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INFLUENCE OF PLANT DENSITY ON YIELD COMPONENTS, YIELD AND QUALITY OF SEED AND FORAGE YIELDS OF ALFALFA VARIETIES

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ABSTRACT

Increasing yield and yield stability of alfalfa seed in South-east Europe is of high importance. Alfalfa seed is an expensive and competitive commodity market, as well as alfalfa for forage, which is an important and often scarce commodity in this region.

The research was done in four years, in two localities with different environmental conditions: Zajecar and Nis. In Zajecar, four cultivars (NS-Slavija, NS-H-11, Zajecarska-83 and Europe) were examined. The following three plant density were examined: spacing of 20 cm and seed amount of 18 kg ha^{-1}, the spacing of 50 cm and seed amount of 9 kg ha^{-1} and the spacing of 80 cm and seed amount of 4.5 kg ha^{-1}. In Nis four local varieties (NS-Slavija, NS-H-11, Zajecarska-83 and Krusevacka-28) were used, and three plant density were tested: row spacing of 20 cm and the amount of seeds in the sowing of 15 kg ha^{-1}, row spacing of 40 cm and the amount of seeds for sowing from 7.5 kg ha^{-1}, row spacing of 60 cm and seed amount of 5 kg ha^{-1}. The influence of density and cultivar on the total number of plants m^{-2}, the stem number m^{-2} (vegetative + generative), the number of generative stems m^{-2}, seed yield calculated in kg ha^{-1}, seed germination (%) and 1000 seed weight (g) was examined. There was no effect of density and cultivar on dry matter yields (t ha^{-1}) obtained from the first and third growth. For the combined use of alfalfa, where the first and third cut was used for fodder, and the second for the production of seed, the best result was obtained with the spacing of 40-50 cm and seed amount of 7.5-9 kg ha^{-1}, where the relationship between seed yield and forage yield was optimal, at both sites. Under the environmental conditions of Zajecar, the highest potential for seed yield was obtained by the NS Slavija, and for forage yield by Zajecarska-83. Domestic varieties were superior to the French cultivar Europe for forage, but not for seed yield. Under the environmental conditions of Nis cultivar Krucevacka 28 showed highest potential for yield, and NS Slavija for forage yield.

Key words: alfalfa for combined use, components of seed yield, seed yield, seed quality, forage yield.

INTRODUCTION

In the region of South-east Europe, alfalfa (*Medicago sativa* L.) is highly adaptable to the environmental conditions, giving high yields and fodder of excellent quality. In this region seed production has a secondary importance because of variable quality and yield (Iannucci et al., 2002; Karamanos et al., 2009).

Alfalfa is the most important perennial forage legume: in Serbia it is grown on about 200 thousand hectares (Radović et al., 2010), in Romania on 350 thousand hectares (Moga and Schitea, 2005), in Croatia on 40-50 thousand hectares (Stjepanović and Popović, 2009).

Using appropriate breeding methods a number of cultivars that are characterized by improved forage quality were created (Schitea et al. 2007; Petolescu et al., 2010; Stjepanović and Popović, 2009; Radović et al., 2010). Improving the production of alfalfa seed is possible by creating high yielding varieties and by improved cultural practices. According to Bolanos-Aguilar et al. (2000, 2002) research on genetics and breeding showed that progress in achieving higher seed yield in alfalfa is limited.

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On the other hand, the optimal use of mineral nutrition (Gossen et al., 2004; Terzić, 2011), growth regulators (Dragovoz et al., 2002; Zhang et al., 2009), pollinators (Jevtić et al., 2005; Andjelković et al., 2010), row distance, the amount of seed used for sowing and plant density (Al-Noaim and Koriem 1992; Zhang et al., 2008; Ionova and Shishela, 2008) can have a significant impact on yield components and yield formation, and it seems like it could be a better route to achieving higher and more stable seed yields.

The density of alfalfa crop is the product of row spacing, distances in row and quantity of seed per unit area, which have direct impact on yield. Determination of optimum density of alfalfa is complex, because alfalfa is the perennial crop (4-5 years), giving lower yield over time (Beković, 2005; Stanisavljević, 2006). Therefore, it is important to determine the appropriate agronomic production technology which will optimise seed yield.

Production of alfalfa seeds is successful in regions where during the growing period there is low humidity and moderately high temperature, allowing periods of pollination, a small occurrence of diseases and favourable conditions for harvest. On this assumption, many regions in South-east Europe are eligible, but the seed yield that is achieved is generally low and highly variable.

In this region, alfalfa seed production is almost always done from the second cut, and the first and third cuts are used for forage production, which is significant and competitive commodity market (Erić, 1995; Stjepanović and Popović, 2009; Moisuc and Djukić, 2002).

The main objective of this study was to: (1) evaluate the effect of different plant densities on yield and yield stability of alfalfa seed for combined production, and (2) study influence of cultivars on yield and yield stability of alfalfa seed production.

MATERIAL AND METHODS

The trials were conducted in two environmental conditions, at Zajecar and Nis.

Under the environmental conditions of Zajecar (43° 53' N; 22° 17' E and 159 m a.s.l.), with appropriate primary treatment sowing was done in mid-April 2002 (A_0). The experimental design was a randomised-block design with four replications.

During four years of using alfalfa (2003-A_1, 2004-A_2 2005-A_3 2006-A_4), we examined the following crop densities: the row spacing of 20 cm and seed amount of 18 kg ha^{-1} (B_1), the row spacing of 50 cm and seed amount of 9 kg ha^{-1} (B_2); row spacing of 80 cm and seed amount of 4.5 kg ha^{-1} (B_3). Cultivars: NS-Slavija (C_1); NS-Novosadjanka H-11 (C_2); Zajecarska-83 (C_3) and the French cultivar Europe (C_4), were investigated.

The following characteristics were analysed: total number of plants per m^2; number of stems per m^2 (total: vegetative + generative), the number of generative stems per m^2, and seed yield calculated in kg ha^{-1}. The following parameters of seed quality were determined: germination (%) and 1000 seed weight (g). Second cut was used for seed production, and for the first and third cuts forage yield was determined in dry forage matter (t ha^{-1}).

Under the environmental conditions of Nis (43°19' N; 21°51' E and 206 m a.s.l.), the experiment was based on the same system and monitored the same parameters as in Zajecar. Sowing was performed one year earlier than in Zajecar (2001-A_0).

The following densities were used in this region: the spacing of 20 cm with the seed amount of 15 kg ha^{-1} (B_1), the spacing of 40 cm and seed amount of 7.5 kg ha^{-1} (B_2), the spacing of 60 cm and seed amount of 5 kg ha^{-1} (B_3). In this region domestic cultivars NS-Slavija (C_1), NS-Novosadjanka H-11 (C_2), Zajecarska-83 (C_3) and Krusevacka 28 (C_4) were used.

Based on the average of agrochemical soil analysis, vertisol in Zajecar was determined to have low $CaCO_3$ content, being slightly acidic (pH 5.7 to 6.3), low to high amounts of humus (2.4% to 2.8%), high amounts of total nitrogen content (0.16% to 0.20%), and mainly high amounts of easily accessible phosphorus (9.8% to 14.7% in mg/100 g soil) and potassium (12.8% to 18.2% in mg/100 g soil). In Nis, the type of soil was alluvial with neutral pH (7.10 to

7.30), medium humus (2.8% to 3.0%) and medium supply of total nitrogen (15% to 20%). The soil is well supplied by phosphorus content (17.5 to 19.2% in mg/100 g soil) and potassium (19.5 to 23.2% in mg/100 g soil).

The experiment was conducted for four consecutive years (A_1, A_2, A_3 and A_4) in the two locations. Mean values and coefficient of variation (CV) were calculated. Years, two treatments (crop density, cultivars), and their interactions were analysed using a standard *F* test. Data from each year were also analysed separately to determine the among-years variations. Analysis of differences between crop density and cultivar averages was performed using Fisher's protected LSD test at a $p \leq 0.05$ significance level. These analysis procedures were performed using the STATISTICA for windows software (Stat Soft 8.0).

RESULTS AND DISCUSSION

During the four-year study, climatic conditions were quite variable (Table 1). During 2003, in the most important months for the production of alfalfa seed (June, July and August), in Zajecar the amount of precipitations was 100 l m^{-2}, and in Nis 92 l m^{-2}. Sufficient rainfall was recorded in Nis in 2005, and in June and July 96 l m^{-2}. In August precipitation was 85 l m^{-2}, but only after harvest.

The other years were characterized by more rainfall especially in June, July and August (Zajecar: 2004 - 192 l m^{-2}; 2005 - 309 l m^{-2}; 2006 - 243 l m^{-2}, and Nis: 2002 - 267 l m^{-2}; 2004 - 176 l m^{-2}; 2005 - 181 l m^{-2}). Due to high rainfall, pollination was reduced and there were an increased number of vegetative stems. In these agro-ecological conditions, precipitations during the summer were usually accompanied by increased wind and this influenced the yield of seed and forage.

Alfalfa for seed production in Southeast Europe is mainly grown in row spacing of 12.5 to 25 cm and with the seed norm of 16-24 kg ha^{-1} (Erić, 1995; Stjepanović and Popović, 2009; Moisuc and Djukić, 2002). Seed rate and row spacing give the sowing density of crops, which is the most important component of seed yield and forage. Crop density influences all the other yield components and affects seed yield and the forage yield (Beković, 2005; Stanisavljević, 2006).

Table 1. Precipitations (l m^{-2}) and average temperatures (T °C) in Zajecar and Nis

		Year	Month											
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Environmental conditions of Zajecar	Precipit. l m^{-2}	2003	53	19	8	9	61	43	56	1	68	15	27	36
		2004	91	42	47	46	29	81	49	62	36	46	79	35
		2005	53	76	26	50	73	25	115	169	21	62	50	61
		2006	28	47	62	40	25	97	53	123	27	20	9	32
	T, °C	2003	0.4	6.1	5.5	10.3	17.8	22.6	23.8	19.8	15.5	10.6	6.6	-2.2
		2004	-0.2	-3.2	4.1	9.9	18.9	22.5	22.3	24.3	15.5	9.3	6.7	1.0
		2005	-1.0	2.2	6.6	11.9	14.8	19.5	21.9	20.5	15.9	12.2	6.6	2.1
		2006	1.4	-2.8	3.9	10.8	16.7	19.1	20.8	19.7	16.2	10.5	3.7	2.0
Environmental conditions Nis	Precipit. l m^{-2}	2002	6	16	48	91	52	52	76	139	56	44	35	70
		2003	84	16	8	42	75	34	32	26	43	132	111	2
		2004	78	66	25	36	56	107	44	25	63	57	127	46
		2005	49	61	70	89	104	51	45	85	21	38	46	76
	T, °C	2002	-1.0	8.1	9.9	12.0	18.9	20.3	25.0	21.9	16.7	12.6	9.0	0.9
		2003	0.0	1.5	6.5	11.7	20.6	25.3	25.4	25.0	17.1	11.9	9.5	4.1
		2004	-0.5	2.8	7.3	13.3	15.0	19.7	22.5	21.6	16.8	14.7	6.5	12.0
		2005	-0.8	5.1	11.8	16.8	19.0	22.3	20.5	17.9	12.3	5.7	3.5	11.2

Alfalfa crop with a larger row-space and with smaller quantities of seed for planting a smaller number of plants, are favourable conditions for root development, but also more favourable conditions for the emergence and development of weeds (Moyer et al., 1991).

Significant interaction year x plant density suggests that between year differences did not have the same effect on the density: the number of plants in treatment B_3 varied significantly less from year to year in relation to treatments B_2 and B_3 (Table 2). Under the environmental conditions of Zajecar cultivar Slavia over three years had significantly more plants per m^2 than Europe. NS Slavia had significantly more plants than varieties Krusevačka 22 and Zajecarska 83, at the site of Nis during a year of research (Table 3). The biological characteristics of alfalfa is to have the crown of a plant developing a number of stems that may be vegetative (important for forage, but not for seed production) and generative (that give seed yield).

Table 2. Significance according to *F* test for years, density and cultivar main effects, and their interactions on seed yield, plant number, stem number, generative stems, seed germination, 1000 seed weight, and forage dry matter yield

Source		df	Seed yield (kg ha^{-1})	Plant number (m^{-2})	Stem number (m^{-2})	Generative stems (m^{-2})	Seed germination (%)	1000 seeds masss	Forage dry matter yield (t ha^{-1})
Year (Y)	Z	3	**	**	**	**	**	**	**
	N	3	**	**	**	**	**	**	**
Density (G)	Z	2	**	**	**	**	**	**	**
	N	2	**	**	**	**	**	**	**
Cultivar (C)	Z	3	**	**	*	*	NS	NS	**
	N	3	**	*	*	*	NS	NS	**
Y x G	Z	6	**	**	**	**	NS	NS	**
	N	6	**	**	**	**	NS	NS	**
G x C	Z	9	**	**	**	**	NS	NS	**
	N	9	**	*	*	*	NS	NS	**
Y x G x C	Z	18	**	**	**	**	NS	NS	**
	N	18	**	**	**	**	NS	NS	**

(Z - environmental conditions Zajecar; N - environmental conditions Nis).
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. †NS, not significant.

Treatment (as average of all cultivars) of B_1 at both sites in all years had the highest number of plants per m^2, but in the first and second year differences between treatments B_1 and B_2 were significant, while in the third and fourth year differences were not significant ($p \leq 0.05$). Treatment B_3 on both sites in the first two years had a significantly lower number of plants in relation to the treatment of B_2 (Table 3).

Table 3. Effect of sowing density on plant number per m^2 of different alfalfa cultivars in two agro-ecological conditions

Factor	Treat-ment	Plant number per m^2							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	193a	134a	89a	67a	205a	143a	95a	72a
	B_2	99b	79b	70ab	46ab	97b	83b	88ab	60ab
	B_3	77c	59c	46b	30b	77c	59c	50b	42b
Cultivar	C_1	133a	96a	72a	54a	127a	95a	81a	60a
	C_2	134a	94ab	70ab	52a	129a	97a	78ab	57a
	C_3	133a	90ab	66ab	43a	128a	98a	75b	57a
	C_4	90b	82b	64b	41a	121a	89a	75b	58a
Year	A	A_1 123; A_2 91; A_3 68; A_4 48 CV=39.0%				A_1 126; A_2 95; A_3 78; A_4 58 CV=32.3%			

Values followed by different letters within columns are significantly different ($p \leq 0.05$) according to the LSD test.

In the first year of utilization of alfalfa (A_1), plant number was directly dependent on the quantity of seed for sowing (Table 3). As expected, in the following years there has been a loss of plants, but not equal on different densities.

There was a significant difference for the total number of stems between treatments B_1 and B_2 and between B_2 and B_3 in all years at both sites (Table 4). As for the number of generative stems, treatment B_1 also had the greatest total number of stems, but there was no significant difference ($p \leq 0.05$) between treatments B_1 and B_2 (Table 5).

Increased rainfall enforces vegetative growth of new stems. In the environmental conditions at Zajecar in 2004 on average over all densities, we determined 517 stems per m^2 of which only 213 or 40% were generative stems, while in 2003 there were 410 stems per m^2 of which 245 or 60% were generative. In agro-ecological conditions of Nis, in 2002 (A_1) there were a total of 529 stems per m^2, of which 173 or 33% were generative stems, while in 2003 (A_2) from a total number of 417 stems per m^2, 164 or 39% were generative stems. The unfavourable relationship between the number of vegetative and generative stems in Nis, in relation to Zajecar can be explained by better soil fertility at Nis, which led to more intense growth of vegetative stems. As expected, under the influence of biological aging, diseases of the root and other factors the number of plants and number of stems was reduced in time (Tables 4 and 5).

Table 4. Effect of sowing density on total stem number per m^2 of different alfalfa cultivars in two agro-ecological sites

Factor	Treat-ment	Total number of stems per m^2							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	641a	842a	542a	294a	820a	668a	527a	237a
	B_2	345b	416b	301b	204b	442b	357b	219b	136b
	B_3	244c	295c	197c	182c	324c	226c	124c	76c
Cultivar	C_1	436a	521ab	354a	235ab	522a	417a	299a	159a
	C_2	419ab	530a	358a	225ab	518a	380b	259b	128b
	C_3	405b	521ab	372a	246a	515a	409ab	291ab	145ab
	C_4	378c	497b	303b	201b	512a	417a	291ab	144ab
Year	A	A_1 410; A_2 518; A_3 347; A_4 227 CV=32.3%				A_1 529; A_2 417; A_3 290; A_4 150 CV=42.9%			

Values followed by different letters within columns are significantly different ($p \leq 0.05$) according to the LSD test.

Table 5. Effect of sowing density on number of generative stems per m^2 of different alfalfa cultivars in two agro-ecological sites

Factor	Treat-ment	Number of generative stems per m^2							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	368a	316a	188a	167a	203a	196a	157a	156a
	B_2	212b	191b	164a	135ab	180ab	169ab	104b	101b
	B_3	154c	133c	111b	101c	136b	126b	84c	69c
Cultivar	C_1	249a	220a	168a	140ab	185ab	159a	113a	117a
	C_2	240a	209ab	145b	127ab	170ab	144ab	112a	106a
	C_3	244a	204b	148b	126b	161b	137b	112a	106a
	C_4	251a	211ab	155ab	144a	198a	157ab	122a	104a
Year	A	A_1 245; A_2 213; A_3 154; A_4 134 CV=27.6%				A_1 173; A_2 164; A_3 115; A_4 109 CV=23.5%			

Values followed by different letters within columns are significantly different ($p \leq 0.05$) according to the LSD test.

At Zajecar, on average over all densities, cultivar Europe showed a significantly lower total number of stems in all years, but considering the number of generative stems it was the leading cultivar. Under the environmental conditions of Nis in the first year (A_1) of use, alfalfa cultivars impact on the total number of stems was not significant, while in the following years NS Slavia had significantly higher total number of stems. Regarding the number of generative stems in two years the influence of cultivar was not significant, but for the other two years it was.

In addition to significant influence of year, density and cultivar on the total number of plants as well as the number of generative stems, there was a significant interaction between these factors (Table 2). The results obtained about the influence of genotype on the number of plants are consistent with the results of Tucak et al. (2009).

There is a lot of conflicting results in the area of alfalfa seed production: testing of optimal row spacing, row-distances, the quantity of seed for sowing, and plant density (Al-Noaim and Koriem, 1992; Zhang et al., 2008; Ionova and Shishela, 2008). It seems that the optimal cultural practices of alfalfa seed must be adapted to the agro-ecological and soil conditions.

During the four years of testing, average seed yield from three different densities and the four varieties, depending on the year, varied from 631 kg ha^{-1} (2002-A_1) to 235 kg ha^{-1} (2005-A_3) (CV= 47.5%) in ecological conditions of Zajecar, and from 449 kg ha^{-1} (2004) to 258 kg ha^{-1} (2001) (CV= 29.4%) in ecological conditions of Nis (Table 6).

Table 6. Effect of sowing density on seed yield (kg ha^{-1}) of different alfalfa cultivars, in two agro-ecological sites

Factor	Treat-ment	Seed yield (kg ha^{-1})							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	664b	367b	244b	203b	245b	434b	235b	443b
	B_2	684a	425a	260a	242a	282a	493a	294a	483a
	B_3	545c	248c	259a	261a	246b	403c	286a	420b
Cultivar	C_1	690a	378b	260a	236a	259b	452ab	266b	444ab
	C_2	586d	268d	257a	236a	227c	411b	242c	438b
	C_3	603c	307c	249a	234a	260b	466a	297a	454ab
	C_4	644b	434a	247a	235a	284a	444b	287a	458a
Year	A	A_1 631; A_2 437; A_3 254; A_4 235 CV=47.5%				A_1 258; A_2 443; A_3 272; A_4 449 CV=29.4%			

Values followed by different letters within columns are significantly different (p≤0.05) according to the LSD test.

During three years, cultivars at mid plant density (treatment B_2) had significantly higher seed yield than the yield achieved on the treatment of B_1 and B_3 (p≤0.05) in ecological site of Zajecar. During two years (2005 and 2006) yields of treatments B_2 and B_3 were not significantly different, but yield of B_2 was significantly higher than the yield with treatment B_1.

Under the environmental conditions of Nis in the period A_1 to A_4, B_2 treatment (in three years) had significantly higher seed yield than other treatments, while in one year (2004) yield of treatments B_2 and B_3 did not differ. During two years (2002 and 2005) there were no significant differences (p≤0.05) between treatments B_1 and B_3.

Under the environmental conditions of Zajecar, NS Slavija produced the highest yield of seeds during three years, but only in 2003 yield was significantly higher than the second yielding cultivar, Europe. In the years unfavourable for the production of alfalfa seed (2005 and 2006) yield potential was not expressed and the influence of cultivar had no significant effect on yield. During two years, the lowest potential for grain yield was found in the cultivar Novosadjanka H-11, which was significantly lower than in other varieties ($p \leq 0.05$). At both sites the year, the density, the cultivar and their interactions significantly affected the seed yield (Table 2). However, the reasons why the cultivars showed statistically significant effect for yield in adverse ecological conditions of Nis (2002 and 2004), but not in Zajecar, are unclear.

Germination and 1000 seed weight are basic seed quality indicators. Under the environmental conditions in Zajecar, seed germination was the highest in all cultivars in 2003, regardless of sowing density (Table 7). At both sites the effect of density on seed germination was significant (Table 2). The highest germination was in the years when the yield was the highest (Zajecar 2003, Nis 2003 and 2005). In all years and at both sites the highest germination was obtained in treatment B_3, then the treatment B_2, and the lowest germination was on the treatment B_1. However, the density did not have statistically significant effect on germination ($p \leq 0.05$). Also, neither cultivar nor all of the interactions had significant effect on seed germination.

Table 7. Effect of sowing density on seed germination (%) at two agro-ecological sites

Factor	Treat-ment	Germination (%)							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	91.8a	78.4a	82.8a	80.6a	87.5a	88.9a	80.9a	89.4a
	B_2	92.9a	80.2a	83.2a	83.2a	89.3a	91.3a	81.9a	93.9a
	B_3	93.9a	81.5a	83.6a	83.6a	90.3a	92.0a	82.3a	94.4a
Cultivar	C_1	92.7a	80.3a	83.2a	80.6a	87.5a	90.0a	81.9a	92.0a
	C_2	93.0a	80.2a	83.6a	81.6a	87.4a	90.6a	82.7a	92.4a
	C_3	92.8a	79.8a	82.9a	81.6a	89.1a	91.7a	81.5a	92.6a
	C_4	92.9a	79.9a	83.1a	82.3a	89.4a	90.6a	80.7a	93.1a
Year	A	A_1 92.9; A_2 80.8; A_3 83.2; A_4 82.5 CV=6.4%				A_1 89.0; A_2 90.7; A_3 81.7; A_4 92.6 CV=5.4%			

Values followed by different letters within columns are significantly different ($p \leq 0.05$) according to the LSD test.

Results show that the years had a significant impact on the 1000 seed weight. The weight of 1000 seeds was highest in treatment B_3, but not significantly higher than one obtained from other treatments ($p \leq 0.05$). Also the influence of cultivar, as well as of the interactions, was not significant. Higher seed weight is better for seed production of alfalfa and a highly positive correlation between germination and 1000 seeds weight was reported (Beković, 2005; Stanisavljević, 2006).

Table 8. Effect of sowing density on 1000 seeds weight (g) of different alfalfa cultivars, in two agro-ecological sites

Factor	Treat-ment	1000 seed weight (g)							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	1.78a	1.85a	1.83a	1.93a	2.03	2.00	1.93	1.73
	B_2	1.85a	1.90a	1.95a	2.00a	2.08	2.03	2.00	1.85
	B_3	1.81a	1.91a	2.03a	2.05a	2.13	2.03	2.00	1.85
Cultivar	C_1	1.80a	1.87a	1.92a	2.03a	2.03	2.00	1.97	1.77
	C_2	1.87a	1.83a	1.93a	1.90a	2.13	2.06	1.96	1.77
	C_3	1.77a	1.87a	1.90a	1.97a	2.08	2.00	1.98	1.83
	C_4	1.87a	1.90a	2.00a	2.07a	2.07	2.05	2.00	1.87
Year	A	A_1 1.81; A_2 1.89; A_3 1.94; A_4 1.94 CV=3.2%				A_1 2.08; A_2 2.03; A_3 1.98; A_4 1.81 CV=5.9%			

Values followed by different letters within columns are significantly different ($p \leq 0.05$) according to the LSD test.

In conditions of South-east Europe, forage production of alfalfa is 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} (Erić, 1995, Stjepanović and Popović, 2009, Moisuc and Djukić, 2002). However, most results suggest that the production of seed crops require lower crop density. This raises the question of which density is optimal for seed production in the second cut, if the first and the third are used as forage. As alfalfa ages its fitness is reduced (due to stress from drought or frost, late cut, crown and root diseases etc.), which all causes the reduction of forage yield (Erić, 1995). However, favourable environmental conditions, especially from the point of view of precipitation at the beginning of exploitation may have positive effect on forage yield. In Zajecar higher forage yield was obtained during the 2004 (A2) in comparison with 2003 (A1). As the crop is further used, dry matter yield of forage inevitably decreases. Treatment B1 had the greatest yield of forage dry matter in all years (Table 9).

In the conditions of Zajecar, during the three years, there was no significant difference ($p \leq 0.05$) between treatments B_1 and B_2. At Nis B1 treatment also had the highest yields of forage, while the crop was in better condition. During the first two years of use A_1-A_2 (2002 and 2003) yields were not significantly higher than yield obtained with treatment B_2. Dry forage yield obtained from treatment B_3 was significantly lower in all years and at both sites than the yield from the treatment B_2 (Table 9).

Table 9. Effect of sowing density on forage yield (t ha^{-1}) of different alfalfa cultivars, at two agro-ecological sites

Factor	Treat-ment	Dry forage yield (t ha^{-1})							
		Agro-ecological conditions of Zajecar				Agro-ecological conditions of Nis			
		2003	2004	2005	2006	2002	2003	2004	2005
Density	B_1	6.56a	7.50a	5.60a	3.96a	6.34a	5.72a	5.05a	4.62a
	B_2	6.30a	7.16a	5.09a	3.24b	6.09a	5.38a	4.20b	3.83b
	B_3	3.15b	3.96b	3.21b	2.57c	4.80b	3.89b	3.60c	3.31c
Cultivar	C_1	5.39ab	6.54a	4.74a	3.26b	5.84a	5.14a	4.51a	4.03a
	C_2	5.50a	6.52a	4.86a	3.23b	5.66a	5.03a	4.33ab	3.65b
	C_3	5.62a	6.32a	4.90a	3. 95a	5.72a	4.98a	4.33ab	4.15a
	C_4	4.84b	5.44b	4.02b	2.59c	5.75a	4.83a	3.97b	3.83ab
Year	A	A_1 5.34; A_2 6.21; A_3 4.63; A_4 3.26 CV=25.7%				A_1 5.74; A_2 5.00; A_3 4.27; A_4 3.92 CV=17.1%			

Values followed by different letters within columns are significantly different ($p \leq 0.05$) according to the LSD test.

At Zajecar in three years Zajecarska-83 had the highest yield of dry forage, in only one year 2006 (A_4) being statistically higher than all others (Table 9). This could be attributed to the good adaptability of this cultivar to these conditions, because local populations of alfalfa were used for its creation. In contrast, the cultivar Europe showed significantly lower yield of dry forage than other cultivars, in all investigated years. Results on the influence of cultivar on forage yield correspond with the results of Petcu et al. (2009).

Under the environmental conditions of Nis during the first two years, the cultivar had no significant effect on yield, while during the last two years NS-Slavija produced significantly higher yields than the cultivar H-11 (Table 9). All interactions had a significant effect on dry forage yield.

CONCLUSIONS

Under the environmental conditions of Eastern and Southern Serbia, depending on the year, alfalfa exhibited a high variability of seed yield (Zajecar CV = 47.5%; Nis CV = 29.4%). Variability of dry forage yield was expressed to a lesser extent (Zajecar CV = 25.7%; Nis CV = 17.1%).

The plant density obtained by sowing at 40-50 cm between rows and using 7.5 to 9 kg ha^{-1} of seed is the optimal compromise between the highest seed yield and satisfactory forage yield and can be applied in agronomy practice in South-eastern Europe.

During the four years of investigation under the ecological conditions of Zajecar highest potential for seed yield was observed in the cultivar NS-Slavia, and the highest forage yield was obtained with the cultivar Zajecarska-83.

Domestic varieties in relation to cultivar Europe showed the superiority for forage yield, but not for seed yield.

Under the environmental conditions of Nis highest potential for seed yield was shown by cultivar Krusevacka-28 and for forage by NS-Slavija.

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