RESEARCH ON THE INFLUENCE OF INOCULATION AND FERTILIZATION ON THE MORPHOLOGICAL AND PRODUCTIVE FEATURES OF LUCERNE (*MEDICAGO SATIVA* L.) UNDER CONDITIONS OF MOLDAVIAN FOREST STEPPE

Mihai STAVARACHE [1], Iulian MUNTIANU [1], Vasile VÎNTU [1], Costel SAMUIL [1], Constantin Iulian POPOVICI [1]

[1] University of Agricultural Sciences and Veterinary Medicine, Iaşi

Abstract

The research conducted during March-June 2010, on the Ezăreni farm, under the condition of Moldavian forest steppe, analyzed in the first year of alfalfa crop, at the first cut, the influence of seed inoculation with symbiotic bacteria *Rhizobium meliloti* Dangeard and fertilization with $N_{50}P_{50}$, $N_{75}P_{50}$ and 30 Mg·ha^{-1} manure, on the number of shoots·plant^{-1}, number of shoots·m^{-2}, plant height dynamics, the leaves/stems ratio and dry matter production (DM) per hectare. The experience has been placed on a cambic chernozem soil type containing 41.8% clay, pH 6.68 and 2.24% humus on 0-20 cm layer. The results showed that under experimental conditions, the number of shoots per plant was between 1.16 to 1.45, the number of shoots·m^{-2} fluctuated between 850-921 shoots·m^{-2}, plant height at harvest was between 40.9-50.1 cm, the leaves/stems ratio ranged from 56.5/43.5 and 45.9/54.1 and DM yields ranged from 1873-2661 kg·ha^{-1}. For this stage of observations, seed inoculation had a unsignificant influence on the tracked indicators. Fertilization positively influenced the number of shoots·plant^{-1}, plant height and dry matter production, negative ratio leaves/stems and had a significant influence on the number of shoots·m^{-2}.

Key words: alfalfa, inoculation, fertilization, productivity

Alfalfa (*Medicago sativa* L.) is one of the most valuable trough the higher production of green mass or hay and high quality feed. Optimal nutritional conditions for culture, ensure high yields and high quality. If the soil lacks nitrogen, alfalfa plants take this nutrient from the atmosphere through symbiotic bacterium *Rhizobium meliloti* Dangeard, bacteria existing in soil or as seed inoculation, phosphorus and potassium should be provided from other sources, mineral or organic. A high density of stems/m^2, a greater height of shoots and leaves/stems ratio are more desirable to have a productive and high quality alfalfa (Varga P., et al., 1973; Vîntu V. et al., 2004).

MATERIAL AND METHOD

The research was conducted during March-June 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 has sought influence of inoculation and fertilization on the number of shoots per plant, number of shoots·m^{-2}, plant height dynamics, the leaves/stems ratio and dry matter yeld (DM) per hectare, of alfalfa (*Medicago sativa* L.) seeded in dense rows (12.5 cm between rows), the first year, to first cut. For this purpose 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-$N_{75}P_{50}$ şi b_4-30 t·ha^{-1} manure).

Number of shoots per plant was determined on May 11^{-th}, 2010, at a number of 72 plants located in different areas of each plot, in the same place in each plot. Number of shoots·m^{-2} was determined on June 15^{-th}, 2010, by counting shoots on a linear m of row at 1 m from the edge of the plot. for reporting to Square meter the result was multiplied by eight. For showing the dynamics of plant height was measured the same plants from each plot in succesive dates of May 11-th, May 27^{-th}, June 1^{-st}, June 8^{-th}, June 14^{-th} and June 21^{-st}, 2010. 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. Yeld production was determined by weighing the yeld harvested from a area of 10 m^2 then reported per hectare. Dry matter was determined by treating samples at 105 ° C for 3 hours.

Uses biological material was represented by Sandra alfalfa variety (F 660-94) registered in 2003 to I.N.C.D.A. Fundulea. Seed from inoculated variant were treated, 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% şi K_2O-0,695%. Fertilizers were applied, and incorporated into soil before seeding.

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

Table 1

Physico-chemical characteristics of soil that was placed experience

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

RESULTS AND DISCUSSIONS

Number of shoots per plant

Analyzing the influence of inoculation, fertilization and interaction between inoculation and fertilization on the number of shoots per plant (*table 2, table 3, figure 1*), note that inoculation had significant influence on the number of shoots per plant, but fertilization with surplus brought food plants, increased the value of this indicator is highly significant differences in all three plots of fertilization. On the interaction between the two factors the number of shoots per plant was between 1.16 (the control a_1b_1) and 1.47 (the plot a_1b_3). $N_{75}P_{50}$ fertilized plots were obtained higher values of number of shoots per plant.

Under the insufficiency of phosphorus alfalfa shoots will lower heights, as showed research results conducted by Volenec J.J. et al.(2008).

Table 2

Influence of inoculation on the number of shoots per plant, alfalfa culture in the first year, first cut

Experimental plot	Number of shoots per plant	Percentage as reffered to control (%)	Diferences	Statistical significance
a_1- uninoculated (control)	1.30	100.0	control	-
a_2- inoculated	1.28	98.5	0.02	-
LSD 5 %-0.03 shoots per plant; LSD 1 %-0.06 shoots per plant; LSD 0.1 %-0.18 shoots per plant.				

Table 3

Influence of fertilization on the number of shoots per plant, alfalfa culture in the first year, first cut

Experimental plot	Number of shoots per plant	Percentage as reffered to control (%)	Diferences	Statistical significance
b_1- unfertilized (control)	1.18	100.0	control	-
b_2- $N_{50}P_{50}$	1.27	109.0	0.09	***
b_3- $N_{75}P_{50}$	1.45	124.2	0.27	***
b_4- 30 $Mg \cdot ha^{-1}$ manure	1.26	108.0	0.08	***
LSD 5 %-0.04 shoots per plant; LSD 1 %-0.06 shoots per plant; LSD 0.1 %-0.08 shoots per plant.				

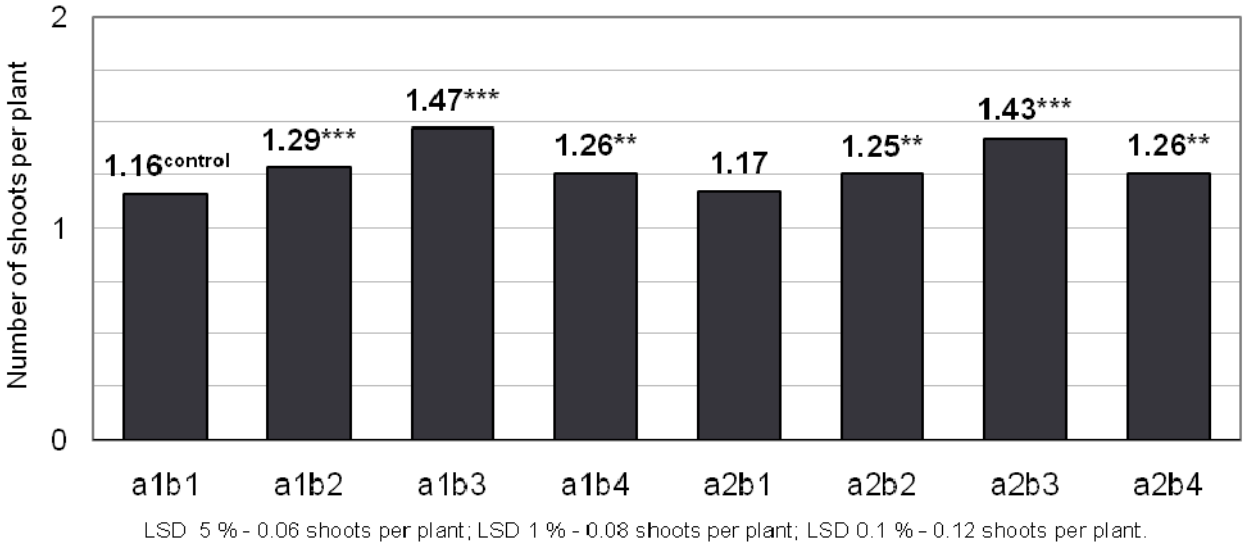

Figure 1 **Influence of interaction between inoculation and fertilization on the number of shoots per plant, alfalfa culture in the first year, first cut**

Number of shoots·m^{-2}

Both inoculation, fertilization and inoculation-fertilizer interaction did not affect significantly the number of shoots·m^{-2}. The values of this indicator varied between 850 shoots·m^{-2} at a_2b_1 plot and 921 shoots·m^{-2} at a_2b_3 plot. Varga P., et al. (1973), shows that the higher number of shoots·m^{-2} is the bigger, the leaves/stems ratio is more favorable to leaves.

Plant height dynamics

From the the seeding date (March 24^{-th}) until plant germination ocurrance (April 9^{-th}) a total of 16 days have passed, the banner appeared on April 22-th (13 days after germination), the first leaf with three leaflets appeared after 5 days (April 27^{-th}), and three leaflets leaves 2 and 3 occurred after 4 days (May 1^{-st} and May 5^{-th}). *Figure 2* shows the dynamics of plant height after plant germination date until the first cut (average values of plant height) and number of days after plant germination, to achieve phenological phases of alfalfa in the first year, first cut. Between May 11-th to May 27^{-th} the growth rate was 1.35 cm per day, from 27^{-th} May to 8^{-th} June growth rate was 0.95 cm per day, from June 8 to June 14 the growth rate was of 0.42 cm per day and between June 14^{-th} to June 21^{-st} growth rate was of 0.25 cm per day.

Table 4, *table 5* and *figure 3* shows the results of the influence of fertilization and interaction between inoculation and fertilization on plant height of alfalfa at harvest. In this case inoculation did not result in significant increases in the height of the plants. Significant increases were shown separately for alfalfa fertilization with 30 Mg·ha^{-1} manure and $N_{50}P_{50}$ and very significant increases for $N_{75}P_{50}$ fertilization. Interaction between two factors led to gains maximum height of 9.2 cm (for plot a_2b_3). Differences in height is due to phosphorus, whilst nitrogen has an insignificant positive impact on plant height, as shown by Delgado I. et al. (2001), which were obtained from the application of a dose of N_{50} in alfalfa, an average increase of plant height of 0.9 cm.

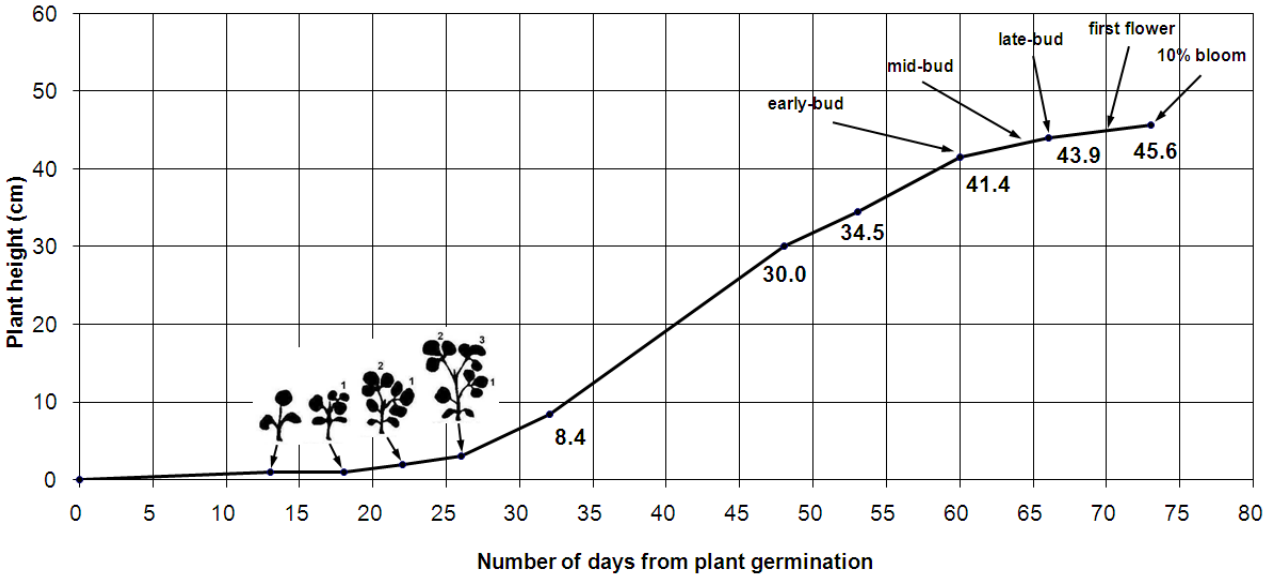

Figure 2 **Plant height dynamics, alfalfa culture in the first year, first cut**

Table 4

Influence of inoculation on the plant height, alfalfa culture in the first year, first cut

Experimental plot	Plant height (cm)	Percentage as reffered to control (%)	Diferences	Statistical significance
a_1- uninoculated (control)	44.9	100.0	control	-
a_2- inoculated	46.4	103.3	1.5	-
LSD 5 % - 7.2 cm; LSD 1 %-16.6 cm; LSD 0.1 %-52.9 cm.				

Table 5

Influence of fertilization on the plant height, alfalfa culture in the first year, first cut

Experimental plot	Plant height (cm)	Percentage as reffered to control (%)	Diferences	Statistical significance
b_1- unfertilized (control)	42.2	100.0	control	-
b_2- $N_{50}P_{50}$	45.7	108.3	3.5	**
b_3- $N_{75}P_{50}$	49.3	116.8	7.1	***
b_4- 30 Mg·ha^{-1} manure	45.4	106.6	3.2	**
LSD 5 % - 2.2 cm; LSD 1 %-3.1 cm; LSD 0.1 %-4.3 cm.				

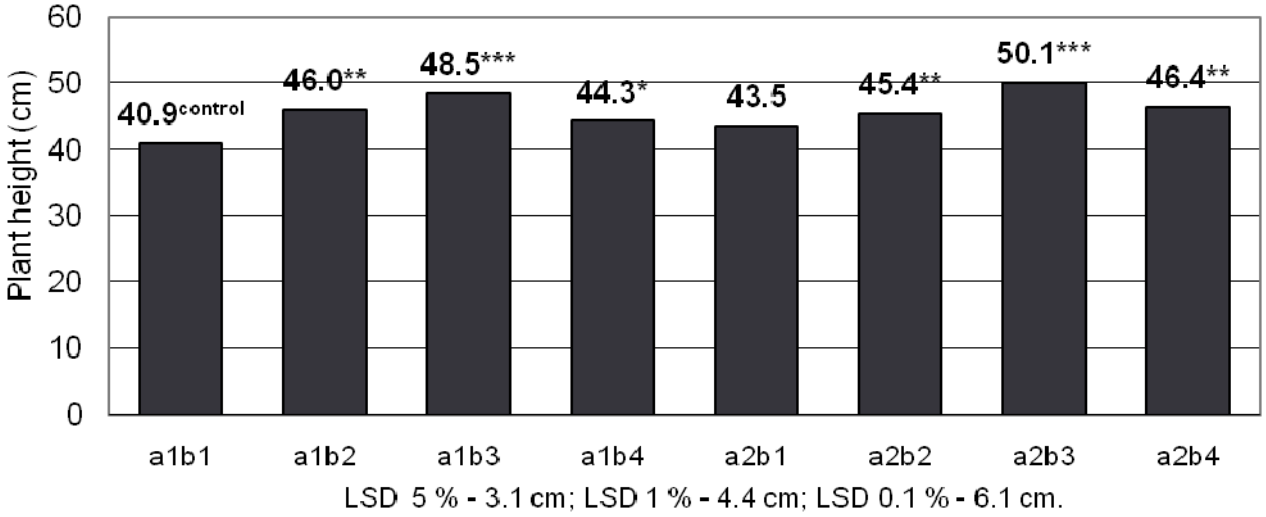

Figure 3 **Influence of interaction between inoculation and fertilization on the plant height, alfalfa culture in the first year, first cut**

Leaves/stems ratio

In figure 4 it is noted that the percentage of leaf is lower in mineral fertilized plots (between 44.9 to 49.2%) due to better developed shoots (greater diameter and length of internodes).

Sheaffer, C.C. et al (2000) shows that the leaves/stems ratio depends on the phenological phase in which the alfalfa harvest. In mid bud the leaves/stems ratio is 53.5/46.5 in early flowering of 47.5/52.5, at the full flowering leaves will represent 37.5% and stems 62.5%.

A higher percentage of leaves is desirable, as they have double the protein and mineral content compared with stems (Varga, P, et al., 1973).

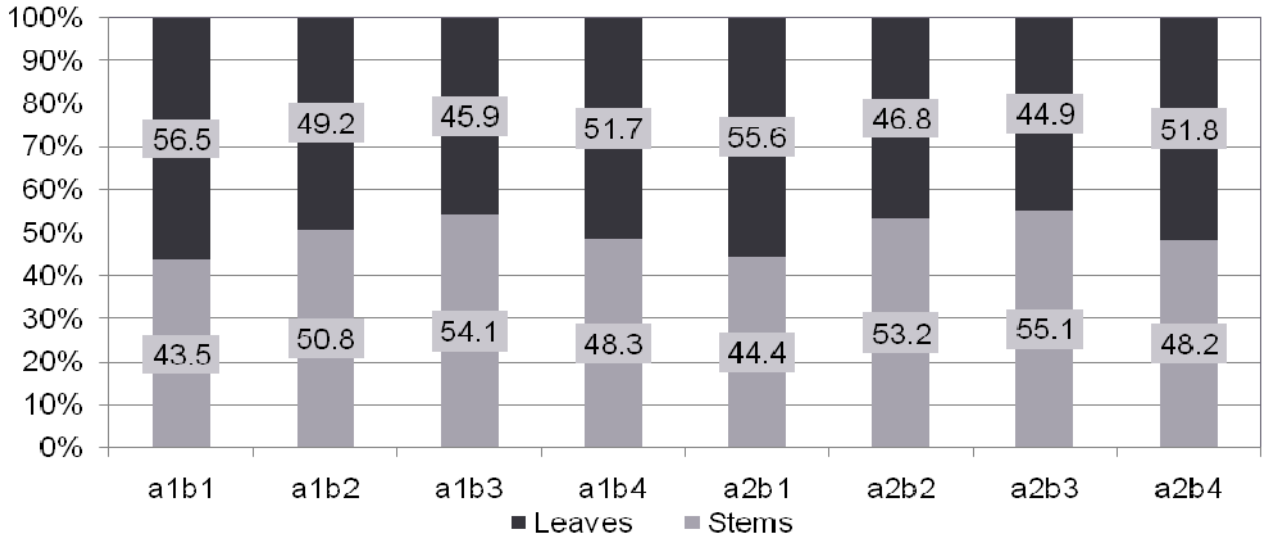

Figure 4 **Leaves/stems ratio, alfalfa culture in the first year, first cut**

Dry matter yeld

Analyzing the influence of inoculation, fertilization and interaction between inoculation and fertilization on DM production per hectare (*table 6*, *table 7* and *figure 5*), it appears that inoculation had no significant influence on the production of DM·ha^{-1}, the same result reached and Dragomir Carmen and Moisuc A. (2007).

Fertilization resulted in significant production increases from 386-937 kg·ha^{-1}, the highest production was obtained in plot b_3, fertilized with $N_{75}P_{50}$, in this case it was a very significant production growth, and an increase due to the supply of both nitrogen and phosphorus.

Nitrogen fertilization increases production when the plants have not form the root nodes of nitrogen assimilation (Moga Elisabeta et al. (1983).

Interaction between inoculation and fertilization resulted in productions ranging from 1873 kg·ha^{-1} DM, the control plot a_1b_1, and 3021 kg·ha^{-1} DM in plot a_2b_3, in this case the increase is very significant.

Table 6

Influence of inoculation on the dry matter production, alfalfa culture in the first year, first cut

Experimental plot	Dry matter production ($kg \cdot ha^{-1}$)	Percentage as reffered to control (%)	Diferences	Statistical significance
a_1- uninoculated (control)	2271	100.0	control	
a_2- inoculated	2460	108.3	189	
LSD 5 % - 543 $kg \cdot ha^{-1}$; LSD 1 %-1253 $kg \cdot ha^{-1}$; LSD 0.1 %-3989 $kg \cdot ha^{-1}$.				

Table 7

Influence of fertilization on the dry matter production, alfalfa culture in the first year, first cut

Experimental plot	Dry matter production ($kg \cdot ha^{-1}$)	Percentage as reffered to control (%)	Diferences	Statistical significance
b_1- unfertilized (control)	1938	100.0	control	
b_2- $N_{50}P_{50}$	2324	119.9	386	**
b_3- $N_{75}P_{50}$	2875	148.3	937	***
b_4- 30 $Mg \cdot ha^{-1}$ manure	2325	120.0	387	**
LSD 5 % - 236 $kg \cdot ha^{-1}$; LSD 1 %-331 $kg \cdot ha^{-1}$; LSD 0.1 %-468 $kg \cdot ha^{-1}$.				

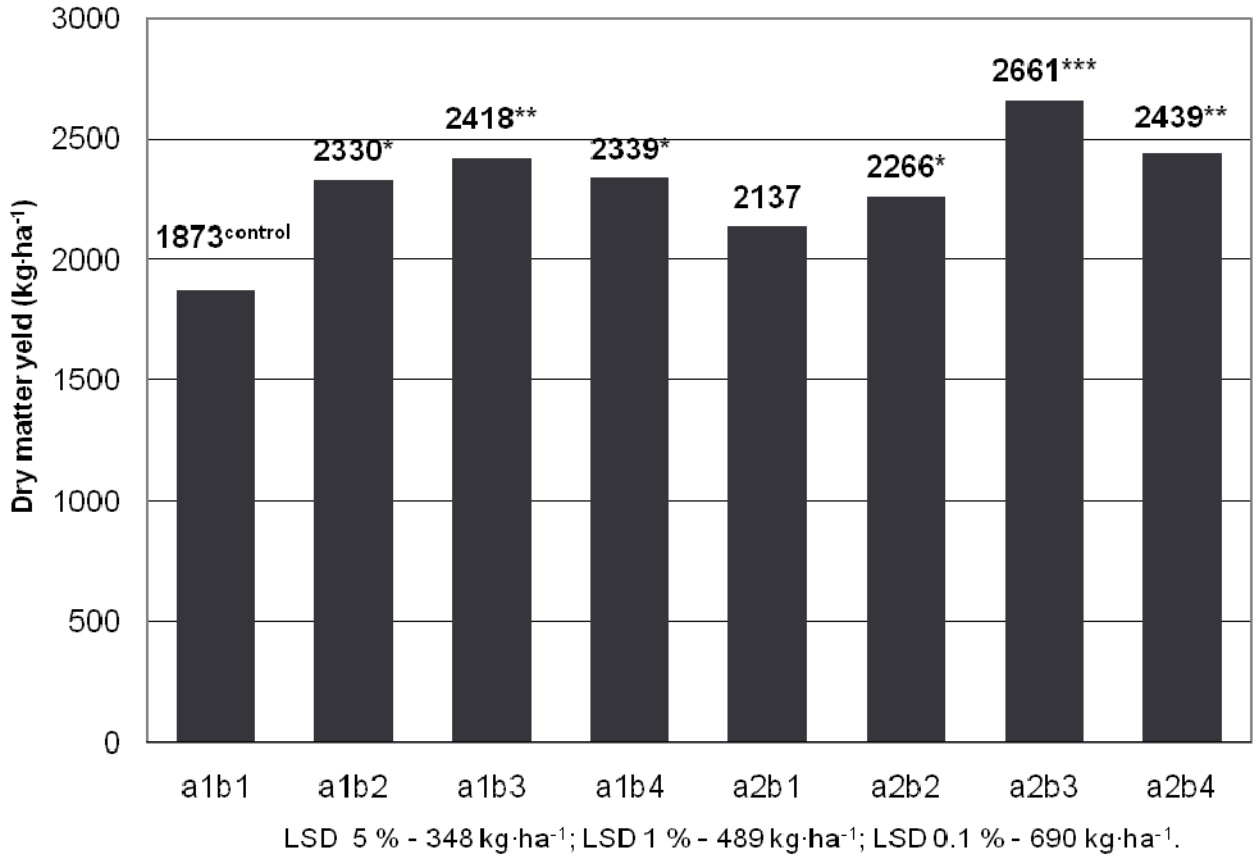

Figure 5 **Influence of interaction between inoculation and fertilization on dry matter production, alfalfa culture in the first year, first cut**

Inoculation treatmests of alfalfa seeds with improved strains of *Rhizobium meliloti* Dangeard are ineficient on chernozem soils which could be explained by favorable conditions encountered by these bacteria who can survive indefinitely in these soils. 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.

Experimental results shows that inoculation of alfalfa seeds with *Rhizobium meliloti* Dangeard strains is very efficient on fined podzolic soil and inefficient on chernozem (Moga I. et al., 2007).

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 with selected strains of *Rhizobium meliloti* Dangeard prior to seeding (Varga P., et al., 1973).

Inoculation efficiency is influenced by the used alfalfa variety (Miller, R. W. and Sirois, J.C., 1982).

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.

CONCLUSIONS

The number of shoots per plant at alfalfa culture in the first year, first cut, was influenced by fertilizer application, each dose of fertilizers causing significant distinguishing differences.

Seed inoculation with *Rhizobium meliloti* Dangeard and application of mineral or organic fertilizers did not influence significantly the number of stems·m^{-2}.

Alfalfa had a rapid and relatively uniform growth until the early bud stage, after this stage growth rate was increasingly lower.

In mineral fertilization leaves/stems ratio have lower values compared to organic fertilization or unfertilized plot (control plot).

All variants of fertilization led to DM production increases (kg·ha^{-1}) distinguishing significantly and very significant. Inoculation did not result in significant differences in DM production, because the symbiotic bacterium *Rhizobium meliloti* Dangeard is present in the soil.

BIBLIOGRAPHY

Delgado, I. et al., 2001 - *Effect of nitrogen fertilisation on alfalfa (Medicago sativa L.) regrowth and production*, Options Méditerranéennes, Série A: Séminaires Méditerranéens, Vol. 45, pag. 141-143, ISSN: 1016-121X.

Dragomir, Carmen, Moisuc, A., 2007 - *Bacterial inoculation effect upon yield capacity in alfalfa and orchard grass*, Lucrări Ştiinţifice, Facultatea de Agricultură USAMVB Timişoara Vol. 38, pag. 275-278, ISSN: 1221-5279.

Miller, R. W., Sirois, J.C., 1982 - *Relative Efficacy of Different Alfalfa Cultivar - Rhizobium meliloti Strain Combinations for Symbiotic Nitrogen Fixationt*, Applied and Environmental Microbiology, Vol. 43 (4), pag. 764-768, ISSN: 0099-2240.

Moga, Elisabeta, et al., 1983 - *Comportarea lucernei şi golomăţului semănate primăvara, în cultură pură şi amestec, în depresiunea Jijiei superioare,* Cercetări Agronomice în Moldova, Vol. 1 (61), pag. 65-70, ISSN: 0379-5837.

Moga, I. et al., 2007 - *Cercetări privind agrotehnica plantelor furajere la fundulea şi în reţeaua experimentală, Anual I.N.C.D.A. Fundulea*, Vol. LXXV, Volum Jubiliar, pag. 318-341, ISSN 2067-7758.

Sheaffer, C.C. et al., 2000 - *Leaf and Stem Properties of Alfalfa Entries*, Agronomy Journal, Vol. 92, pag. 733-739, ISSN: 0002-1962.

Varga, P., et al., 1973 - *Lucerna*, Editura Ceres, pag. 34-35, 135-136.

Vîntu, V. et al., 2004 - *Cultura pajiştilor şi a plantelor furajere*, Editura „Ion Ionescu de la Brad" Iaşi, pag 389-406, ISBN: 973-7931-47-X.

Volenec, J.J. et al., 2008 - *P and K Management Strategies for Optimal Alfalfa Production*, Purdue University, West Lafayette, Proceedings of the 2006 Indiana CCA Conference, Indianapolis, IN 47907-2054.