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VARIATIONS IN THE ALFALFA FORAGE YIELD PER CUTS IN THE FIRST VEGETATION YEAR

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ABSTRACT

Understanding the effects of seeding rate and row spacing on fresh and dry matter yield may help obtain optimal and cost-effective alfalfa production (*Medicago sativa* L.). The objective of this study was to determine the optimal seeding rate and row spacing when sowing different alfalfa genotypes. A field experiment was set up in the fall of 2020 in Zlokukjani of Skopje settlement, North Macedonia, according to a split-split plot design in a randomized complete block in three replications. The treatments in the experiment include two seeding rates (8 and 16 kg ha^{-1}), two row spaces (20 and 40 cm), and four alfalfa cultivars. The obtained results show a significant influence of the sowing method (row spacing) on the two examined traits (fresh and dry forage yield) compared to the seeding rate, which did not show significant differences in yield. The highest yield of fresh (26 t ha^{-1}) and dry (7.04 t ha^{-1}) forage is achieved in the first cut at a sowing distance of 20 cm. Our one-year research recommends that when applying appropriate agro-technical methods in crop establishment and temperate climatic conditions, there is no justification for applying high sowing norms (over 16 kg ha^{-1}). The selection of an appropriate commercial cultivar and row spacing are inevitable items within an agronomic production technology to obtain optimal alfalfa forage production.

Key words: alfalfa, seeding rate, row spacing, cultivar, fresh forage, and dry matter yield.

INTRODUCTION

Alfalfa is a leading forage crop due to high agro-climatic adaptability, high yield potential, and high nutrition value (Li & Brummer, 2012; Undersander et al. 2011; Abdel-Rahman & Abu Suwar 2012; Baxevanos et al. 2021; Jefferson & Cutforth 1997). It is most common in temperate climate worldwide and is grown on approximately 32 million hectares (Bouton, 2012). According to the State Statistical Office data in R. North Macedonia in 2020, 517.039 ha of arable land were registered, and 41.318 ha were established under fodder crops. As the most common forage crop in the same year, alfalfa was grown on 19.096 ha with an average hay yield of 5.455 kg ha^{-1} (SSO, 2021).

The yield and quality of alfalfa fresh forage and hay depend on a series of agro-technical measures, such as soil cultivation, seedbed preparation, seeding rate, sowing season etc., as well as on ecological conditions (air temperature, amount of precipitation, photoperiod and soil fertility). The proper seeding rate is crucial for successful alfalfa establishment (Atis et al. 2019; Lu et al. 2019). The question of what is the accurate seeding rate to use is of practical importance, especially when introducing glyphosate-tolerant varieties, which have higher prices compared to the commercial alfalfa varieties used so far (Berti & Samarappuli, 2018). Their irrational use, i.e., the use of high seeding rates, can significantly increase the costs of alfalfa establishment, and among other things, there may be increased competence between plants, which leads to plant mortality and natural thinning of the crop (Hall et al. 2004; Hall et al. 2012). Numeral research on alfalfa production state that the seeding rate can vary in the range of 4 to 40 kg ha^{-1} (Bolger & Meyer, 1983; Lloveras et al., 2008; Atis et al., 2019; Hakl et al., 2021). Several authors consider the seeding rate of 15-18 kg ha^{-1} to be the upper limit for obtaining optimal stand density, fresh and dry matter yield (Hansen & Krueger 1973; Hall et al. 2004; Abdel-Rahman & Abu Suwar 2012; He et al. 2018; Katanski et al. 2020). The use of high seeding rates, mainly above 17 kg ha^{-1}, increases the plant density and the competitiveness among plants, but in the long run, they have a limited effect on the forage yield (Hall et al. 2004, 2010; Lloveras et al. 2008). However, Glaspie et al. (2011) obtained the highest dry matter yield when using 18 kg ha^{-1}, compared to the other examined lower seeding rates. Yan-Hua et al. (2017) recommend the seeding rate to be in the range of 22.5 - 30.0 kg ha^{-1}, resulting from an increased number of plants when using higher seeding rates, especially in the first vegetation year. Similar results are presented by Hui-Gang et al. (2019), according to which, the total alfalfa hay yield increased with the increase of the seeding rate, and the highest yield was achieved at 22.5 kg ha^{-1}. In determining the optimal seeding rate, the water supply in the region, the irrigation method, the purity of the seed material, the seedbed preparation, the soil type, etc., have a crucial impact. Thus, considering these factors, many studies show an increase or decrease in seeding rates from the recommended optimum (Suttie 2000; Katanski et al. 2020; Hakl et al. 2021).

The basis for successful alfalfa production is good management and coordination of production factors that can be controlled and play an essential role in its productivity. In addition to determining the optimal seeding rate, an important parameter that can be controlled and is directly related to plant productivity is row spacing. In practice, sowing alfalfa in wider rows is closely related to seed production, while sowing in narrow rows is consistent with highly productive forage production. Hui-Gang et al. (2019) confirm the same having in mind that during the three-year research they obtained the highest total hay yield at the row spacing of 15 cm, compared to the spacing of 30 and 40 cm, which caused a yield decrease. These results are in line with Zhang et al. 2008 and Chocarro & Lloveras 2014, where a statistically significant difference between the row spaces was detected, and a higher dry mass yield was obtained when sowing in narrowest rows.

Due to alfalfa high adaptability to different geographical and climatic conditions, the recommended seeding rates and sowing methods vary widely. As a consequence of climate-ecological changes, determining the optimal seeding rate, row spacing, and selection of the appropriate cultivar is constantly a challenge for scientific research and a source of debate among alfalfa growers. The aim of this research is to investigate the impact of seeding rate and row spacing on fresh and dry matter yield per cuts of four alfalfa cultivars in the first productive year. We have set two null hypothesis (H_0), where the first says that the seeding rate does not significantly impact FFY and DMY, and the second one says that the row spacing does not have significant impact on both traits.

MATERIAL AND METHODS

Design and preparation of the experiment

The field experiment is set up in Zlokukjani of Skopje settlement, North Macedonia (42˚00´ North, 21˚22´ East, 258 m above sea level) according to a split-split-plot design in a randomized complete block design in three replications. The variants of the seeding rate as the main factor were distributed in main plots (largest) and have the lowest degree of precision. The row spaces are distributed in subplots, and the varieties as a tertiary factor are distributed in sub-sub plots (smallest plots) and have the highest degree of precision. The sowing was performed manually on 12 September 2020. The following treatments were included in the experiment: a) two seeding rates: 8 and 16 kg ha^{-1}; b) two row spaces: 20 and 40 cm and c) four alfalfa cultivars. The basic plot at a row spacing of 20 cm and 40 cm was 1 m × 3 m and 1.6 m × 3 m, respectively.

Figure 1. Geographic location of the experiment (Source: Google Earth)

The area before the experiment was uncultivated land with the presence of low and high grass vegetation. During the preparation of the experimental field, primary and pre-sowing tillage was carried out. Due to the low rainfall in September 2020, several sprinkler irrigations were carried out soon after sowing. Weeds that appeared after sowing were removed mechanically.

Plant material

The research involved four commercial alfalfa cultivars: Debarska, Banat VS, NS Jelena, and Nijagara. Debarska is a domestic cultivar created at the Agricultural Institute in Skopje. It is characterized by high yield, good quality, strong regenerative ability, longevity and resistance to low temperatures (Ивановски, 2000). Banat VS, NS Jelena, and Nijagara are foreign cultivars created at the Institute of Field and Vegetable Crops in Novi Sad, R. Serbia. Banat VS is a cultivar developed by selecting local Pannonian alfalfa populations and is suitable for growing in fertile soils and neutral pH reaction. It is known as a forage high-yielding cultivar and shows a high degree of tolerance to drought, low temperatures, and lodging. Genotype NS Jelena originates from crosses between Greek and Serbian populations and is primarily intended for breeding in Mediterranean regions. It is characterized as a highly drought-tolerant cultivar and is suitable for intensive cutting regimes. Cultivar Nijagara is created using interspecies hybridization between *M. sativa ssp. sativa* and *M. sativa ssp. falcata* gene pools in order to create germplasm more tolerant to acid soils. This cultivar is recommended to be grown in hilly and mountainous regions on heavy hydromorphic soils (Milić et al. 2014; Rhouma et al. 2017; Milić et al. 2019).

Climate and soil conditions

The experiment was set up on alluvial soil type with pH 7.71, 9.16 % $CaCO_3$, 247 mg kg^{-1} P_2O_5, 225.9 mg kg^{-1} K_2O, and 2.47 % organic matter at a depth of 30 cm. Before sowing, the basic fertilization with 400 kg ha^{-1} of 8:16:24 NPK fertilizer was performed. In addition to the basic fertilization, in the spring of 2021, 200 kg ha^{-1} of nitrogen fertilizer UNIKO 25.5 % N was applied.

Weather conditions for the Skopje region are shown on Figure 2 (UHMR). The average temperature in September 2020 was 21˚C and is higher compared to the long-term average (19.3˚C). The drastically lower amount of precipitation in September (24.5 mm) and October

(43.4 mm) caused deterioration of soil moisture, especially in the surface soil layer, and delayed the germination of plants. The winter period that followed was characterized by higher average temperatures compared to the LTA, which enabled a successful wintering of the crop. The annual amount of precipitation (521.5 mm) in the first productive year (2021) was higher compared to 2020 (502.4 mm) and LTA (502.4 mm), which benefits forage production.

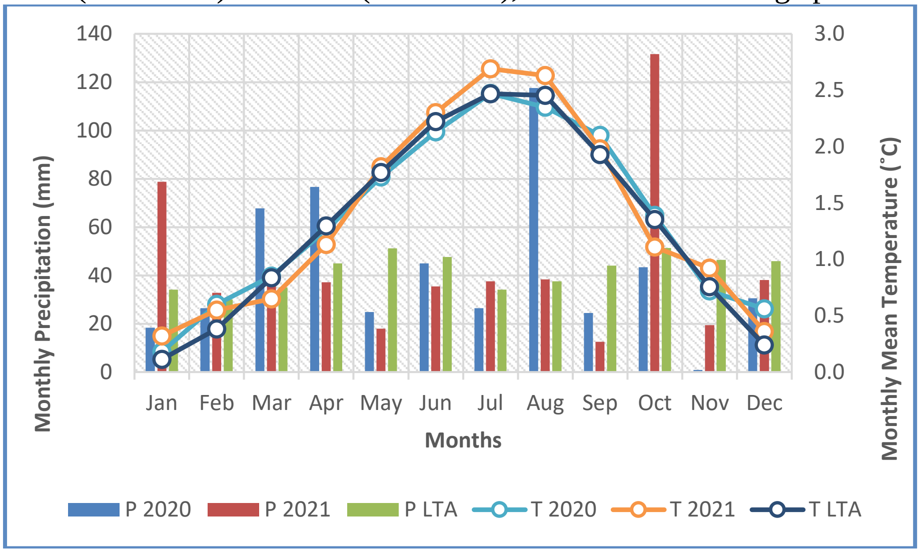

Figure 2. Meteorological data for the examined period and LTA (1991-2020)

Measurements

The effect of the applied treatments on the fresh (FFY) and dry (DMY) matter yield (t ha^{-1}) was monitored in the spring of 2021. The mowing was done manually (with a sickle) at an approximate height of the plant stem of 5 cm. According to the Kalu & Fick (1981) classification, plants were cut at an early stage of flowering (approximately 10% of plants with flower). The FFY was determined on the field immediately after mowing using a digital scale. After measuring the FFY, a sample of 300 g was taken and dried at room temperature until constant weight. After determining the percentage of dry matter, the dry matter yield was calculated. In the first vegetation year, five cuttings were performed, of which four were for forage production (I, III, IV and V), and one (II cut) was intended for seed production. Due to the unfavorable weather conditions (heavy and frequent rains after the I cut), plant lodging, and root rot occurred. At the same time, plants re-growth started, causing that the second cut was not fully realized and recorded, so it had to be eliminated.

Statistical analysis

Data were analyzed using RStudio software v. 1.4.1717, using a linear mixed model. In order to determine the impact of the fixed effects (replication, seeding rate, row spacing, variety, and interactions between them) on the fresh (FFY) and dry (DMY) matter yield, ANOVA was conducted using the Kenward-Roger method (method for approximate calculation of degrees of freedom). The emmeans (Estimated Marginal Means) package was used to compare the factor levels. The significance of the differences between the mean values was determined by the Tukey test at a P = 0.05 significance level.

RESULTS AND DISCUSSION

In all cuts the seeding rate did not significantly impact FFY and DMY, while the row spacing showed significant impact on both traits (Table 1 and 2). The initial or null hypothesis (H_0) for the seeding rate is accepted, i.e., p> .05, and the obtained results indicate that there is no significant difference in the obtained yield between two seeding rates. The following null hypothesis concerning the row spacing is rejected because p <.05 for the two traits investigated and results in a significant difference in the yields obtained between two row spaces.

Table 1. Significance of the examined effects on the total fresh forage yield (FFY) per cut, according to the *F*-test

Source of variation	I cut			III cut			IV cut			V cut		
	df	F-test	Pr(>F)	df	F-test	Pr(>F)	df	F-test	Pr(>F)	df	F-test	Pr(>F)
Replication	2	3.17	0.23943	2	0.52	0.655932	2	2.41	0.2924638	2	2.68	0.271379
Seeding rate (SR)	1	0.43	0.57571	1	0.01	0.90729	1	0.02	0.8910299	1	0.1	0.77941
Row spacing (RS)	1	50.51	0.00207**	1	54.53	0.001793**	1	83.9	0.0007885***	1	27.29	0.006409**
Cultivar (C)	3	4.11	0.01722*	3	3.84	0.022277*	3	2.48	0.0848876.	3	21.01	6.719e-07***
SR x RS	1	0.56	0.49543	1	0.96	0.38262	1	4.82	0.0929120.	1	1.34	0.310082
SR x C	3	3.96	0.01978*	3	0.54	0.654503	3	1.04	0.3907824	3	4.81	0.009166**
RS x C	3	0.47	0.70123	3	0.99	0.411911	3	0.6	0.6186802	3	4.44	0.012810*
SR x RS x C	3	0.08	0.96571	3	0.01	0.998303	3	0.28	0.8358565	3	2.73	0.065992.

*** Significant at the P=0.001 level, ** Significant at the P=0.01 level, * Significant at the P=0.05 level

The obtained results are consistent with those of Lloveras et al. (2008); Abdel-Rahman & Abu Suwar (2012); Chocarro & Lloveras (2014); Berti & Samarappuli (2018); Atis et al. (2019); Katanski et al. (2020) and Xu et al. (2021) according to which the seeding rate has no significant impact on the forage yield. The initial results in our research have shown that there is no justification for using a higher seeding rate in the forage production in the examined cultivars. The obtained FFY and DMY in all cuts did not show significant difference between two seeding rates (Table 3 and 4). When increasing the seeding rate, the plant density increases, and thus the yield, but to a certain extent, i.e., until establishing an optimal seeding rate depending on the agro-climatic conditions in a region. When the set optimum is exceeded, the plant density increases, and thus the yield decreases, as a result of the increased internal competitiveness and the reduced branching of the plants, which leads to a reduction of the biomass per plant, and thus the total yield (Hall et al. 2004; 2010; He et al. 2018).

Table 2. Significance of the examined effects on the total dry matter yield (DMY) per cut, according to the *F*-test

Source of variation	I cut			III cut			IV cut			V cut		
	df	F-test	Pr(>F)	df	F-test	Pr(>F)	df	F-test	Pr(>F)	df	F-test	Pr(>F)
Replication	2	3.61	0.216789	2	0.13	0.8828836	2	3.08	0.2447088	2	1.31	0.432555
Seeding rate (SR)	1	0.01	0.913848	1	0.01	0.9173621	1	0.1	0.7797922	1	0.05	0.836064
Row spacing (RS)	1	33.29	0.004477**	1	95.16	0.0006186***	1	121.68	0.0003839***	1	28.1	0.006081**
Cultivar (C)	3	3.76	0.023875*	3	4	0.0191676*	3	4.01	0.0190073*	3	23.68	2.368e-07***
SR x RS	1	0.26	0.63572	1	0.7	0.4472535	1	4.79	0.0936528.	1	1.44	0.295977
SR x C	3	5.24	0.006307**	3	0.96	0.4267071	3	1.12	0.3571884	3	4.4	0.013255*
RS x C	3	1.43	0.257202	3	1.53	0.2319163	3	1.22	0.3234677	3	3.71	0.025132*
SR x RS x C	3	0.4	0.750108	3	0.26	0.849909	3	0.23	0.8698714	3	3.58	0.028513*

*** Significant at the P=0.001 level, ** Significant at the P=0.01 level, * Significant at the P=0.05 level

Unlike the seeding rate, the row spacing has a significant impact on the forage yield, which is confirmed in the studies of Zhang et al. (2008); Chocarro & Lloveras (2014); Hui-Gang et al. (2019). Determining the appropriate row spacing is an inevitable issue in order to obtain optimal alfalfa forage production (Stanisavljevic et al. 2012; Mattera et al. 2013). According to the results, in all cuts a significantly higher alfalfa FFY and DMY was obtained when sowing at a distance of 20 cm, compared to 40 cm (Table 3 and 4). Comparing by cuts, the highest average FFY and DMY was obtained in the first cut – 26.0 t ha^{-1} and 7.0 t ha^{-1}, and the lowest in the fifth cut - 6.5 t ha^{-1} and 1.5 t ha^{-1}, respectively. In a similar direction are the results of research conducted in Korea, wherein the first vegetation year at a row spacing of 15 cm and seeding rate of 20 kg ha^{-1}, the highest yields of fresh forage (23.3 t ha^{-1}) and hay (6.3 t ha^{-1}) are recorded from the first cut, and the lowest in the last cut - 14.8 t ha^{-1} and 2.3 t ha^{-1}, respectively (Geun et al. 2021). Abdel-Rahman and Abu Suwar (2012) at a seeding rate of 15 kg ha^{-1} under irrigation conditions, the highest average yield of fresh (9.83 t ha^{-1}) and dry (2.25 t ha^{-1}) matter yield were obtained in the second cut, and the lowest in the last (sixth cut), 5.78 t ha^{-1} and 1.52 t ha^{-1}, respectively. Celebi et al. (2010) also observed a decrease in the yield from the first to the third cut in two production years when sowing at a row spacing of 20 and 40 cm. When cutting alfalfa at an early flower stage, for all production years, from the first to the fourth cut, an average yield of 33, 29, 22, and 16% of the total annual hay yield was obtained (Djaman et al. 2020). It can be noticed a declining trend in the alfalfa production (fresh and dry mass) from the first to the last cut in the first production year. Reducing the time interval between cuts results in a shorter time required for alfalfa re-growth and biomass production, which negatively affects yield (Brink et al. 2010; Rimi et al. 2012). Another reason for the consequent decline in the yield is the difference in the average daily temperature and the number of daylight hours at the beginning and end of a production year.

Table 3. The influence of the seeding rate and row spacing on a FFY (t ha^{-1}) of four alfalfa cultivars per cuts

Cuts	Seeding rate (kg ha^{-1})	FFY (t ha^{-1})	Row spacing (cm)	FFY (t ha^{-1})
I cut	8	21.0^{a}	20	26.0^{b}
	16	22.0^{a}	40	17.0^{a}
III cut	8	20.1^{a}	20	22.9^{b}
	16	20.2^{a}	40	17.5^{a}
IV cut	8	13.9^{a}	20	16.0^{b}
	16	14.0^{a}	40	11.8^{a}
V cut	8	7.6^{a}	20	8.9^{b}
	16	7.8^{a}	40	6.5^{a}

* The values followed by the different letters in a column looking individually per cut indicate a significant statistical difference ($p<0.05$)

Table 4. The influence of the seeding rate and row spacing on a DMY (t ha^{-1}) of four alfalfa cultivars per cuts

Cuts	Seeding rate (kg ha^{-1})	DMY (t ha^{-1})	Row spacing (cm)	DMY (t ha^{-1})
I cut	8	5.8^{a}	20	7.0^{b}
	16	5.8^{a}	40	4.6^{a}
III cut	8	5.9^{a}	20	6.8^{b}
	16	5.9^{a}	40	5.1^{a}
IV cut	8	3.8^{a}	20	4.4^{b}
	16	3.8^{a}	40	3.2^{a}
V cut	8	1.8^{a}	20	2.1^{b}
	16	1.8^{a}	40	1.5^{a}

* The values followed by the different letters in a column looking individually per cut indicate a significant statistical difference ($p<0.05$)

According to Table 1 and 2, the interactions (seeding rate x cultivar) and (row spacing x cultivar) in the fifth cut have a significant impact on both examined traits (FFY and DMY). The lowest average yield of fresh forage and hay in all analyzed treatments (except at a seeding rate of 16 kg ha^{-1}) was recorded in the Nijagara cultivar, and it is significantly lower than the cultivars NS Jelena and Debarka (Table 5 and 6). The highest average yield (FFY and DMY) in all treatments, except for the seeding rate of 16 kg ha^{-1}, is achieved in the cultivar Debarka. The average FFY according to row spacing in this cultivar varies from 7.1 to 10.3 t ha^{-1} and hay from 1.7 to 2.4 t ha^{-1}. In contrast, in the research of Milić et al. (2014) and Katanski et al. (2020) with several alfalfa genotypes, the highest average hay yield was obtained from the Nijagara cultivar - 21.7 t ha^{-1} and 15.6 t ha^{-1}, respectively. The obtained results from the research confirm that the achieved yield is a variable that directly depends on the genetic factors (selection of the appropriate cultivar) and the applied agro-technical measures (fertilization, irrigation, seeding date, harvesting regime, and sowing method).

Table 5. Fresh forage yield – FFY (t ha^{-1}) in the fifth cut of alfalfa under the interaction of the seeding rate and the cultivar, and the row spacing and the cultivar

Cultivar	Seeding rate (kg ha^{-1})	FFY (t ha^{-1})	Cultivar	Row spacing (cm)	FFY (t ha^{-1})
Nijagara	8	6.1^{a}	Nijagara	20	7.6^{a}
Banat VS		7.8^{b}	Banat VS		7.7^{a}
NS Jelena		7.9^{b}	NS Jelena		9.9^{b}
Debarka		8.7^{b}	Debarka		10.3^{b}
Nijagara	16	6.8^{a}	Nijagara	40	5.3^{a}
Banat VS		6.6^{a}	Banat VS		6.7^{b}
NS Jelena		8.9^{b}	NS Jelena		7.0^{b}
Debarka		8.7^{b}	Debarka		7.1^{b}

* The values followed by different letters in a column indicate a significant statistical difference ($p<0.05$)

Table 6. Dry matter yield – DMY (t ha^{-1}) in the fifth cut of alfalfa under the interaction of the seeding rate and the cultivar, and the row spacing and the cultivar

Cultivar	Seeding rate (kg ha^{-1})	DMY (t ha^{-1})	Cultivar	Row spacing (cm)	DMY (t ha^{-1})
Nijagara	8	1.4^{a}	Nijagara	20	1.8^{a}
Banat VS		1.8^{b}	Banat VS		1.8^{a}
NS Jelena		1.8^{b}	NS Jelena		2.3^{b}
Debarka		2.0^{b}	Debarka		2.4^{b}
Nijagara	16	1.6^{a}	Nijagara	40	1.3^{a}
Banat VS		1.6^{a}	Banat VS		1.5^{b}
NS Jelena		2.1^{b}	NS Jelena		1.6^{b}
Debarka		2.0^{b}	Debarka		1.7^{b}

* The values followed by different letters in a column indicate a significant statistical difference ($p<0.05$)

CONCLUSIONS

The results of the conducted research indicate that in the first year of production of the examined alfalfa genotypes, the seeding rate does not significantly impact the fresh forage and dry matter yield.

Unlike the seeding rate, the row spacing showed significant differences in yield. When sowing at a row spacing of 20 cm in all cuts, a higher yield of forage mass was achieved compared to sowing at a distance of 40 cm in the four examined cultivars. The lowest average yield of fresh forage and hay in all analyzed treatments (except at a seeding rate of 16 kg ha^{-1}) was recorded in the cultivar Nijagara and the highest in the cultivar Debarka.

From the conducted analysis, we can notice a declining trend in the alfalfa production (on fresh and dry mass) from the first to the last cut.

The obtained data are based on the monitoring of the vegetation in the first production year and provide information only for the initial yield of alfalfa, which will be monitored in the following production years. However, from these results, although annual, we can conclude that in regions with a temperate climate, there is no justification for applying high seeding rates (over 16 kg ha^{-1}). When applying advanced agro-technical methods in stand establishment, it is recommended to use lower seeding rates without significant yield losses, and thus the production would be more economically profitable.

The selection of an appropriate commercial cultivar and row spacing are inevitable issues within an agronomic production technology in order to obtain optimal alfalfa forage production.

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