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The effect of cutting management on seed yield, seed yield components, and seed quality of red clover (*Trifolium pratense* L.)

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Abstract

Red clover (*Trifolium pratense* L.) is the second most important perennial forage legume in the Republic of Serbia, cultivated on 90-120.000 ha. The objective of this study was to determine the effects of cutting management on seed yield, some seed yield components, and seed quality of the NS red clover variety Una during the second and third year of growing. Five cutting managements with variable dates of initial cutting were tested in the 2019-2020 period. The first cutting was used for seed production in the C1 management and the second growth in the C2-C5 managements (C2-budding, C3-start of flowering, C4-mid of flowering, and C5-full flowering). In all cutting systems, significantly higher seed yields were achieved in the second year of growing. Unfavourable environmental conditions for pollinators as well as thinning of red clover crops in the third year of growing are the main reasons for low seed yields. The highest seed yield (539 kg/ha) was achieved in the C2 cutting management in the second year of growing. The cutting management had no significant effects on red clover seed quality.

Key words: Trifolium pratense L., cutting management, seed yield, seed yield components, seed quality

Introduction

Red clover (*Trifolium pratense* L.) is one of the leading forage legumes around of the world, primarily used for the production of hay, silage, and grazing (Annicchiarico et al., 2015). Nowadays red clover is considered as a very valuable raw material for the production of pharmaceutical supplements based on phytoestrogens (Bursać et al., 2011; Vlaisavljevic et al. 2014, 2016). From the agro-ecological aspects its importance is reflected in being the source of nitrogen in crop rotation on farming systems and also as a feed resource for pollinating insects. Red clover is a valuable rotational crop, especially in organic agriculture where no synthetic N fertilizers are used (Taylor & Quesenberry, 1996).

In the Republic of Serbia, red clover is the second most important perennial forage legume. Compared to alfalfa, it better tolerates acid soils. In addition, many years of breeding work in research institutes (Institute of Field and Vegetable Crops, Novi Sad and the Institute for Forage, Kruševac) have created numerous domestic high-yield varieties of red clover with good quality (Radović et al., 2010). Despite that, areas under red clover in the Republic of Serbia in the last two decades have significantly decreased (halved) from 120,000 to about 60,000 ha, primarily due to the reduction in the volume of livestock production in Serbia.

Currently, domestic seed production (85t-139 t over the 2015-2017 period - http://www.escaa.org) is not sufficient to meet the demand (about 300 t), so import of seeds from other countries is necessary. However, foreign cultivars tend to have low adaptability to local conditions and have lower seed yield. This is a great loss for our national economy, bearing in mind that in some regions of Serbia, there are very favourable conditions for seed production of red clover. On a global scale this production is mainly concentrated in regions with temperate climates, on areas for combined use (hay-seeds). Yields of 500 kg seed/ha are considered satisfactory in large-scale production (Karagić et al., 2010), while the potential seed yield is over 1000 kg/ha (Vasiljevic et al., 2005). In commercial production 50 % of the potential seed yield is rarely realized (Wang et al., 2010). In Western and Central Europe diploid red clover cultivars typically produce around 400–500 kg seed/ha, depending on the harvest year, location, and cultivar (Boller et al., 2010). In Oregon (USA), seed yields are usually higher; between 600 to 1200 kg/ha (Anderson et al., 2019). In the agro-ecological conditions of Serbia seed yield of red clover is 150-300 kg / ha (Lugic et al., 1999). Management practices to increase seed yield include fertilization, adequate weed and pest control, the synchronization of flowering through pre-cutting in spring, and the application of plant growth regulators to prevent lodging. The main reasons of low seed yield are inadequate pollination, genetic problems, or a combination of such traits (Vleugels et al., 2019). One of the most important factors that influence synchronization of flowering and guarantee high yields and quality of red clover seeds is cutting management, heavily influenced by environmental conditions.

In seed production it is very important to obtain seeds of high quality that have good physiological and biochemical characteristics (Boller et al., 2010). Germination of red clover seeds is complicated by the presence of hard seeds. Generally, in legumes, hard seeds are characterized as seeds that cannot receive water and gases (Matilla et al., 2005; Baskin et al., 1998), and these seeds germinate subsequently when the seeds become permeable (Stanisavljević et al., 2021). The development of hard seeds may be due to various factors, including properties of hilum, nature of seed coat, level of seed maturity, some genetic factors, and environmental conditions during seed development.

The objective of this study was to determine the effects of five different cutting managements on seed yields, some seed yield component, and seed quality of the NS red clover variety Una in the second and third year of growing.

Material and Methods

The trial with the red clover cultivar Una was set up on the experimental field of the Institute of Field and Vegetable Crops in Bački Petrovac according to a randomized block design with three replicates. Sowing was done with a machine on 10 April 2018 at an interrow spacing of 25 cm with a seeding rate of 15 kg/ha. The size of the elementary plot unit was 15 m2 (3x5 m).

The first factor (C) was the cutting management. There were five treatments. The first cutting was used for seed production in the cutting management 1. The second cutting was used for seed production in four followed cutting managements with variable dates of the first cutting, depending on the growing stage (C2-budding, C3-start of flowering, C4-mid of flowering, and C5-full flowering). The second factor (Y) included the environmental conditions in the second (2019) and third year (2020) of red clover growing. Red clover seed was harvested in a single passage of a Hege harvester, following desiccation with diquat performed when about 80% of pods on normally developed plants were at the stage of physiological maturity.

Analysis of seed yield components (number of flower heads per m2, number of flowers per head, number of seeds per head, % fertility, and seed yield per m2) was performed on a sample of 1 m2 at the stage of full pod maturity. Seed quality parameters were determined one year after harvest, in order to avoid a dormant period of seed. The obtained results were processed by the statistical method of analysis of variance and tested by the LSD test, with the use of the GenStat software.

Meteorological conditions

Bački Petrovac and the surrounding area have a temperate continental climate. Table 1. shows the two main meteorological parameters (rainfall and temperature) recorded during the 2019-2020 growing seasons as compared to the long-term average (1964-2018). During the growing season, the long-term average rainfall (1964-2018) was 376.4 mm and the mean temperature 18.2oC.

Tab. 1. Rainfall and mean monthly temperatures during the period (2019-2020) and long-term average (1964-2018) - location Bački Petrovac

Month	Rainfall (mm)			Temperature °C		
	2019	2020	1964-2018	2019	2020	1964-2018
I	45.8	22.9	38.4	-0.1	0.4	-0.1
II	17.0	45.5	35.7	4.2	6.2	1.9
III	15.9	53.8	39.8	9.8	7.8	6.6
IV	54.1	11.1	47.6	13.4	12.9	11.8
V	147.6	47.3	67.6	14.7	16.1	17.0
VI	63.7	161.9	88.6	23.2	20.7	20.1
VII	21.0	77.3	66.7	23.3	22.4	21.8
VIII	79.1	137.5	58.1	24.4	23.2	21.4
IX	53.1	31.4	47.8	18.2	19.1	17.0
X	20.0	93.0	47.2	13.8	13.0	11.7
XI	53.7	14.2	49.1	11.2	6.5	6.2
XII	61.1	37.3	46.2	4.6	5.0	1.6
Total-Year	632.1	733.2	632.8			
Growing season	418.6	466.5	376.4			
Average-Year				13.4	12.8	11.4
Growing season				19.5	19.1	18.2

In the two years of the study, the mean monthly air temperature during the growing season was about 1°C higher than the long-term average (Table 1.). Generally, large amounts of rainfall during the summer months in 2020 had a negative impact on seed production. Extremely high rainfall deficits were observed in the months of March and July in 2019, as well as in the months of April and September in 2020 (Table 1.).

Cutting management

Date of seed harvest depended on the cutting management and environmental conditions in the year of growing (Table 2.). It has been noticed that the harvesting period in both years lasted about one month, but in the second year of growing higher amount of precipitation in May delayed the start of the harvest by about one week compared to the third year.

Tab. 2. Date of the first cutting for hay and seed harvesting depending on the cutting management and year of growing

Cutting management	the second year of growing (2019)		the third year of growing (2020)	
	date of the 1st cutting	date of the seed harvesting	date of the 1st cutting	date of the seed harvesting
C1-1st cutting for seed	-	24 July 2019	-	16 July 2020
C2- 2nd cutting for seed (1st cutting in the stage of budding)	27 May 019	31 July 2019	18 May 2020	28 July 2020
C3- 2nd cutting for seed (1st cutting in the stage of the start of flowering)	03 June 2019	07 Aug 2019	25 May 2020	03 Aug 2020
C4- 2nd cutting for seed (1st cutting in the stage of the mid of flowering)	17 June 2019	13 Aug 2019	29 May 2020	07 Aug 2020
C5- 2nd cutting for seed (1st cutting in the stage of full of flowering)	24 June 2019	30 Aug 2019	04 June 2020	12 Aug 2020

Results and Discussion

Results of our study on the effects of cutting management and environmental conditions of the year of growing on red clover seed yield components and seed yield are presented in the Table 3. The cutting management had a strong influence on all seed yield components, particularly on the number of flower heads per m2 in the second year of growing (Table 3.). The number of inflorescences per m2, number of flowers per inflorescence, and number of seeds per inflorescence were significantly higher in 2019 due to the fact that growing conditions during seed yield formation were more favourable than in the third year of cultivation. It is interesting to note that significant differences in seed number per inflorescence were observed among the treatments only in the third year of growing (Table 3.). The cutting management 2 (C2), which involved the earliest first cutting (in the stage of budding) in the second year of growing achieved the highest number of inflorescences per m2 (848) and seed yield (539 kg ha-1). Hejduk (2006) and Vasiljevic et al. (2007) point out that the red clover seed yield becomes progressively lower as the cutting of the first growth for hay is delayed.

Unfavourable weather conditions for pollinators as well as thinning of red clover crops in the third year of growing are the main reasons for low seed yields. Higher yield of red clover seeds in the second growth of the second year of cultivation compared to the first year of establishment was found by Barać et al. (2011) and Vasiljevic et al. (2007).

Tab. 3. Red clover seed yield components and seed yield (kg/ha) depending on the cutting management and year of growing

Traits	NI/ m^2		NF/I		NS/I		F(%)		SY (kg/ha)	
Year/cut.	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
C1	524	423	100	74	82	34	83.0	44.7	341	135
C2	848	177	115	106	89	87	78.0	82.0	539	109
C3	582	240	120	107	93	86	77.3	80.0	363	166
C4	769	191	127	106	87	84	69.0	78.7	465	121
C5	188	181	130	105	87	78	66.6	77.0	73	52
Average	582	242	118	99	88	74	74.8	72.5	356	117
LSD	C	Y	C	Y	C	Y	C	Y	C	Y
0.05	119.3	164.4	12.5	20.8	10.8	23.4	4.1	8.3	72.5	116.5
0.01	106.2	244.9	17.2	47.9	14.9	54.0	5.6	19.1	99.8	268.7

NI-number of inflorescences per m^2; NF/I-number of flowers per inflorescence; NS/I- number of seeds per inflorescence; F- % fertility; SY- seed yield per ha

Over the two-year period (2019-2020), both of the factors significantly affected red clover seed yield. Interaction between the cutting management and environmental conditions of the year of growing was significant for almost all analyzed traits except the number of flowers per head (Table 4).

Tab. 4. Red clover seed yield components and seed yield mean squares from the ANOVA across five cutting managements and two years

Source of variation	df	Mean squares				
		NI/ m^2	NF/I	NS/I	F(%)	SY (kg/ha)
Cutting (C)	4	***	***	***	***	***
Year (Y)	1	NS	NS	NS	NS	**
C x Y	4	***	NS	***	***	***
Residual	16	NS	NS	NS	NS	NS
CV %		23.6	9.4	11.0	4.5	25.0

NI-number of inflorescences per m^2; NF/I-number of flowers per inflorescence, NS/I- number of seeds per inflorescence; F- % fertility; SY- seed yield per ha *, **, *** Significant at the 0.05, 0.01 and 0.001 levels of probability, respectively; NS - not significant

The results of the impact of the cutting management and climate conditions in the year of growing on red clover seed quality parameters are presented in Table 5. Climate conditions in the year of growing had a significant influence on some of the quality parameters, such as typical seedlings (%); HS- hard seed (%); DS-dead seed (%), and total germination.

Seed germination energy can be considered as the basic criterion for germination in field conditions. If soil conditions are almost ideal, germination obtained in laboratory conditions is a good indicator of viability (Petkovic et al., 2017). Rapid germination and improved germination parameters such as germination energy enable better crops establishment by shortening the period of seed exposure to the attack of soil pathogens (Beckstead et al., 2007; Dalling et al., 2011). Germination energy average ranged from 56.5 % (in the second year) to 60.9 (in the third year - Table 5.), which is in accordance with the results of Petkovic et al. (2017).

Tab. 5. Red clover seed quality parameters depending on the cutting management and climatic conditions in the year of growing

Traits	GE (%)		G (%)		AS (%)		HS (%)		DS (%)		TG (G+HS)	
Year/cut.	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
C1	55.3	70.0	66.3	71.0	20.0	3.7	10.0	4.7	3.6	21.0	76.3	75.6
C2	56.7	67.7	68.7	70.7	18.7	5.7	7.0	2.3	5.6	21.3	75.7	73.0
C3	56.7	60.3	65.3	72.3	18.7	5.7	13.0	4.3	3.0	18.7	78.3	77.3
C4	55.3	57.7	62.3	66.7	22.0	5.3	10.7	3.7	5.0	24.7	73.0	70.3
C5	58.3	49.0	66.0	54.7	18.7	8.0	11.3	3.0	4.0	34.7	77.3	57.7
Average	56.5	60.9	65.7	67.1	19.6	5.7	10.4	3.6	4.3	24.0	76.1	70.8
LSD	C	Y	C	Y	C	Y	C	Y	C	Y	C	Y
0.05	7.3	4.7	6.4	2.5	2.5	2.2	3.0	1.6	7.2	3.1	7.3	3.7
0.01	10.6	6.7	9.4	3.6	3.6	3.1	4.3	2.3	10.4	4.5	10.6	5.2

GE-germination energy (%); G- germination (%); AS-. atypical seedlings (%); HS- hard seed (%); DS-dead seed (%). TG-total germination (%)

Total germination was 76.3% in the second and 70.8% in the third year of growing (Table 5.). In both years the highest total germination (78.3% and 77.3%) was achieved in the cutting management 3 (c3). Hampton and Hill (2002) point out that the decrease of seed germination may be affected by adverse conditions before the harvest, as well as conditions during storage.

The presence of hard seeds in our study was significantly higher in the second year of growing (on average 10.4 %) than in the third (on average 3.6% - Table 5.). In both years, the cutting management had a strong influence on hard seed presence (Table 5.). The lowest hard seed presence was recorded in the cutting management 2 (7.0 % and 2.3%). Studying the presence of hard seeds in varieties and populations of red clover, Vasiljević et al. (1997) found that it varied from 0.5 to 10.3%.

The appearance of atypical seedlings was particularly pronounced in the second year of growing and on average reached 19.6 % (Table 5.).

However, the cutting management and climatic conditions in the year of red clover growing had no significant effect on seed germination energy (Table 6.).

Tab. 6. Red clover seed quality parameters mean squares from the ANOVA across five cutting managements and two years of growing

Source of variation	df	Mean squares					
		GE (%)	G (%)	AS (%)	HS (%)	DS (%)	TG (%)
Cutting (C)	4	NS	*	NS	NS	NS	NS
Year (Y)	1	NS	NS	***	***	***	**
C x Y	4	*	**	NS	NS	*	*
Residual	16	NS	NS	NS	NS	NS	NS
CV %		9.9	4.7	21.4	28.7	27.3	6.1

GE-germination energy (%); G- germination (%); AS-. atypical seedlings (%); HS- hard seed (%); DS-dead seed (%); TG-total germination (%) *, **, *** Significant at the 0.05, 0.01 and 0.001 levels of probability, respectively; NS - not significant

Conclusion

The cutting management significantly affected red clover seed yields. The highest number of heads per m2 (848) and total seed yield (539 kg ha-1) were obtained in the cutting management 2 with the earliest cutting for hay (the stage of budding) in the second year of growing. Unfavourable weather conditions in the third year of growing were the main reason for low seed yields. The cutting management had no significant effect on total germination of seed. The lowest hard seed presence in both years of growing was recorded in the cutting management 2 (7.0 % and 2.3%).

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Утицај система косидбе на принос семена, компоненте приноса семена и квалитет семена црвене детелине (*Trifolium pratense* L.)

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Сажетак

Црвена детелина (Trifolium pratense L.) је друга по значају вишегодишња крмна легуминоза у Републици Србији, која се гаји на око 90-120.000 ха. Циљ овог истраживања био је да се утврди ефекат различитих система косидбе на принос семена, најважније компоненте приноса семена и квалитет семена НС сорте црвене детелине Уна током друге и треће године живота. У периоду 2019-2020, тестирано је пет различитих система косидбе са променљивим датумима првог откоса. Први откос је коришћен за производњу семена у систему косидбе К1, а други откос у системима косидбе од К2 до К5 (са променљивим датумима првог откоса за производњу сена, зависно од фазе развоја: К2-бутонизација, К3-почетак цветања, К4-средина цветања, К5-пуно цветање). У свим системима косидбе у другој години живота црвене детелине остварени су знатно већи приноси семена. Неповољни еколошки услови за опрашиваче, као и проређивање усева црвене детелине у трећој години живота су главни разлози ниских приноса семена. Највећи принос семена (539 кг/ха) остварен је у систему косидбе К2 у другој години живота црвене детелине. Систем косидбе није имао значајан утицај на квалитет семена црвене детелине.

Кључне ријечи: Trifolium pratense L., систем косидбе, принос семена, компоненте приноса семена, квалитет семена

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