# Effect of Bacterial Inoculation in some Mixtures of Grassland Legumes and Gramineae

**Claudiu Ghiocel[1]*, Roxana Vidican[2], Neculai Dragomir[1], Dorin Camen[1], Nicoleta Moraru[1], Dorin Rechiţean[1], Anca Sala[1], Gherasim Voichiţa[1]**

*[1]Faculty of Animal Science and Biotechnologies, 300645, Timişoara, Calea Aradului, no. 119, România*
*[2]University of Agricultural Sciences and Veterinary Medicine,400372, Cluj-Napoca, Calea Manastur, no. 3-5, România*

**Abstract**
The paper presents the influence of bacterial inoculation of perennial legumes (alfalfa, bird's-foot trefoil) in pure culture and cultivated in association with perennial gramineae on fodder yield, fodder quality, and atmospheric nitrogen-fixing ability. Results point out an increase of 10-12% of the yield of dry matter in the variants inoculated. Bacterial inoculation with specific bacterial stems (Sinorhizobium meliloti and Mezorhizobium loti) has a positive influence on fodder quality too, materialised in the raw protein content and the amount of nitrogen fixed biologically.

**Keywords**: perennial gramineae and legumes; bacterial inoculation; dry matter; quality; nitrogen fixation.

## 1. Introduction

Temporary grasslands are an important source of fodder and, due to their contribution to the soil nitrogen supply from symbiotic fixation; they make the entire fodder crop rotation effective. Research shows that in mixtures of grassland gramineae and legumes the amount of fixed nitrogen is determined by the following factors: the legume species in the mixture and their share, the yield, the competitiveness and persistence of the legumes, soil nitrogen supply, cultivation and valorisation technology (bacterial inoculation of legumes nitrogen fertilisation, mowing or grazing) [1-8]. The paper points out the influence of bacterial inoculation and nitrogen fertilisation on dry matter yield in certain perennial legumes sowed in pure culture or in mixture with some perennial gramineae.

## 2. Materials and methods

Research was carried out at the Centre for Research in Grasslands and Fodder Plants of the B.U.A.S.V.M. of Timisoara, Romania, during 2012-2013, on a salinised chernozem. The experiment consisted of a polyfactorial trial with the following variants: A – bacterial inoculation of the legumes ($a_1$ = no inoculation, $a_2$ = inoculation); B – nitrogen fertilisation ($b_1 = N_0$, $b_2 = N_{100}$); C – perennial legume and gramineae species ($c_1$ = Medicago sativa, $c_2$ = Lotus corniculatus, $c_3$ = Medicago sativa 50% + Dactylis glomerata 50%, $c_4$ = Lotus corniculatus 50% + Lolium perenne 50%, $c_5$ = Medicago sativa 25% + Lotus corniculatus 25% + Dactylis glomerata 25% + Lolium perenne 25%).
Bacterial inoculation of legume species was done by treating the seeds before sowing with selected bacteria stems multiplied in a liquid medium on an agar substratum from two bacteria species: *Sinorhizobium meliloti* for Medicago sativa and *Mesorhizobium loti* for Lotus corniculatus.

* Corresponding author: Claudiu Ghiocel,
Email: ghiocelc2013@yahoo.com

## 3. Results and discussions

Fixing atmospheric nitrogen is one of the most spectacular and effective natural phenomena that could be, in the future, a source of great scientific and practical interest for the increase of agricultural production.

Economic and environmental benefits of nitrogen fixation could be summed up as follows: profitability, energetic efficacy, plant nutrition, humankind security, environmental quality, and sustainable agriculture.

Increasing the atmospheric nitrogen fixation process can be done through bacterial inoculation of legume species based on specific improved bacterial stems. Natural and technological factors have a strong impact on the process of atmospheric nitrogen fixation in the legumes on permanent or temporary grassland ecosystems or cultivated as monoculture in different systems of crop rotation [2].

Results from the two production years show that, in the first vegetation year (2012) nitrogen fertilisation ($N_{100}$) had a stronger negative influence on dry matter yield in the legume species sowed in pure culture, particularly in Lotus corniculatus.

In 2012, the conjugated effect of experimental factors had negative produced negative values of the dry matter yields if compared to the results of the Medicago sativa yields.

The unilateral mean of the factors points out the contribution of bacterial inoculation of the legumes to the dry matter yield, i.e. a mean increase of 0.31 t of dry matter/ha, which is statistically significant (Table 1).

In the first production year, if we take into account only the effect of bacterial inoculation, there were significant increases of the production compared to the control variants (no inoculation):

- in *Medicago sativa*, the increase produced by bacterial inoculation was 1.27 t of dry matter/ha (18.6%);
- in *Lotus corniculatus*, the yields were almost identical;
- in the mixture of *Medicago sativa* + *Dactylis glomerata*, the increase was of 0.30 t of dry matter/ha (5.6%);
- in the mixture of *Lotus corniculatus* + *Lolium perenne*, the increase was of 0.44 t of dry matter/ha (7.5%);
- in the complex mixture of *Medicago sativa* + *Lotus corniculatus* + *Dactylis glomerata* + *Lolium perenne*, the increase was of 0.95 t of dry matter/ha (13%).

In the second production year (2013), bacterial inoculation had a positive influence on dry matter yield in all five legume cultures (sowed in pure culture or in mixture), including in the variants fertilised with nitrogen (Table 2).

Thus, the variant cultivated with *Medicago sativa* (sowed in pure culture) yielded, compared to the control variant (no inoculation, no nitrogen fertilisation) and to the variant with inoculation but with no nitrogen fertilisation, yielded a surplus of 1.18 t of dry matter/ha (14%).

In the same conditions, the complex mixture of *Medicago sativa* + *Lotus corniculatus* + *Dactylis glomerata* + *Lolium perenne* yielded, compared to the control variant (no inoculation, no nitrogen fertilisation), an increase of 1.06 t of dry matter/ha (12%) as a result of bacterial inoculation (Table 2).

If we compare the variants with inoculation and nitrogen fertilisation ($N_{100}$) with the control variants (no inoculation, no fertilisation), we can see there is a mean increase of 2.35 t of dry matter/ha (20%).

The complex mixture inoculated and fertilised ($N_{100}$) yielded the highest increase (23%) because of the gramineae species that benefited from the nitrogen supply.

Of the three types of cultures tested in the second production year, we noted the simple mixture of *Medicago sativa* (50%) + *Dactylis glomerata* (50%), which yielded 11.01 t of dry matter/ha under the influence of the studied factors.

**Table 1.** Dry matter yield (2012)

| Variant | | | t/ha | Dif. t/ha | % | Significance[1)] |
|---|---|---|---|---|---|---|
| Inoculation | Fertilization | Species | | | | |
| No inoculation | $N_0$ | *Medicago sativa* (*Ms*) | 6.81 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 5.00 | -0.81 | 73 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 5.39 | -1.42 | 79 | 0 |
| | | *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | 5.89 | -0.92 | 86 | 0 |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 7.30 | 0.49 | 107 | |
| | $N_{100}$ | *Medicago sativa* (*Ms*) | 6.71 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 5.17 | -1.54 | 77 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 5.83 | -0.88 | 87 | 0 |
| | | Lc + *Lolium perenne* (*Lp*) (50% + 50%) | 6.42 | -0.29 | 96 | |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 6.80 | 0.09 | 101 | |
| Inoculation | $N_0$ | *Medicago sativa* (*Ms*) | 8.08 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 4.89 | -3.19 | 61 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 5.69 | -2.39 | 70 | 0 |
| | | *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | 6.33 | -1.75 | 78 | 0 |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 8.25 | 0.17 | 102 | |
| | $N_{100}$ | *Medicago sativa* (*Ms*) | 7.61 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 4.78 | -2.83 | 63 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 5.61 | -2.00 | 74 | 0 |
| | | *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | 6.11 | -1.50 | 80 | 0 |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 7.02 | -0.59 | 92 | |
| The one-sided media factors | | | | | | |
| No inoculation | | | 6.13 | - | 100 | |
| Inoculation | | | 6.44 | 0.31 | 105 | * |
| $N_0$ | | | 6.36 | - | 100 | |
| $N_{100}$ | | | 6.21 | -0.15 | 98 | |
| *Medicago sativa* (*Ms*) | | | 7.30 | - | 100 | |
| *Lotus corniculatus* (*Lc*) | | | 4.96 | -2.34 | 68 | 000 |
| *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | | | 5.63 | -1.67 | 77 | 000 |
| *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | | | 6.19 | -1.11 | 85 | 000 |
| *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | | | 7.35 | 0.05 | 101 | |

[1)] DL 5%: A = 0.15; B = 0.52; C = 0.40; A x B x C = 0.80 (t/ha)

**Table 2.** Dry matter yield (2013)

| Variant | | | t/ha | Dif. t/ha | % | Significance[1)] |
|---|---|---|---|---|---|---|
| Inoculation | Fertilization | Species | | | | |
| No inoculation | $N_0$ | *Medicago sativa* (*Ms*) | 8.40 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 7.37 | -1.03 | 88 | |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 10.30 | 1.90 | 124 | * |
| | | *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | 9.69 | 1.29 | 115 | * |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 9.17 | 0.77 | 109 | |
| | $N_{100}$ | *Medicago sativa* (*Ms*) | 9.73 | - | 100 | |
| | | Lotus corniculatus (Lc) | 8.27 | -1.46 | 85 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 12.40 | 2.67 | 127 | * |
| | | *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | 9.85 | 0.12 | 101 | |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 10.37 | 0.64 | 107 | |
| Inoculation | $N_0$ | *Medicago sativa* (*Ms*) | 9.58 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 7.82 | -1.76 | 82 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 9.03 | -0.55 | 94 | |
| | | *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | 9.97 | 0.39 | 104 | |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 10.23 | 0.65 | 107 | |
| | $N_{100}$ | *Medicago sativa* (*Ms*) | 10.58 | - | 100 | |
| | | *Lotus corniculatus* (*Lc*) | 9.38 | -1.20 | 89 | 0 |
| | | *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | 12.33 | 1.75 | 117 | * |
| | | Lc + *Lolium perenne* (*Lp*) (50% + 50%) | 10.18 | -0.40 | 96 | |
| | | *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | 11.29 | -0.71 | 107 | |
| The one-sided media factors | | | | | | |
| No inoculation | | | 9.55 | - | 100 | |
| Inoculation | | | 10.04 | 0.49 | 105 | * |
| $N_0$ | | | 9.15 | - | 100 | |
| $N_{100}$ | | | 10.44 | 1.29 | 114 | *** |
| *Medicago sativa* (*Ms*) | | | 9.57 | - | 100 | |
| *Lotus corniculatus* (*Lc*) | | | 8.21 | -1.36 | 86 | 000 |
| *Ms* + *Dactylis glomerata* (*Dg*) (50% + 50%) | | | 11.01 | 1.44 | 115 | *** |
| *Lc* + *Lolium perenne* (*Lp*) (50% + 50%) | | | 9.92 | 0.35 | 104 | |
| *Ms* + *Lc* + *Dg* + *Lp* (25% + 25% + 25% + 25%) | | | 10.27 | 0.70 | 107 | * |

[1)] DL 5%: A = 0.31; B = 0.32; C = 0.55; A x B x C = 1.10 (t/ha)

## 4. Conclusions

Bacterial inoculation of the studied legumes in the two production years recorded a mean increase of the dry matter yield of 5% compared to the variants with no inoculation. Alfalfa, sowed in pure culture or in mixture with gramineae species, contributed the most to the increase of the dry matter yield when the seeds are inoculated with nitrogen-fixing bacteria and the culture is nitrogen-fertilised.

## References

1. Dragomir C.,– Cercertări privind contribuţia unor leguminoase furajere perene la aprovizionarea cu azot a pajiştilor, în condiţiile fixării pe cale biologică. Teză de doctorat, USAMVB Timişoara, 2009, p. 344
2. Dragomir N, Dragomir C.,– Fixarea azotului în ecosistemele de pajişti şi leguminoase perene, Editura Eurobit, Timişoara, 2012
3. Kristensen E. S. et al., A simple model for estimation of atmospherically – derived nitrogen in grass – clover systems. Biological Agriculture and Horticulture, 1995, -12, 263-276
4. Ledgard, S.F., Sprosen, M.S. & Steele, K.W., - Nitrogen fixation by nine white clover cultivars in grazed pasture, as affected by nitrogen fertilization. Plant and Soil, 1996, 178, 193-203
5. Loges R. And Taube F., - Methodological aspects of determination of biological N – fixation of different forage legumes (http://org/00002151/archiviert) 1995
6. Loges R. et al., - Yield, forage quality, residue nitrogen and nitrogen fixation of different forge legumes. 13th International IFOAM Scientific Conference – The World Grows Organic, Basel, 2000, 28-31 August
7. Sanders I. et al., - Nitrogen fixation and soil nitrogen in organic ley arable rotations. In: UK Organic Research (Eds. Powell et al.), Proceeding of the COR Conference, 26-28 march 2002, pp. 85-88
8. Vinther, F. P., - Biological nitrogen fixation in grass-clover affected by animal excreta. Plant and Soil, 1998, 203, 207-215