# Direct reseeding of degraded grass stands with various fodder species and genotypes of perennial legumes

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### Abstract

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During the period 2018-2021 in the ecological conditions of the Central Northern Bulgaria, a comparative test was conducted in terms of share and stability in the grass stand of different types and genotypes of perennial self-sown legumes, directly reseeded on a degraded pasture grass stand. The following variants were studied: *Trifolium pratense* L. Nika 11(2n) var. 1, Astur(2n) – var. 2 and a selection population obtained from the subspecies crossings *T. pratense* ssp. *pratense* and *T. pratense* ssp. *nivale* – var. 3; *Onobrychis viciifolia* Scop., Vishnovski – var. 4 and local population – var. 5; *Lotus corniculatus* L., Targovishte 1 – var. 6 and local pasture ecotype – var. 7; *Anthyllis vulneraria* L., Pamir cultivar – var. 8. According to the results, the type and genotype of the reseeded grasses cause significant differentiation regarding the share of the legume component in the degraded grass stand. The best results were obtained in reseeding with a selection population of red clover (*Trifolium pratense*) obtained from the subspecies crossings *T. pratense* ssp. *pratense* ssp. *nivale*, as well as with sainfoin (*Onobrychis viciifolia*) Vishnovski cultivar. The study of the mixed reseeding of the two species and genotypes under the conditions of Central Northern Bulgaria is well founded. The species of bird's-foot-trefoil (*Lotus corniculatus*) and kidney vetch (*Anthyllis vulneraria*), under conditions of early spring direct reseeding, fail to establish themselves in the grass stand, regardless of the adaptive potential of the used genotypes – cultivars and populations. Other methods of successful reseeding should be evaluated and selected.

Keywords: legumes; direct reseeding; Trifolium pratense; Lotus corniculatus; Onobrychis viciifolia; Anthyllis vulneraria

#### Introduction

Direct reseeding with legumes is a rational method of improving and restoring pastures and meadows. It is applied through different technologies adapted to specific climatic and edaphic conditions, to the type and mode of use of grass stands (Mocanu & Hermemean, 2009; Skládanka et al., 2016; Gaweł & Grzelac, 2020; Gaweł et al., 2022). A significant number of studies indicate that the inclusion of legume components by this method extends the longevity of economically profitable use of the grass stand, increases the productivity and quality of the obtained fodder (Golka et al., 2016; Lazarev et al., 2020; Vasileva & Vasilev, 2020; Grebennikov et al., 2021; Bozhanska & Iliev, 2021). The success of direct reseeding depends largely on the type of legume reseeded. It is also related to the availability of seeds of suitable cultivars. When selecting a cultivar for this purpose, it is necessary to assess its adaptability to the habitat, including the existing grass community, as well as to the regime of use of the grass stand (Abusuwar, 1993; Riday, 2008). Main fodder-grass crops, such as red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) have a high success rate in direct reseeding (Lazarev & Avdeev, 2018), but because of their low grazing sustainability, they again fall out from the grass stand when are used for a pasture or combined (Georgieva & Nikolova, 2012; Mantino et al., 2016; Marinova, 2017). This imposes a selection interest in local populations and wild species/subspecies of legumes or in non-traditional for Bulgaria species that have specific biological characteristics serving the purposes of direct reseeding. The subterranean clover, common vetch, red clover, arrow-leaf clover, black medick have been defined as such in previous Bulgarian studies (Naydenova & Vasileva, 2016; Vasileva, 2017).

Red clover (*Trifolium pratense* L.) is the most commonly used legume for direct reseeding, because of its rapid seedling growth under competitive conditions, shade tolerance and good compatibility with the most common fodder grass species (Riday, 2008). This species is a major legume component of grass mixtures for combined use in Northern Bulgaria (Bozhanska & Churkova, 2019). Direct reseeding appears to be a successful method for its maintenance as a significant component of the grass stand (Skládanka et al., 2016).

Bird's-foot-trefoil and sainfoin are other major legume crops for Bulgarian conditions. They have higher longevity and resistance in grass stands in combined and pasture use compared to red clover (Churkova & Churkova, 2021), but there is no data on their performance in direct reseeding.

Studies on genotypic effects on species performance in direct reseeding are also lacking. At the same time, a large number of our studies of local ecotypes and subspecies of red clover, sainfoin and bird's-foot-trefoil (Naydenova & Vasileva, 2019b; Naydenova et al., 2015) found a large intraspecific variability in biological and morphological characteristics of interest for the selection of cultivars suitable for direct reseeding. In this connection, the current comparative test is also being conducted regarding share and stability in the grass stand of different species and genotypes of perennial legumes, directly reseeded on degraded pasture grass stand under the ecological conditions of the Central Northern Balkan.

## **Material and Methods**

Eight reseeding variants of a semi-natural grass stand used for unregulated grazing in a hilly area in the Central Northern Bulgaria were studied. The experiment was started on March 15, 2018, using the method of long plots with an experimental plot size of 30 m<sup>2</sup>. Reseeding was conducted with the following types and genotypes of legumes:

• *Trifolium pratense* L. (TP) – 3 genotypes, including: the diploid cultivar Nika 11, the tetraploid cultivar

Astur and a selection population obtained from the subspecies crossing *T. pratense* ssp. *pratense* and *T. pratense* ssp. *nivale*;

- Onobrychis viciifolia L. (OV) 2 genotypes: Vishnovski cultivar and local population (from the Central Pre-Balkan region)
- Lotus corniculatus L. (LC) 2 genotypes: Targovishte 1 and local pasture ecotype;
- Anthyllis vulneraria L. (AV) 1 genotype: Pamir cultivar.

For all variants, a sowing rate of 1000/m<sup>2</sup> germinated seeds was used.

From the second to the fourth vegetation after reseeding, the relative share (by weight % of fresh mass) of the reseeded and available legume species in the spring and summer regrowth of the grass stand, respectively, was assessed by taking samples in triplicate per variant and subjecting them to botanical analysis.

### **Results and Discussion**

The best results were obtained in reseeding with red clover (Trifolium pratense). In the second vegetation, the relative share of the species in the grass stand is within the limits of 15–41% in the first regrowth and from 29 to 39% – in the second (Table 1a, 1b). In the third and fourth vegetation, the presence of clover is significantly lowerwith up to 8-9% in the spring regrowth and up to 20% in the summer (Table 2a, 2b, 3). Grown as a monoculture under the conditions of Northern Bulgaria, the species is medium-long-lasting, with maximum development in the second vegetation. Its performance during direct reseeding corresponds to the indicated agrobiological characteristic. In this regard, its positive effect on the productivity and quality of the grass stand in direct reseeding can be defined as short-lived, as it is observed in the second vegetation and in the summer regrowth of the second and third vegetation. This makes it worthwhile to investigate mixtures of legume species to compensate for their biological limitations. The study by Gawel & Grzelac (2020), who sowed different seed mixtures involving red, white, hybrid clover and alfalfa indicated compensatory interactions between alfalfa and clovers to maintain productivity across seasons and years, but best results in terms of botanical composition and corresponding quality of the grass stand was achieved in reseeding with a mixture of the three types of clover.

When assessing the benefits of reseeding with red clover, its high levels of nitrogen fixation should also be taken into account. They amount to 373 kg  $N \cdot ha^{-1} \cdot year^{-1}$  (Carlsson & Huss-Danell, 2003) which implies a significant effect on the

Reseeding variants	Weight percentage of reseeded spe-	CV	VC	ML	MS	MF	Legumes
	cies/genotype I cut / II vegetation						total
TP cv. Nika 11	35	3	-	3	-		41
TP cv. Astur	15	5	-	3	-	5	28
TP Breeding population	41	5	_	2	_	11	59
OV cv. Vishnovski	17	2	_	-	2	-	21
OV Local population	9	5	-	_	-	6	20
LC cv. Targovishte 1	3	2	6	-	-	-	11
LC Local population	3	6	5	-	_	11	25
AV cv. Pamir	0	11	11	-	_	8	30
Mean	15.4	4.9	7.3	2.7	2.0	8.2	40.5
CV, %	85.6	59.5	43.8	21.7		33.8	50.3

Table 1a. Relative share of legumes in the grass stand, which are reseeded and self-sown in the spring regrowth of the second vegetation, weight %

Legend: TP – Trifolium pratense; OV – Onobrychis viciifolia; LC – Lotus corniculatus; AV – Anthyllis vulneraria; CV – Coronilla varia; VC – Vicia cracca; ML – Medicago lupulina; MS – Medicago sativa; MF – Medicago falcata

Table 1b.	. Relative share	e of legumes in th	e grass stand	- reseeded a	and self-sown	in the	summer	regrowth	of the	second
vegetatio	n, weight %									

Reseeding variants	Weight percentage of reseeded spe- cies/genotype II cut / II vegetation	CV	VC	ML	MS	MF	Legumes total
TP cv. Nika 11	29	11	-	-	3	15	57
TP cv. Astur	35	8	-	-	-	23	66
TP Breeding population	39	3	-	-	-	30	72
OV cv. Vishnovski	15	3	-	-	-	30	48
OV Local population	3	3	-	-	-	23	29
LC cv. Targovichte 1	0	11	-	-	-	15	26
LC Local population	0	11	8	-	-	23	42
AV cv. Pamir	0		15	-	-	15	30
Mean	15.1	7.1	11.5		3.0	21.8	46.3
CV, %	111.5	56.2	43.0			28.9	38.0

trophic relationships in the grass stand and, accordingly, on the restoration and maintenance of its biodiversity.

The red clover genotype/cultivar used for reseeding also significantly affected the relative share of the species in the mowed biomass. The diploid hay cultivar Nika 11 has a higher share in the spring regrowth, whereas the tetraploid cultivar Astur in the summer. The selection population obtained from the subspecies crossing T. pratense ssp. pratense X T. pratense ssp. nivale stands out with seasonally stable high share in the grass stand and relatively best longevity (Table 3). The low percentage of clover in the fourth vegetation can also be associated with insufficient summer and autumn moisture reserve in 2020 compared to the average for the region (Figure 1). The presence of the tetraploid cultivar Astur is two times lower than that of the diploids, which confirms our previous results on the lower drought tolerance and, accordingly, longevity of the tetraploid germ plasm of the species (Naydenova & Vasileva, 2019a).

According to Belanger &Tremblay (2010), good moisture availability before sowing and in the year after direct reseeding favored establishment and yield of reseeded grasses. The high share of the clover genotypes studied by us can also be connected with the large amount of late spring precipitation in the first and second vegetation. On the other hand, this raises the question whether to reseed more drought-resistant species, such as *Onobrychis viciifolia, Lotus corniculatus* and *Anthyllis vulneraria* in areas with unstable spring moisture supply over the years. These species were included as variants in the present study, with contrasting results observed regarding their suitability for direct reseeding.

The share of sainfoin (*Onobrychis viciifolia*) in the mowed biomass in the second vegetation is up to 17% in the first regrowth and up to 15% in the second one. In the third vegetation, when the regrowths were formed in significantly drier conditions, the relative share of the species was up

to 18 and 24%, respectively, for the spring and summer regrowth. In the fourth vegetation, despite its higher drought resistance and longevity, sainfoin had an equal share in the grass stand with that of clover (Table 3). When comparing both species, it is important to note that the share of sainfoin is more stable in years and seasons. A significant effect of genotype on reseeding performance was also observed in this species. Except for the fourth vegetation, Vishnovski

Table 2a. Relative share of legumes in the grass stand – reseeded and self-sown in the spring regrowth of the third veg-etation, weight %

Reseeding variants	Weight % of reseeded species/	CV	VC	ML	MS	MF	Legumes
	genotype I cut / III vegetation						total
TP cv. Nika 11	9	-	-	8	—	—	17
TP cv. Astur	8	-	-	12	-	-	20
TP Breeding population	8	-	-	9	-	-	17
OV cv. Vishnovski	18	-	-	6	-	-	24
OV Local population	11	_	-	8	-	-	19
LC cv. Targovishte 1	0	5	-	15	-	-	20
LC Local population	0	6	-	12	-	-	18
AV cv. Pamir	0	11	-	18	-	-	29
Mean	6.8	7.3		11.0			20.5
CV, %	95.3	43.8		36.7			20.0

Table 2b. Relative share of legumes in the grass stand – reseeded and self-sown in the summer regrowth of the third vegetation, weight %

Reseeding variants	Weight % of reseeded species/ genotype II cut / III vegetation	CV	VC	ML	MS	MF	Legumes total
TP cv. Nika 11	8	11	_	_		15	34
TP cv. Astur	16	_	-	_	2	9	27
TP Breeding population	20	6	17	-	-	7	50
OV cv. Vishnovski	24	8		-	-	9	41
OV Local population	8	3	2	-	—	8	21
LC cv. Targovishte 1	0	3	15	-	-	6	24
LC Local population	0	6	6	-	-	8	20
AV cv. Pamir	0	8	26	-	-	3	37
Mean	9.5	6.4	13.2		2.0	8.1	31.8
CV, %	100.5	44.8	71.8			41.8	33.4

# Table 3. Relative share of legumes in the grass stand – reseeded and self-sown in the spring regrowth of the fourth vegetation, weight %

Reseeding variants	Weight % of reseeded species/genotype I cut /	CV	VC	ML	MS	MF	Legumes total
	IV vegetation						
TP cv. Nika 11	7	3	-	8	-	-	18
TP cv. Astur	3	-	-	9	-	-	12
TP Selection population	8	9	8	12	-	-	37
OV cv. Vishnovski	8	5	-	3	_	-	16
OV Local population	6	6	3	6	_	_	21
LC cv. Targovishte 1	0	5	3	9	-	-	17
LC Local population	0	8	3	15	-	-	26
AV cv. Pamir	0	6	5	12	_	-	23
Mean	4.0	6.0	4.4	9.3			21.3
CV, %	91.6	33.3	49.8	40.8			36.3



# Fig. 1. Monthly precipitation amounts (mm) for the vegetation period of the experimental years

cultivar had several times higher percentage share in the biomass than that of the local population.

The reseeded genotypes of bird's-foot-trefoil (Lotus corniculatus), such as Targovishte and local pasture population participate in the grass stand only in the first regrowth of the second vegetation with the same weight share of 3% (Table 1a). Despite the favourable conditions, the bird's-foot-trefoil did not manage to establish itself in the grass stand by direct reseeding, thus repeating the results of our previous study conducted in the period 2013-2015. Similar results are also presented by Lazarev et al. (2020). Bird's-foot-trefoil has a high natural capacity for self-sowing. Its seeds preserve germination rate in the gastrointestinal tract of grazing animals, which also helps its spread and maintenance in the meadows. Adapted cultivars were used in the present study, and it can be considered that seed ecology is not the cause of the failure of the species under direct reseeding. In our other studies for the area, a high abundance of bird's-foot-trefoil was found in grass stands degraded as a result of fire (Navdenova et al., 2022). The above relates the failure of the species in direct reseeding to the low competitive ability of seedlings in the existing grass communities. In this regard, in order to use the significant characteristics of bird's-foot-trefoil for the restoration of degraded grass stand (such as drought resistance, grazing sustainability, high levels of nitrogen fixation, optimal content of condensed tannins) other methods for its successful reseeding should be evaluated and selected.

Anthyllis vulneraria – kidney vetch – was not recorded in the samples subjected to botanical analysis in any of the experimental years. The conditions in the area correspond to the ecological requirements of the species, respectively the genotype (Pamir cultivar). When this cultivar is sown as a pure crop in the grass stands in the hilly regions of Northern Bulgaria it is distinguished by very good development and excellent forage characteristics (Athar et al., 2019). But according to the results of the present experiment, successful reseeding of *Anthyllis vulneraria* in degraded grass stands probably also requires a specific method of grass stand preparation.

In accordance with what has been presented so far, the variation coefficient shows a stronger differentiation in terms of legume component of the grass stand depending on the reseeding variant in the second and fourth vegetation (Table 1-3).

Of the self-sown legumes, black medick (*Medicago lupulina*) has the largest share in the grass stands in the first regrowth, whereas sickle alfalfa in the second one (*Medicago falcata*). The average weight percentage of black medick increased from 2.7 in the second vegetation to 11.0% in the fourth one. A high share of this species was observed in plots unsuccessfully sown with bird's-foot-trefoil and kidney vetch with up to 18.0%. Gorski et al. (1984) found high allelopathic activity of black medick. According to the authors, it contains up to 2.5% hemolytic saponins of the dry matter, which exhibit an allelopathic effect, suppressing the germination and growth of other types of fodder grasses. Such a reason for the failure of reseeded bird's-foot-trefoil and kidney vetch can also be assumed.

The sickle alfalfa, is the other established valuable local pasture grass (Naydenova et al., 2022). According to the results, its presence in the resulting fodder mass is not affected or limited by the reseeded grasses. The highest share of *Medicago falcata* in the summer regrowths in the second vegetation reached up to 30% in the reseeding variants with the selection population of red clover and with the Vishnovski sainfoin cultivar. Synergistic interactions between these genotypes and the local sickle alfalfa genotype can be hypothesized. These interactions result in a significant increase in the share of the functional group of legumes in the degraded grass stand.

Another self-established legume species in the improved grass stand is the crown vetch (*Coronilla varia*), which has a stable average share in the biomass over the years and regrowths with up to 7.1%. Crown vetch is a legume with low fodder quality and its weaker presence in the plots reseeded with red clover and sainfoin underlines the potential of these species to improve the quality of the directly reseeded grass stands.

A significant share (up to 13.2%) of bird vetch (*Vicia cracca*), which is a rhizomatous species with high resistance in degraded grass stands by overgrazing or fire, was also observed in grass stands with unsuccessfully reseeded bird's-foot-trefoil and kidney vetch. Allelopathic activity has also been observed for many species of the genus *Vicia* (Geddes et al., 2015; Kitiş et al., 2016).We are not aware of such data

for *Vicia cracca*, but there are well-founded studies on how this wild species affects the legume cultivars and genotypes reseeded in the grass stand.

#### Conclusions

The type and genotype of the reseeded grasses caused significant differentiation regarding the share of the legume component in the degraded grass stand. The best results were obtained in reseeding with a selection population of red clover (*Trifolium pratense*) obtained from the subspecies crossing *T. pratense* ssp.*pratense* X *T. pratense* ssp.*nivale*, as well as with sainfoin (*Onobrychis viciifolia*) Vishnovski cultivar. The species, such as bird's-foot-trefoil (*Lotus corniculatus*) and kidney vetch (*Anthyllis vulneraria*), under conditions of early spring direct reseeding, failed to establish themselves in the grass stand, regardless of the adaptive potential of the used genotypes – cultivars and populations. Other methods of successful reseeding should be evaluated and chosen.

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