RESEARCH ON THE INFLUENCE OF INOCULATION AND FERTILIZATION ON THE ALFALFA *(MEDICAGO SATIVA* L.), IN FIRST YEAR OF VEGETATION, UNDER CONDITIONS OF MOLDAVIAN FOREST STEPPE

M. Stavarache, I. Muntianu, V. Vîntu, C. Samuil, C.I. Popovici

University of Agricultural Sciences and Veterinary Medicine, Iaşi
e-mail: mihaistavarache@uaiasi.ro

Abstract

The research conducted during March-October 2010, on the Ezăreni farm, under the climatic condition of Moldavian forest steppe, analyzed in the first year of alfalfa crop, the influence of seed inoculation with symbiotic bacteria Rhizobium meliloti Dangeard and fertilization, on the number of shoots·m^{-2}, plant height dynamics, the leaves/stems ratio and dry matter production (DM). The results registered over three cuts showed that under local climatic conditions, the number of shoots·m^{-2} fluctuated between 704-989 shoots·m^{-2}, plant height at the moment of harvesting was on average 45.6 cm at the fist cut, 68.5 cm at the second cut and 27.1 cm at the third cut. The leaves/stems ratio was 50.31/49.69 at the first cut, 44.23/55.77 at the second cut and 63.68/36.32 at the third cut. Total DM yields ranged from 5.04 to 7.75 Mg·ha^{-1}. Data obtained showed that seed inoculation had a unsignificant influence on the studied indicators. Fertilization influenced positively the height of plants and dry matter production, negatively the leaves/stems ratio and did not have a significant influence on the number of shoots·m^{-2}. First cut represented 37.6% of the total DM, the second cut was 48.4%, and the third cut 14.0%.

Key words: alfalfa, inoculation, fertilization, productivity

INTRODUCTION

Alfalfa *(Medicago sativa* L.*)* is one of the most valuable forage plants trough high yields that it offers, and especially the nutritional value as feed. Through symbiosis with bacterium *Rhizobium meliloti* Dangeard, high levels of atmospheric nitrogen are fixed annually. Productivity and quality of alfalfa is directly dependent on soil conditions, climatic conditions and cultivation technology used.

High yields and quality are obtained when feeding conditions are optimal. If the soils are poor in nitrogen, alfalfa take this nutrient from the atmosphere by symbiosis, while phosphorus and potassium should always be provided from other sources, mineral or organic [2].

Under certain conditions, seed inoculation and application of organic and mineral fertilizers ensure high production increases [6] [7].

MATERIAL AND METHODS

The research was conducted during March-October 2010, on the Ezăreni farm (47°05'-47°10' North latitude and 27°28'-27°33' East longitude), farm belonging to the University of Agricultural Sciences and Veterinary Medicine Iaşi.

The soil from the region is a cambic chernozem characterized by the indicators presented in *Table 1*.

Research followed the influence of inoculation and fertilization on the number of shoots·m^{-2}, plant height dynamics, the leaves/stems ratio and dry matter yeld (D.M.) per hectare, of alfalfa (*Medicago sativa* L.), the first year.

Table 1 Physico-chemical characteristics of the soil on Ezareni Farm

Horizon	Clay (<0.002)	pH	Humus (%)	N total (%)	P-Al (ppm)	K mobile (ppm)	Ca exch. (me)
Ap 0-20 cm	41.8	6.68	2.24	0.178	26.00	242	15.21
Atp 20-28 cm	38.8	6.78	2.40	0.149	10.43	178	15.38

A bifactorial experiment was established, with a design type 2x4, arranged in subdivided plots in three replications, which have a 10 m^{-2} harvesting area (2m x 5m). The factors were as follows: A-seed inoculation with two graduations (a_1-uninoculated and a_2-inoculated) and B-fertilization with four graduations (b_1-unfertilized, b_2-$N_{50}P_{50}$, b_3-$N_{75}P_{50}$ and b_4-30 t·ha^{-1} manure).

The number of shoots·m^{-2} was determined by counting the shoots on a line of 1m in length from rows distanced at 1 m away of the plot margins, in four repetitions, afterwards calculating the shoots number per square meter.

In order to demonstrate the dynamics of plant height, measurements of the same plants from each plot in succesive dates, were made throughout the growing season.

The leaves/stems ratio was determined by separating the stem, leaflets, buds and flowers by the stem, weighing them separately and report their amount to the amount of strain (leaves/stems ratio).

Yield production was determined by weighing the yield harvested from an area of 10 m^{-2} then reported per hectare.

Dry matter was determined by treating samples at 105°C for 3 hours.

The biological material used was represented by Sandra alfalfa variety (F 660-94) registered in 2003 to I.N.C.D.A. Fundulea. The inoculation of seeds happened one hour before sowing, with selected strains of *Rhizobium meliloti* Dangeard. Manure used had the following chemical composition: N-0.445%, P_2O_5-0.212% and K_2O-0.695%.

Fertilizers were applied, and incorporated into the soil before seeding. Harvesting was performed with Bertolini 411 harvester, at a height of 7 cm from the ground. The timing of the first cut was at 10% flowering, second cut was harvested at full flowering and third cut at 50% flowering.

In the area where research was conducted, the 2009-2010 crop year, monthly average temperatures had no signifficant deviations from the multiannual mean values.

During the growing season shortages of water was recorded in the following periods: third decade of April and May, the second and third decade of July, and the first decade of August.

In the third decade of June there were a quantity of 132 mm rainfall, well above normal.

The results were interpreted statistically by analysis of variance and calculation of least square difference (LSD).

RESULTS AND DISCUSSION

Number of shoots·m^{-2}

The investigations carried out showed that inoculation, fertilization as well as the interaction between the two did not influence significantly the number of shoots·m^{-2},except a_1b_4 and a_2b_4 plots at the second harvest, where differences were significant (*Table 2*).

At all three cuts the value of this indicator was different. At the first cut the number of shoots·m^{-2} varied between 850-921 shoots·m^{-2}, at the second cut varied between 935-1074 shoots·m^{-2} and at the third cut varied between 676-727 shoots·m^{-2}. Mean values are shown in *Graphic 1*.

Table 2 Influence of inoculation and fertilization on the number of shoots·m^{-2}, alfalfa culture in the first year

Experimental plot			Number of shoots·m^{-2}		
			First cut	**Second cut**	**Third cut**
a_1- uninoculated (control)	b_1- unfertilized (control)		853	935	676
	b_2- $N_{50}P_{50}$		885	951	707
	b_3- $N_{75}P_{50}$		892	985	713
	b_4- 30 Mg·ha^{-1} manure		878	1024*	709
a_2- inoculated	b_1- unfertilized		850	958	684
	b_2- $N_{50}P_{50}$		884	974	698
	b_3- $N_{75}P_{50}$		921	1010	727
	b_4- 30 Mg·ha^{-1} manure		900	1074**	720
	LSD	*5 %*	*101*	*78*	*83*
		1 %	*142*	*109*	*116*
		0.1 %	*201*	*154*	*164*

Graphic 1 Number of shoots·m^{-2} at three cuts, alfalfa culture in the first year

Plant height dynamics

The results show that inoculation did not significantly affect plant height. Fertilization resulted in maximum height increases in the $N_{75}P_{50}$ plot.

Analyzing the influence of interaction between inoculation and fertilization on plant height, the first year alfalfa crop (*Table 3*) observed that, at the first cut, plant height ranged from 40.9 to 50.1 cm, at the seccond cut ranged from 62.4 to 74.0 cm and at the third cut ranged from 23.8 to 30.0 cm. Differences in height between control and other plots have been statistically insured.

Among all, nitrogen causes a larger increase in plant height [3].

Table 3 Influence of inoculation and fertilization on the plant height, alfalfa culture in the first year

Experimental plot			Plant height (cm)		
			First cut	**Second cut**	**Third cut**
a_1- uninoculated (control)	b_1- unfertilized (control)		40.9	62.4	23.8
	b_2- $N_{50}P_{50}$		46.0**	66.3***	26.4***
	b_3- $N_{75}P_{50}$		48.5***	70.5***	28.9***
	b_4- 30 Mg·ha^{-1} manure		44.3*	67.2***	27.2***
a_2- inoculated	b_1- unfertilized		43.5	66.8***	25.3**
	b_2- $N_{50}P_{50}$		45.4**	70.4***	27.7***
	b_3- $N_{75}P_{50}$		50.1***	74.0***	30.0***
	b_4- 30 Mg·ha^{-1} manure		46.4**	70.7***	27.7***
	LSD	*5 %*	*3.1*	*1.7*	*1.1*
		1 %	*4.4*	*2.4*	*1.5*
		0.1 %	*6.1*	*3.3*	*2.1*

The vegetation season for alfalfa culture in the first year, in the climate conditions from Ezăreni farm, had a total of 189 days (from the germination ocurrance - April 9^{-th} until the date of last cut - October 15^{-th}).

From germination until the first cut a total of 73 days have passed, until the second cut

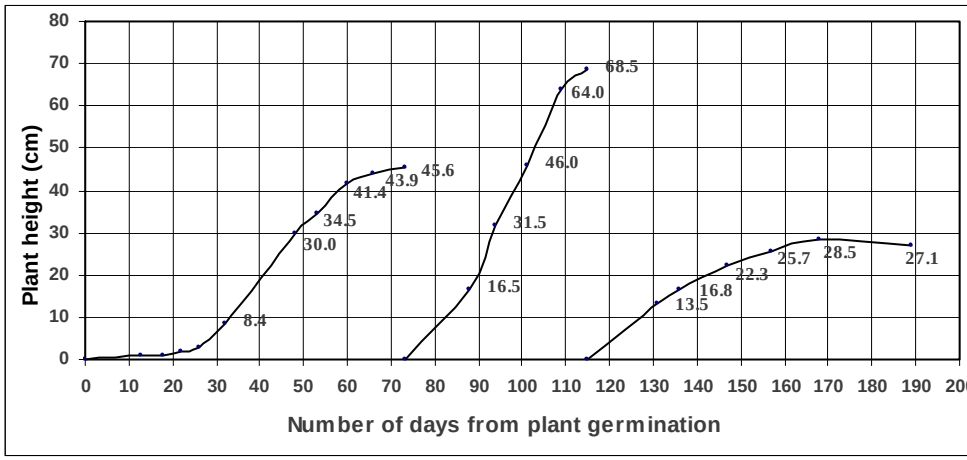
42 days have passed and by the third cut 72 days have passed.

Graphic 2 shows the dynamics of the alfalfa plant height in the first year of vegetation. At the first cut, from May 11^{-th} to June 8^{-th} (the vegetative growing) the growth rate was on average 1.03 cm per day, and from inflorescence emergence to harvest (from June 14^{-th} to June 21^{-st}) the growth rate droped to 0.42 to 0.25 cm per day.

At the second cut the growth rate was lower immediately after cut, from 1.10 cm per day (from June 21^{-st} to July 6^{-th}), then increased to an average of 2.27 cm per day in the vegetative season (from July 6^{-th} to July 26^{-th}). With the emergence of inflorescence the growth rate has dropped to 0.76 cm per day.

At the third cut the growth rate was lower, only 0.67 cm per day from August 2^{-nd} to September 3^{-rd}, then declined to 0.30 cm per day from September 3^{-rd} to September 24^{-th}, and with the advent of flowers plants have suffered a decline of growth rate.

Growth rate and the time needed for completion of each cut has been influenced by climatic conditions during the growing season.



Graphic 2 Plant height dynamics, alfalfa culture in the first year

Leaves/stems ratio

The leaves/stems ratio was different as influenced by experimental factors but also by harvest cycle. For each of the three cuts, the leaves/stems ratio was higher (meaning a higher percentage of leaves) for the organic fertilized and unfertilized plots (*Table 4*).

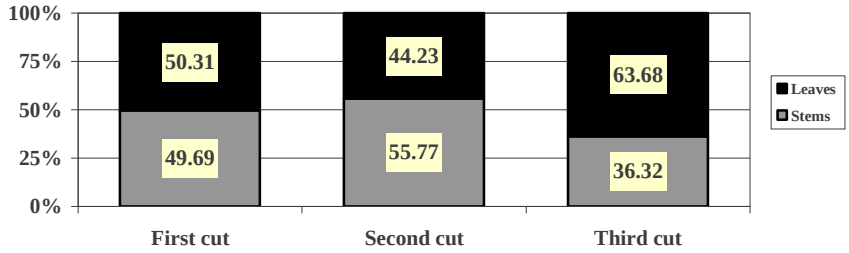
Table 4 Influence of inoculation and fertilization on the leaves/stems ratio, alfalfa culture in the first year

Experimental plot		**First cut**		**Second cut**		**Third cut**	
		Leaves (%)	**Stems (%)**	**Leaves (%)**	**Stems (%)**	**Leaves (%)**	**Stems (%)**
a_1- uninoculated (control)	b_1- unfertilized (control)	56.52	43.48	47.27	52.73	65.08	34.92
	b_2- $N_{50}P_{50}$	49.23	50.77	46.43	53.57	64.38	35.62
	b_3- $N_{75}P_{50}$	45.92	54.08	41.44	58.56	63.28	36.72
	b_4- 30 Mg·ha^{-1} manure	51.68	48.32	46.93	53.07	63.79	36.21
a_2- inoculated	b_1- unfertilized	55.62	44.38	45.29	54.71	63.80	36.20
	b_2- $N_{50}P_{50}$	46.78	53.22	42.46	57.54	64.42	35.58
	b_3- $N_{75}P_{50}$	44.90	55.10	40.01	59.99	61.95	38.05
	b_4- 30 Mg·ha^{-1} manure	51.82	48.18	44.00	56.00	62.74	37.26

Percentage of leaves was lower in the mineral fertilized plots due to more vigorous stems (thicker stems with longer internodes). The leaves/stems ratio varied by each cut. At

the first cut, the average percentage of leaves was almost equal to that of stemns, at the second cut leaves were 10% less than the stems, and the third cut the percentage of leaves was 27% higher than stems (*Graphic 3*).

The leaves/stems ratio vary by the number of shoots·m^{-2} and the growth stage when alfalfa is harvested. As the number of shoots·m^{-2} is lower, the higher the percentage of leaves, with the advent of the inflorescence the percentage of leaves decreases, which leads to a reduction in feed quality obtained [5].



Graphic 3 Leaves/stems ratio at three cuts, alfalfa culture in the first year

Dry matter yeld

The investigations carried out showed that the inoculation did not significantly affect dry matter production, the highest yields were obtained using mineral fertilization, where the differences were statistically assured (*table 5*).

Recorded data showed that production increases ranged from 7.7 to 53.8%, the largest production differences (2.19 and 2.71 Mg·ha^{-1} D.M.) were recorded in a_1b_3 and a_2b_3 plots. In all plots of fertilization in both uninoculated and inoculated plots, the production differences were statistically assured.

Table 5 Influence of inoculation and fertilization on dry matter production, alfalfa culture in the first year

Experimental plot			**Dry matter production (Mg·ha^{-1})**				
			First cut	**Second cut**	**Third cut**	**Total**	**% as reffered to control**
a_1- uninoculated (control)	b_1- unfertilized (control)		1,87	2.39	0.77	5.04	100.0
	b_2- $N_{50}P_{50}$		2,25*	2.79***	0.88	5.92**	117.5
	b_3- $N_{75}P_{50}$		2,73***	3.53***	0.97*	7.23***	143.6
	b_4- 30 Mg·ha^{-1} manure		2,24*	3.04***	0.88	6.15***	122.2
a_2- inoculated	b_1- unfertilized		2,00	2.63**	0.79	5.42	107.7
	b_2- $N_{50}P_{50}$		2,41**	3.03***	0.88	6.31***	125.4
	b_3- $N_{75}P_{50}$		3,02***	3.73***	0.99*	7.75***	153.8
	b_4- 30 Mg·ha^{-1} manure		2,41**	3.27***	0.89	6.57***	130.5
	LSD	*5 %*	*0.33*	*0.16*	*0.17*	*0.46*	
		1 %	*0.47*	*0.22*	*0.24*	*0.64*	
		0.1 %	*0.66*	*0.31*	*0.34*	*0.91*	

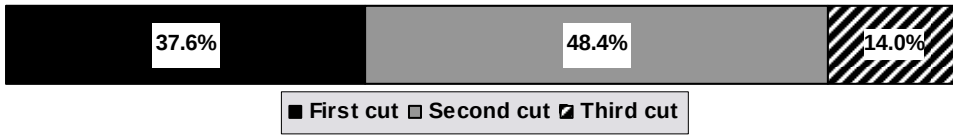
Production increases are caused by fertilizer application and less by inoculation. Nitrogen affects the production of D.M. in a greater degree at the first cut, in the first year of vegetation, when plants have a reduced ability to fix atmospheric nitrogen, which is 25-30%, at the following cuts percentage of fixed nitrogen increases above 60% [4] [8]. Under the insufficiency of phosphorus in the soil, application of this element generates significant production increases throughout the growing season [1] [2].

The reduced efficiency of seed inoculation on the chernozem soil, is explained by favorable conditions encountered by these bacteria, which here can survive unlimited in soil. In this situation, immediately after seeding the seed

is inoculated naturally by strains of *Rhizobium meliloti* Dangeard existing in the soil, which are adapted to local conditions. There is a close relationship between the efficiency of alfalfa seed inoculation and soil content of exchangeable calcium. Only where the soil content of exchangeable calcium is less than 2 ‰, it is indicated that alfalfa seed should be inoculated. When the exchangeable calcium content in the soil is more than 2 ‰ inoculation is ineffective [9].

Exchangeable calcium content of the chernozem cambic soil at Ezăreni Farm in the top 20 cm (Ap horizon) is 15.21 milliequivalents, which coresponds to 3 ‰, explaining the negligible inoculation influence on the indicators analyzed.

The recorded data show that in the first year of alfalfa culture, under the climatic conditions in the area of study, first harvest was 37.6% of the total D.M. production, second harvest was 48.4%, and third harvest was 14.0% (*Graphic 4*).



Graphic 4 The procentage of each cut from total dry matter production, alfalfa culture in the first year

CONCLUSIONS

In alfalfa culture, the first year, under the farm Ezăreni conditions, year 2010, inoculation, fertilization and interaction between them did not influence significantly the number of shoots·m^{-2}, this indicator varies only according to the vegetation cycle.

Plant height was positively influenced by fertilization and insignificant by inoculation.

Fastest rate of growth of the plants was recorded in the second harvest, as a result of specific climatic conditions of the period studied.

The percentage of stems was positive influenced by mineral fertilization and less by organic fertilization.

Production increases were caused by fertilizer and less by inoculation.

Inoculation did not result in significant differences in the indicators examined, because under the farm Ezăreni conditions (soil with a calcium content of 3 ‰) *Rhizobium meliloti* Dangeard symbiotic bacteria is present in the soil.

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