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EFFECTS OF ROW SPACING AND PLANT GROWTH REGULATION ON ALFALFA SEED YIELD (*MEDICAGO SATIVA* L.)

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Abstract

The influence of sowing method, growth regulator and harvest method on alfalfa seed yield was examined in three years (2015-2017), in locality near Belgrade, with using alfalfa variety NS Banat ZMS II. Research showed the statistically very significant effect of sowing method on seed yield, and the best average way for all three years period was 60 cm between rows of sowed seed. The growth regulator proved to be effective only in the rainy year, while harvesting crops previously treated with desiccant proved to beuseful, with the exception in the year of establishment when this difference was not statistically significant. Meteorological conditions had the highest influence on the seed yield, amount of precipitation in the vegetation period mostly, and the researched factors have proved to be useful in suppressing the consequences of poor weather. In years suitable for seed production, the recommendation for the production practice is an intermediate distance of 60 cm and the use of a desiccant.

Key words: Alfalfa, Row spacing, Growth regulator, Harvest method, Production.

Introduction

Forage grasslands represent 26% of the land area in the world, and 70% of agricultural area (Anon., 2010). Lucerne or alfalfa (*Medicago sativa*) is the most widely cultivated as it can be grown with both temperate and tropical grasses (Capstaff & Miller, 2018) and grown successively for 3 or more years (Bélanger *et al.*, 2006).

Alfalfa (*Medicago sativa* L.) is a perennial flowering plant in the legume family *Fabaceae*. Alfalfa is the most famous fodder crop with high yield of biomass that has excellent nutritive value, which is ideal for the production of milk and meat (Veronesi *et al*., 2010). Alfalfa is the most important perennial forage legume. In Serbia it is grown on about 200 thousand hectares (Stanisavljević *et al.*, 2012). In our conditions, alfalfa is the main forage crop, and because of it, the presence of alfalfa seed production in agricultural production is necessary. The availability of high-quality seed material in the required quantities is one of the conditions for successful production of fodder on sown grasslands. Today, domestic production of lucerne seeds most often does not meet our needs, although we were important producers and exporters of alfalfa seed. Imported foreign varieties are not adapted to our climatic conditions in many cases, or they have poor quality. In addition, increased imports of alfalfa seed have a negative impact on the country's payments balance. The alfalfa seed has a high market value, and seed production can be carried out in all areas where alfalfa is successfully grown as a crop. Vučković (2003 and 2004) states that in our country, the best conditions for the production of alfalfa seed are in Banat, the northeast Bačka and eastern Serbia. The favorable climatic and soil conditions of our country give us a good basis for successful and economically justified production of alfalfa seed.

The yields of alfalfa seeds in our country range from 100-1500 kg ha^{-1}, usually 300 to 500 kg ha^{-1}. Row spacing is one of the most important factors in the production of alfalfa seed (Vučković, 2004). Improving the production of alfalfa seed is possible by creating high yielding varieties and by improved cultural practices. Genetics and breeding showed that progress in achieving higher seed yield in alfalfa is limited (Bolanos-Aguilar *et al.*, 2002) on the other hand, the optimal use of mineral nutrition (Gossen *et al*., 2004), growth regulators (Dragovoz *et al.*, 2002; Zhang *et al.*, 2009), pollinators (Andjelković *et al*., 2010), row distance, the amount of seed used for sowing and plant density (Zhang *et al.*, 2008) can have the significant impact on yield components, yield formation, and it seems like it could be a better route to achieve higher and more stable seed yields (Stanisavljević *et al.*, 2012). The seed production of alfalfa in Serbia takes place in the majority of cases on crops of alfalfa that are primarily used for the production of fodder. Lush vegetative growth of alfalfa is desirable in the production of fodder, but there is a problem in seed production when, due to a large amount of above-ground mass, the alfalfa stem falls, which negatively affects on the yield and seed quality. Growth regulators are opening up new opportunities for increasing seed yield of grasses and legumes (Lorenzetti, 1993). Treatment with etephon induces changes in the metabolism of phenolic substances, the activity of peroxidase, and causes a significant decrease in alfalfa growth (Cvirkova *et al.*, 1994). Chen *et al*., (2016) raport that plant growth regulators Naphthylacetic acid, gibberellic acid and brassinolide consistently increased seed yields.

For successful forage biomass production is very important optimal nutrient and water supply (Dawson *et al.*, 2004). The ability to re-grow alfalfa is affected by the previous N status (Meuriot *et al.*, 2004 and 2005). Plant growth has been illustrated to be sensitive to salinity (Soylemez *et al*., 2017). Growth of alfalfa may be improved by combined inoculation of alfalfa with arbusculrmycorrhizae fungi and rhizobium (Zhu *et al.*, 2016).

The main scientific goal of this research is to find, in certain agroecological conditions, the most suitable model

for achieving high and stable yield of lucerne seed of excellent quality based on the examination of the influence of sowing methods, growth regulator and harvest method on seed yield.

Materials and Methods

The study with investigated alfalfa cultivar was the field experiment in municipality of Surčin, near Belgrade (44º48' N; 20º17' E and 117 m a.s.l.). The experiment included the research of the influence of three different factors on the quality and yield of alfalfa seed. The sowing was carried out manually with 18 kg ha^{-1} of seed. We used domestic variety of alfalfa, NS Banat ZMS II (Institute of Field and Vegetable Crops, Serbia). The experiment was set up as a three factor experiment in a randomised-block system with four replications. Plot size was 10.8 m^2. Insect pests are suppressed with products based on chlorpyrifos and cypermethrin. Herbicides that are used for weed control are based on quizalofop-P-tefuril.

During three years of using alfalfa (2015-2017), we examined the following three factors: 1. crop densities (A) - the row spacing of 15 cm (A_1), 30 cm (A_2); 45 cm (A_3) and 60 cm (A_4); 2. treatment with growth regulator (B) - without treatment (B_1),treatment with Cerone preparations (active substance ethephon) at the start of flowering with the amount of 1 l ha^{-1} (B_2); and treatment with Cerone preparations (active substance ethephon) at the start of flowering with the amount of 2 l ha^{-1} (B_3); 3. harvest method (C) - with the use of desiccation (Sirius preparation, active substance glufosinate – ammonium) (C_1) and - without the use of desiccation (C_2), were investigated.

All experimental data were processed statistically by using the computer software STATISTICA (Stat Soft 12.0). Analysis of differences between row spacing, treatment with growth regulator and harvest methods averages was performed using Fisher's protected LSD test at a $p \leq 0.05$ significance level.

Meteorological condition: Meteorological conditions vary from year to year (Popović *et al.*, 2013; 2015, Janković *et al.*, 2018). In the first year of experiment, the average temperature was 14.1ºC with total precipitation of 684.2 mm. Average temperatures in second year was 13.5ºC with total precipitation of 759.8 mm and in the third year was 13.9ºC and 508.8 mm (Figs. 1a and 1b).

Meteorological conditions in 2015, characterized by extremely high temperatures and average precipitation, had positive influence on growth and development of the plant, and thus on the seed yield too. Large amounts of precipitation in 2016 had an adverse impact on the seed production of alfalfa. The best year for the production of alfalfa seed was 2017. Although this year in Belgrade was characterized as very dry, the amount of precipitation in the vegetation period had positive effect on seed yield.

Results and Discussion

The average yield of alfalfa seed in 2015, depending on the way of sowing, the growth regulator and the method of harvest (Table 1), was 189.0 kg ha^{-1}. The effect of sowing on the yield of lucerne seed proved to be statistically very significant. In this year with a small amount of precipitation, the highest yield was achieved at the smallest spacing between rows (A_1), 214.3 kg ha^{-1}. Slightly lower yield achieved on the row spacing of 60 cm (204.6 kg ha^{-1}), while sowing method A_2 had the yield of 175 kg ha^{-1}. Variant that gave the smallest yield of alfalfa seed per hectare was the variant A_3. The seed yield of variant A_3 was 162 kg ha^{-1}, that is 24.4% lower than the best result (A_1) and 14.3% lower than the average yield of alfalfa seed.

The effect of the growth regulator on lucerne seed yield was not statistically significant. As compare to the control variant (189.4 kg ha^{-1}), the plants treated with the growth regulator in the amount of 1 l ha^{-1} give only slightly higher yield (190.1 kg ha^{-1}), while the yield of 187.5 kg ha^{-1} was recorded in treatment with 2 l ha^{-1}, which is 1.9 kg ha^{-1} less than the control variant. Harvesting method also didn't have a statistically significant effect on seed yield. The control variant achieved a yield of 186.2 kg ha^{-1}, while on treated plots the average yield was slightly higher (191.8 kg ha^{-1}). In 2016, under the effects of applied treatments, the average yield of lucerne seed (Table 2) was 248.5 kg ha^{-1}.

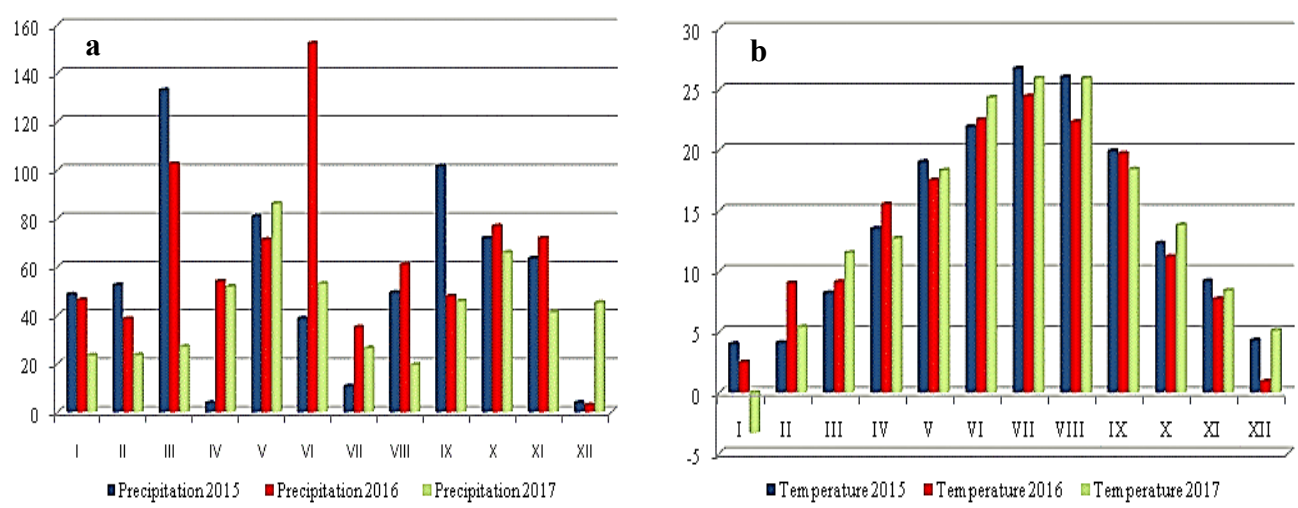

Fig. 1. Monthly precipitation (mm), a, and average monthly air temperatures (ºC), b, Belgrade, Serbia, 2015-2017.

Table 1. Seed yield in 2015 (kg ha $^{-1}$).

Row spacing	Harvest method	Growth regulator			Average
		B_1	B_2	B_3	
A_1	C_1	221.8	222.3	215.1	219.7
	C_2	208.3	210.1	208.5	208.9
Average		**215.0**	**216.2**	**211.8**	**214.3**
A_2	C_1	177.0	178.4	176.2	177.2
	C_2	172.5	172.7	173.1	172.7
Average		**174.7**	**175.6**	**174.6**	**175.0**
A_3	C_1	163.8	165,0	162.6	163.8
	C_2	159.7	161.2	159.5	160.2
Average		**161.7**	**163.1**	**161.1**	**162.0**
A_4	C_1	207.5	206.8	204.6	206.3
	C_2	204.7	204.1	200.1	202.9
Average		**206.1**	**205.4**	**202.4**	**204.6**
Growth regulator		**B_1**	**B_2**	**B_3**	**Average**
Harvest method	C_1	192.5	193.1	189.7	191.8
	C_2	186.3	187.0	185.2	186.2
Average		**189.4**	**190.1**	**187.5**	**189.0**

Parameter	Level	A	B	C	AB	AC	BC	ABC
F-test		**	ns	ns	ns	ns	ns	ns
LSD	0.05%	16.5	9.4	6.6	22.2	14.7	12.4	32.4
	0.01%	22.3	12.4	8.7	32.3	20.6	17.0	53.7

Table 2. Seed yield in 2016 (kg ha $^{-1}$).

Row spacing	Harvest method	Growth regulator			Average
		B_1	B_2	B_3	
A_1	C_1	214.5	221.8	225.5	220.6
	C_2	206.0	210.2	207.4	207.9
Average		**210.2**	**216.0**	**216.5**	**214.2**
A_2	C_1	244.7	256.3	267.0	256.2
	C_2	231.1	238.8	249.6	239.8
Average		**237.9**	**247.5**	**258.5**	**247.9**
A_3	C_1	220.3	232.7	239.3	230.7
	C_2	210.1	218.2	228.1	218.7
Average		**215.1**	**225.5**	**233.6**	**224.7**
A_4	C_1	306.0	314.2	319.6	313.2
	C_2	287.7	304.8	309.4	300.7
Average		**296.9**	**309.5**	**314.5**	**306.9**
Growth regulator		**B_1**	**B_2**	**B_3**	**Average**
Harvest method	C_1	246.4	256.2	262.9	255.2
	C_2	233.7	243.0	248.6	241.8
Average		**240.0**	**249.6**	**255.8**	**248.5**

Parameter	Level	A	B	C	AB	AC	BC	ABC
F-test		**	**	**	ns	ns	ns	ns
LSD	0.05%	8.8	7.1	8.1	16.7	17.9	15.1	39.6
	0.01%	11.9	9.4	10.6	24.3	25.2	20.8	65.7

The effect of the sowing method proved to be statistically very significant in this year. The alfalfa seed sown in rows at a width of 60 cm gave a yield of 306.9 kg ha^{-1}, which was the highest yield, in this year with high precipitation. Stjepanovićand Popović (2009) conclude in their research that in a wet vegetation period, significantly higher yield was achieved with wider inter-row space. With a yield that was 19.3% lower than the previous variant, variant A_2 achieved the second best yield this year, which amounted to 247.9 kg ha^{-1}. Lower yields recorded with the inter-row distance of 45 cm (224.7 kg ha^{-1}), while the lowest yield was obtained with the inter-row distance of 15 cm, and it was 214.2 kg ha^{-1}, which was 30.2% lower than the variant with highest yield (A_4). Interestingly, the variant with sowing at 15 cm in the last year gave the highest yield, and agroecological conditions in 2016. In that year primarily precipitation in the vegetation period influenced on the height and the lushness of the plants. Under these conditions we have plant lodging, and the bad influence of plant lodging was particularly pronounced on plants at the smallest vegetation area. Many processes such as pollination and seed maturation were conducted more difficult due to plant lodging and lushness on a small vegetation area.

Also, the rainy period during the time of flowering and harvesting led to weaker pollination of the plants resulting in a lower number of pods, as well as rotting the seeds on the plant during harvest due to high humidity and temperature. This is highly expressed on plants in the smallest vegetation area due to poor ventilation caused by high plant density.

Treatment with growth regulator had statistically very significant influence on the yield of lucerne seed. The highest yield was obtained on plots treated with growth regulators in the amount of 2 l ha^{-1} and it was 255.8 kg ha^{-1}. A variant with treatment of 1 l ha^{-1} gave a slightly lower yield (249.6 kg ha^{-1}). Both variants were more successful than the control variant whose yield was 240 kg ha^{-1}. Treatment with growth regulator influenced the decrease in plants lushness, which also reflected on higher yield of seeds, compared to the control variant, especially plants treated with a dose of 2 l ha^{-1}. Growth regulator performed positively in years with high rainfall, such as 2016. The average seed yield of plants treated with desiccant varied to 255.2 kg ha^{-1} (C_1) compared to 241.8 kg ha^{-1} (C_2). Desiccation beneficially influenced the seed yield under unfavorable harvest conditions due to the high amount of precipitation, equating seed maturation, and also decrease seed degradation caused by adverse climatic conditions.

The average seed yield of lucerne in 2017 (Table 3), depending on the factors tested, amounted to 610.9 kg ha^{-1}. If we compare the average seeds yield from 2015 (189 kg ha^{-1}) and 2016 (248.5 kg ha^{-1}) with yield from 2017, we come to the conclusion that the average seeds yield from 2017 was significantly higher.

Table 3. Seed yield in 2017 (kg ha $^{-1}$).

Row spacing	Harvest method	Growth regulator			Average
		B_1	**B_2**	**B_3**	
A_1	C_1	696.0	702.1	702.5	700.2
	C_2	678.5	682.2	687.3	682.6
Average		**687.2**	**692.1**	**694.7**	**691.4**
A_2	C_1	568.8	556.7	558.2	561.2
	C_2	551.3	528.2	541.1	540.2
Average		**560.0**	**542.5**	**549.6**	**550.7**
A_3	C_1	508.8	510.5	513.6	510.9
	C_2	494.2	494.2	495.8	494.7
Average		501.5	502.2	504.6	502.8
A_4	C_1	703.7	707.4	710.0	707.1
	C_2	685.3	695.2	692.1	690.8
Average		**694.5**	**701.4**	**701.0**	**698.9**
Growth regulator		**B_1**	**B_2**	**B_3**	**Average**
Harvest method	C_1	619.3	619.2	621.1	619.8
	C_2	602.3	599.9	603.9	602.1
Average		**610.8**	**609.6**	**612.5**	**610.9**

Parameter	Level	A	B	C	AB	AC	BC	ABC
F-test		**	ns	*	ns	ns	ns	ns
LSD	0.05%	19.7	19.5	15.2	45.9	33.7	28.4	74.4
	0.01%	26.6	25.7	19.9	66.7	47.3	39.2	123.4

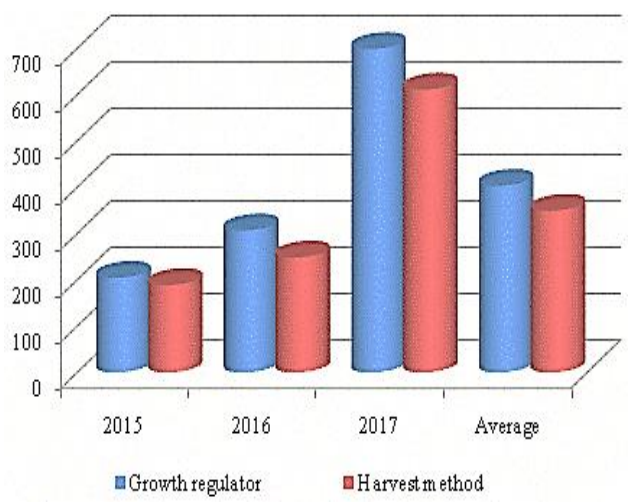

Fig. 2. Effect of year, growth regulator and harvest method on seed yield (kg ha^{-1}).

The effect of the sowing method also proved to be statistically very significant. The smallest seed yield was on variant A_3 - 502.8 kg ha^{-1}. A some what higher yield was recorded with a variant of 30 cm between rows, 550.7 kg ha^{-1}. Variants A_1 and A_4 with a yield of 691.4 kg ha^{-1} and 698.9 kg ha^{-1} had significantly higher yields. This means that the variant of A_4 had the highest yield, 27.5% better compared to the weakest variant A_3. The highest yields were obtained in dry and sunny years (Vučković, 1995). Cash (2002) states that the seed crops require long and hot summers with minimal summer rainfall. This year was characterized by favorable agroecological conditions for seed production, which explains the significantly higher yield compared to previous years.

The growth regulator did not have significant effect on seed yield, table 3, Fig. 2. This is in accordance with the information from Rajala and Peltonen-Sainio (2000), which present that action of growth regulators highly

depends on weather conditions. Rincker *et al.,* (1988) state that growth regulators haven't yielded satisfactory results, as confirmed by our research. The harvest method significantly influenced seed yield of alfalfa. From plots treated with a desiccant 619.8 kg ha^{-1} was obtained, unlike the control one, whose yield was 602.1 kg ha^{-1}.

The grass-legume mixtures had the higher yield of pasture compared to the pure-stand grasses and forage legumes maintained as a control. The total DM yield of all the mixed pastures varied between years, with a progressive increase in total DM yield of the mixtures in the second year of the pasture (Tessema & Feleke, 2018).

Karagić *et al*., (2003) stated that average yield of alfalfa seed in agroecological conditions of Serbia is about 250 kg ha^{-1}, with a large variation depending on the cultivation year from 50 kg ha^{-1} to 700 kg ha^{-1}. Calculating the three-year average, the highest yield was obtained by sowing at a spacing of 60 cm (403.5 kg ha^{-1}), while 373.3 kg ha^{-1} was obtained with 15 cm spacing. Variant A_2 gave 324.5 kg ha^{-1} on average, while the lowest average yield was measured at a spacing of 45 cm - 296.5 kg ha^{-1}. Treatment with growth regulators had the best results with the treatment of 2 l ha^{-1} (351.9 kg ha^{-1}). Plants treated with 1 growth regulator in concentration of l ha^{-1} have achieved an average seed yield of 349.8 kg ha^{-1}, while the control variant averaged 346 kg ha^{-1}. The average seed yield of plants treated with desiccant in the three-year period was 355.6 kg ha^{-1}, with a control of 343.4 kg ha^{-1}.

In conditions of South-East Europe, forage production of alfalfa was mainly based on the spacing of 12.5 cm to 25 cm and the amount of seeds from 16 kg ha^{-1} to 25 kg ha^{-1} was archievedStjepanović and Popović (2009) and Moisuc & Djukić (2002). According to Abrosimova & Fadeeva (2015), Popovic *et al.,* (2016) and Vasileva and Ilieva (2017) the selection work aimed only at creating high yield varieties could lead to the loss of ecological stability of the genotype. These authors considered that since the average value of the attribute and the sensitivity of the genotype to the environment are relatively independent and genetically determined by themselves, the selection work related to environmental stability must be continually controlled.

Conclusion

Based on the obtained results of the influence of sowing methods, growth regulators and harvest methods on lucerne seed yield, we can conclude that the greatest impact on seed yield have meteorological conditions, primarily the distribution and amount of precipitation. The most favorable year for the production of alfalfa seed was 2017, with the average yield of 610.9 kg ha^{-1}, which is higher yield from 248.5 kg ha^{-1} in 2016 and 189 kg ha^{-1} of alfalfa seed obtained in 2015. In 2017, the sowing method significantly affected on the seed yield, and the highest yield was obtained by variants with 60 and 15 cm between rows. In 2016, which had a large amount of precipitation, the variant with 60 cm between rows proved to be the best, while in the year of establishment we have the best results with sowing at 15 cm between rows. The growth regulator did not have the desired effect in years favorable for seed production, while on the basis of this research, its use in years with large amount of rainfall justified. Desiccation did not achieve the desired effect only in the year of establishment, while in other years, and especially in 2016, it had a positive effect on seed yield. General recommendations of this research for the production practice is sowing the seeds of alfalfa with 60 cm of space between rows, using the growth regulators only in wet years and using the desiccants during the harvesting of alfalfa for seed.

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