (5.0), <i>P. lanceolate</i> (4.3), <i>A. millefolium</i> (3.7), <i>A. campestris</i> (3.3) <i>A. angulosum</i> (3.0), <i>G. boreale</i> (3.0), <i>H. perforatum</i> (3.0)
Removed topsoil	<i>A. angulosum</i> (5.0), <i>A. millefolium</i> (5.0), <i>A. campestris</i> (5.0), <i>S. officinalis</i> (4.7), <i>H. perforatum</i> (4.7), <i>P. lanceolata</i> (4.0), <i>G. boreale</i> (3.7)	<i>A. angulosum</i> (6.0), <i>S. officinalis</i> (5.7), <i>A. campestris</i> (5.3), <i>H. perforatum</i> (5.0), <i>G. boreale</i> (4.7), <i>P. lanceolata</i> (4.3), <i>A. millefolium</i> (4.0), <i>C. dubium</i> (3.7)

Table 5. The frequency of occurrence of the species characteristic of *Cnidion dubii* meadows in dependence on the introduction method in 2015 (scale 1-6 points).

Introduction method	<i>Allium angulosum</i>	<i>Cnidium dubium</i>	<i>Galium boreale</i>	<i>Sanguisorba officinalis</i>	Mean
Cutting of sward with straw mulching	2.3a*	1.7a	2.0a	4.5a	2.6B
Cutting of sward without straw mulching	3.0a	3.7a	3.7a	4.0a	3.6B
Cutting of sward without sowing	0.7a	0.0a	0.7a	0.7a	0.5A
Ploughing and rototilling with straw mulching	1.0a	3.0b	2.7b	3.0b	2.4B
Ploughing and rototilling without straw mulching	3.0a	5.0b	3.0a	5.3b	4.1 C
Ploughing and rototilling without sowing	0.7a	0.0a	0.7a	0.7a	0.5A
Removed topsoil with straw mulching	5.0b	1.0a	3.7 b	4.7b	3.6B
Removed topsoil without straw mulching	6.0b	3.7a	4.7ab	5.7b	5.0C
Removed topsoil without sowing	0.7a	0.0a	0.0a	0.7a	0.3A
Mean	2.5AB**	2.0A	2.3AB	3.2B	

*a-c the same letters in rows are not significantly different; **A-C the same letters in rows and in columns (for mean) are not significantly different

Table 6. Population abundance in dependence on seedbed preparation and the methods of sowing in 2015 and 2017 (average values for the plots where the species occurred, according to a 5-point scale).

Introduction method	Cutting of sward		Ploughing and rototilling		Removed topsoil		Mean
	Straw mulching						
	yes	no	yes	no	yes	no	
Species	Year 2015						
<i>A. millefolium</i>	1.0	3.5	1.5	1.0	3.2	3.3	2.2d*
<i>A. angulosum</i>	4.0	3.6	2.0	1.7	2.7	2.4	2.7de
<i>A. campestris</i>	1.0	2.0	1.0	1.0	2.7	2.7	1.7cd
<i>C. dubium</i>	2.0	2.0	1.0	2.5	1.0	1.0	1.6cd
<i>D. deltoides</i>	-	-	-	-	1.0	1.0	0.3a
<i>E. planum</i>	1.0	1.0	1.0	1.0	1.5	1.0	1.1bc
<i>G. boreale</i>	1.0	1.0	1.5	2.0	1.0	1.0	1.2b
<i>H. perforatum</i>	1.0	2.0	3.0	2.0	2.5	2.5	2.2d
<i>L. vulgare</i>	1.0	1.0	-	-	1.0	1.0	0.7ab
<i>P. lanceolata</i>	1.0	2.5	1.0	1.0	1.0	2.5	1.5c
<i>P. erecta</i>	1.0	1.0	1.5	1.0	1.0	1.0	1.1bc
<i>R. acetosa</i>	-	1.0	-	1.0	1.0	1.0	0.7ab
<i>S. officinalis</i>	1.0	3.0	1.0	1.5	1.5	1.5	1.6c
<i>S. ochroleuca</i>	-	1.0	-	1.0	1.0	1.0	0.7ab
<i>T. pratensis</i>	1.0	1.0	-	-	1.0	1.0	0.7ab
<i>V. thapsus</i>	1.0	-	1.0	-	3.2	1.0	1.0b
<i>V. longifolia</i>	1.0	-	-	-	1.0	-	0.3a
Mean	1.1a*	1.5b	0.9a	1.0a	1.7b	1.5b	
Species	Year 2017						
<i>A. millefolium</i>	2.2	2.6	2.3	2.3	3.2	3.9	2.8d

Table 6. Continued.

<i>A. angulosum</i>	-	-	-	-	1.0	-	0.2a
<i>A. campestris</i>	1.0	1.0	1.0	1.2	1.9	2.5	1.4bc
<i>C. dubium</i>	1.2	1.0	1.2	1.0	1.0	-	0.9ab
<i>D. deltoides</i>	-	-	-	-	1.0	1.9	0.5ab
<i>E. planum</i>	1.0	1.4	1.0	1.2	2.0	1.6	1.4bc
<i>G. boreale</i>	1.0	2.0	1.0	1.0	1.5	1.0	1.2b
<i>H. perforatum</i>	3.2	2.3	2.2	1.8	2.1	2.2	2.3d
<i>L. vulgare</i>	2.0	1.0	-	-	1.0	1.2	0.9ab
<i>P. lanceolata</i>	1.6	1.2	1.5	1.5	1.6	1.4	1.9cd
<i>P. erecta</i>	2.2	1.0	1.8	1.2	1.6	1.2	1.5c
<i>R. acetosa</i>	-	1.0	-	-	-	1.0	0.3a
<i>S. officinalis</i>	1.0	1.6	1.2	1.3	1.8	1.6	1.4bc
<i>S. ochroleuca</i>	-	1.0	-	1.0	-	1.0	0.5ab
<i>T. pratensis</i>	1.0	1.0	-	-	-	1.0	0.5ab
<i>V. thapsus</i>	1.0	-	1.0	-	2.5	1.2	0.9ab
<i>V. longifolia</i>	-	-	-	-	1.0	-	0.2a
Mean	1.1b	1.1b	0.8a	0.8a	1.4c	1.3bc	

*a-d the same letters in rows and in columns (for mean) are not significantly different

plough and rototilling a significantly lower frequency was shown by *A. angulosum*, and in variant without mulching also by *G. boreale*. The mentioned species occurred with more than 40% lower frequency than *C. dubium* and *S. officinalis*. In the variants with removed soil *C. dubium* developed in the weakest form, especially on mulching plots. It occurred averagely over twofold seldom than other species in that group (*A. angulosum*, *G. boreale*, *S. officinalis*). On the control areas the species of *Cnidion dubii* meadows occurred sporadically. The analysis of mean values of the species occurrence frequency allowed for the distinction of two groups of the methods of *Cnidion dubii* meadows renovation. The methods of the best effect in the first period after seeding were topsoil removal and plough without mulching. Irrespective of the method, the highest statistical frequency of occurrence was shown by *S. officinalis*, and the lowest by *C. dubium*, which may be connected with species diaspore sizes as the result of previous studies showing that smaller-seeded species tend to have higher light demands for germination. The ones of *C. dubium* are about 5-times smaller and characterized themselves by lesser germination ability than the diaspores of *S. officinalis* [36, 37].

It should be underlined that in the case of *C. dubium* the best effects of restitution are given by ploughing without mulching, which probably originates from that species' reaction on nitrogen release due to the mineralization of the organic substrate after cultivation [38]. The restoration effects of *A. angulosum* and

G. boreale were similar. Both of them showed the best occurrence frequency in the variant with removed sod without mulching.

In 2015 the sown species differentiated themselves not only by occurrence frequency but also by population abundance (Table 6). The best development was shown by one species of *Cnidion dubii* meadows – *A. angulosum*, especially in the variant with cutting (3.6, 4.0 pts. in the scale 0-5), one of the *Arrhenatherion* meadows – *A. millefolium*, and one of dry meadows – *H. perforatum* (2.2 pts. on average). The lower abundances (1.5-1.7 pts. in average) were shown by two species of *Cnidion dubii* meadows (*C. dubium* and *S. officinalis*), one species of *Arrhenatherion* meadows (*P. lanceolata*), and one of dry meadows (*A. compestris*). The significant abundance was noted also in the case of *V. thapsus*, but only on the plots with removed sod and mulching (3.2 points). The recent studies indicated that under dry conditions (e.g., dry grasslands or dry periods) the presence of dead plant tissues had a positive effect on seedling establishment [34]. The remaining species occurred singly. Among them, *D. deltoides* occurred only in the variant with removed sod, but *L. vulgare* and *T. pratensis* occurred in all variants with the exception of ploughing.

The species seeded in the variant with removed sod and cutting without mulching characterized themselves by significantly higher population abundance. The second group of methods were the variants with plough and cutting with mulching

Table 7. Soil coverage (%) depending on the introduction method in 2015 and 2017.

Introduction method	Year 2015			Year 2017			
	<i>Solidago</i> sp.	Other species	Total coverage	<i>Solidago</i> sp.	Introduced species	Other species	Total coverage
Cutting of sward with straw mulching	14.2b*	16.2ab	30.4c	35.0c	7.5ab	52.5c	60.0b
Cutting of sward without straw mulching	14.8b	18.1b	32.9c	7.5a	5.0a	45.0c	50.0b
Cutting of sward without sowing	13.5b	13.7ab	27.2b	11.5b	0.7a	34.3c	35.0a
Ploughing and rototilling with straw mulching	12.4b	13.3ab	25.7b	11.0b	10.5b	29.5b	40.0a
Ploughing and rototilling without straw mulching	12.9b	19.3b	32.2c	5.0a	8.5b	29.0b	37.5a
Ploughing and rototilling without sowing	16.3b	15.7ab	32.0c	11.5b	1.0a	29.0b	30.0a
Removed topsoil with straw mulching	0.2a	18.0b	18.2ab	1.0a	26.7d	15.8a	42.5a
Removed topsoil without straw mulching	0.1a	15.4ab	15.5ab	0.0a	19.0c	26.0b	45.0a
Removed topsoil without sowing	0.0a	6.7a	6.7a	10.0b	7.5	27.5b	35.0a

*a-d the same letters in columns are not significantly different

(1.0 point in average). The relatively low abundance of the seeded species was effected by the lack of introduced species in soil coverage in 2015 (Table 7).

The number of introduced species and their population abundance decreased in the investigation period (Tables 3, 6). In spring 2017 the number of species was about 1-3 species lower on average, and the mean a little bit higher (7 on average, from 5 to 9) than in 2015. Similarly to 2015, the majority of the species sown in 2017 were found on the variant with removed topsoil without straw mulching (14 species), but the minority on the cultivated plots – plough and rototilling (10 species). The significant differences in species number were stated only for the variant with removed sod without mulching and the one with ploughing and mulching. The decrease in the species number in time was stated also by [1]. But in some investigations the increase in the number of the species developed from the diaspores after a longer period of domination was noticed [39]. According to that paper, four years after the transfer of plant material a total of 92 species had successfully been established, among them 25 of the Red List.

Among the species typical of *Cnidion dubii* meadows the distinctive regress from the sward was shown by *A. angulosum*, which could be caused by long drought (Table 6). Despite the significant abundance of that species in the variant with vegetation cutting, after two years its presence was not proved, and in variant with removed sod it occurred singly. Almost twofold lower (in average) population abundance in 2017 was shown by *C. dubium* – the second characteristic species of *Cnidion dubii* meadows. The status of the *G. boreale* and *S. officinale* populations remained on a similar level as two years before.

Beside the species characteristic for *Cnidion dubii* meadows in peculiar variants the regression of

R. acetosa, *S. ochroleuca* and *V. longifolia* was stated. Those species occurred singly in 2015. The difficulties with the introduction and keeping the target species were pointed out by foreign studies [40-43]. Renovation of the wet and fen meadows is especially difficult [22, 44]. The removal of even a 50-cm layer of soil is being proposed for effective introduction of some plant species [31].

In 2017 not only the decrease but also the increase in the population abundance of the introduced species were stated. The species of *Cnidion dubii* (the community in regress) were replaced by the ones of *Arrhenatherion* meadows, especially *A. millefolium* (in variant with ploughing) and *P. lanceolata*. In the variant with removed sod the density of *D. deltoides* increased almost twofold, and the density of *E. planum* population of 30%. In the variant with vegetation cutting and mulching the population of *H. perforatum* and *P. erecta* increased 2-3 fold. Regardless of the individual species, the significantly larger population abundance in 2017 was typical for the areas with removed sod and the ones mowed without mulching. The mentioned methods may be consisted as one homogenic group. In the variant with removed sod that value averaged about 16% less, but the variant with vegetation mowing was about 36% bigger than in 2015. In the second homogenic group of methods, the mean population abundance was equal to 1 point on average and of about 10% lower than in 2015. It is difficult to estimate the obtained results because of a lack of studies concerning the individual species. As a rule the total number of the introduced species and the total coverage are given. For example, the total number of the species increased significantly in the restored semi-natural grasslands between 2001 and 2012, from 51.8 to 62.9 per site [45]. Because of various tolerance of the species for changing environmental conditions, the studies of individual species may

contribute to the success of community restoration [46-48].

The share of introducing species in the sward in the first year after sowing (2015) was low, most often below 1%, sporadically 5-7%. In spring 2017 their share was significant. They constituted from 5% to about 27% on sown areas (Table 7). Statistically the highest share of these plants was typical for the swards on the plots with removed topsoil. The sown species there constituted 64% of soil coverage on treatment with mulch and 42% without mulching. It should be pointed out that the share of introduced species on the plots without sowing was relatively high (7.5%), which testifies to the occurrence of these species diaspores in soil. These species were *A. millefolium*, *H. perforatum*, and *P. erecta*. These are the ones characteristic of drying habitats but not indicative species for *Cnidion dubii* meadows.

The decrease in the invasive species share, especially the high one of *Solidago* sp., increased soil coverage from about 26% to 46% on average (Table 7). The development of *Solidago* was most effectively restrained by removing of surface layer of soil. Besides the introduced species in the sward, the typical meadow species come into sight, grasses (*Arrhenatherum elatius*, *Elymus repens*, *Calamagrostis epigejos*, *Poa pratensis*, *Poa palustris*) and *Fabaceae* plants (*Medicago lupulina*, *Trifolium repens*, *Trifolium pratense*, *Vicia sepium*). The fast development of expansive species like *C. epigejos* and *E. repens*, which gradually increased their shares in the sward, threatens the development of the introduced species. Many target species exhibited low recruitment rates because their ability to disperse in time and space is low.

Conclusions

The investigated methods of meadow renovation on the studied area of the Middle Vistula Valley generally allowed restraining the *Solidago* sp. occurrence in plant communities in order to improve the botanical composition of the sward and to increase the terrain coverage by the meadow plants. The introduced species after three years since sowing covered from 5% to 27% of the area.

The effectivities of meadow species introduction depended on the intensity of seedbed preparation and the method of diaspore sowing. The removal of sod was the best way for the reduction of *Solidago* sp. occupation at the meadows. The number of introduced species was the highest (17) and their occurrence frequency was high.

The species characteristic for *Cnidion dubii* meadows – *Allium angulosum*, *Cnidium dubium*, *Galium boreale*, and *Sanguisorba officinalis* – initially had developed best on the variant with removed sod, with plough and rototilling but without mulching. *A. angulosum* and *C. dubium*, despite pretty well results in the first year, quickly retreated from the sward, which shows that their occurrence at a higher degree depends on keeping

the regime of water conditions than in the case of the rest of the species of that group.

The relatively simply introduction of *Sanguisorba officinalis* in wet sites, *Achillea millefolium* in moist sites, and *Hypericum perforatum* in drying sites shows the higher possibility of enriching the meadows with one or few species.

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Conflict of Interest

The authors declare no conflict of interest.

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