Biomass yield and quality of alfalfa cultivars grown on pseudogley soil

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Abstract
This paper presents results of a three-year study on the effect of seed preinoculation on biomass yield and dry matter quality in alfalfa grown on pseudogley soil. Prior to sowing, the seeds of alfalfa cultivars (including Banat, NS Mediana, K-28, Sinskaja and OS-66) were inoculated with a pH-resistant Rhizobium meliloti strain. The seed preinoculation induced a significant increase in dry matter yield in all cultivars during the first two years. The absence of the positive effect of the inoculant used in this study resulted from its low persistence in the soil having extremely poor physical and chemical properties i.e. from its competition with indigenous strains. Dry matter yield in all years was substantially higher in cv. K-28 than in most other cultivars. The significantly higher dry matter yield obtained with cv. K-28 in the third year as compared to that of the other cultivars suggested its tolerance of lower pH values of soil.

Key words: alfalfa, inoculation, cultivar, yield, quality.

Introduction
Low soil pH is a limiting factor in alfalfa production (Koopman et al., 1995). Low pH and high Al levels seriously affect the Rhizobium-legume symbiosis (Coventry and Evans, 1989) by root system development of the host plant (Brady et al., 1991), nodulation (Pijnenborg et al., 1991) and nitrogenase activity (Paulino et al., 1987). Soto et al. (2004) report that the low productivity of alfalfa grown on acid soils results from the effect of two factors: the inability of alfalfa to attain symbiosis with R. meliloti strains and competition with acid-resistant indigenous strains having low nitrogen-fixation capacity. The symbiotic nitrogen-fixation is dependent upon host plant genotype, Rhizobium strain as well as upon the interaction of these symbionts with pedoclimatic i.e. environmental factors. Extremely low pH affects nodulation by reducing soil and legume rhizosphere colonization by rhizobium. Highly acid soils (pH<4.0) often have low levels of P, Ca and Mo and high concentrations of Al and Mn, which are generally toxic to both symbionts (Bordeleau and Prevost, 1994; Samac and Testaye, 2003; Giller et al, 1998, Al-Falih, 2002), with nodulation being more adversely affected than the development of the host plant and nitrogen-fixation (Bordeleau and Prevost, 1994). As opposed to some rhizobia, the presence of R. meliloti in acid soils can be detected only after its host has developed (Hirsch, 1996) or their presence is negligible (Koopman et al, 1995). This fact necessitates selection of specific effective strains, competitive in terms of nodule formation, and tolerant of low soil pH. As reported by Soto et al. (2004) and Glenn and Dilworth (1994), Rhizobium meliloti strains are highly susceptible and can grow only at pH of above 5.5, but strains exhibiting satisfactory activity at substantially lower pH values have also been selected (Jarak et al., 2003). Given the importance of rhizobial inoculants in agriculture, a large number of studies focus on factors affecting Rhizobium survival in the soil. Many results are contradictory mainly due to the complexity of interaction between different factors. As a substantial acreage of
soils in the Republic of Serbia is acidic, the objective of this study was to evaluate the effect of preinoculation on dry matter yield and quality of alfalfa grown on very acid pseudogley.

**Material and methods**

The trial was set up at the experimental field of the Secondary School of Agriculture and Food Engineering in Kraljevo on very acid pseudogley soil having a pH in KCl of 4.79, a low content of humus (2.56%) and moderate levels of nitrogen, phosphorus and potassium (0.135% N, 7.20 mg100g\(^{-1}\) P\(_2\)O\(_5\) and 11.0 mg100g\(^{-1}\) K\(_2\)O). The soil had poor physical and mechanical properties and, in view of its chemical characteristics, it showed low productive capacity. The trial was established in a randomized block design with three replications, with the plot size being 5 m\(^2\). Five alfalfa cultivars were used in this study. Prior to sowing, cvs. Banat, NS Mediana, K-28, Sinskaja and OS-66 were inoculated with an acid-resistant *Rhizobium meliloti* strain. The three-year study focused on evaluating the following: total dry matter yield (t ha\(^{-1}\)) and quality (the contents of crude proteins - CP, crude ash - CA, crude fats - CFs, crude fibre – CF and the proportion of nitrogen-free extract – NFE).

The obtained results were subjected to a two-factor (inoculation x cultivar) analysis of variance (ANOVA). Individual and interaction differences between the tested properties were determined by t-test.

**Results and discussion**

The use of alfalfa seed preinoculation induced a significant increase in dry matter yields during the first two years as compared to the control in all cultivars (the absence of cultivar x inoculation interaction) (Tab. 1). The average increase in the first and second years was 127.8% and 46.6%, respectively, in all cultivars. As opposed to the first two years, no positive effect of the inoculant used on dry matter yield was observed in the third year.

The results of a pot trial conducted by Rice (1982) suggested that *R. meliloti* strains tolerant of low pH gave rise to a nearly tenfold increase in dry matter yield (soil pH 5.5) as compared to pH-sensitive strains.

The long-term effects of seed inoculation i.e. a substantial increase in forage yield were also reported by Shupam et al. (1996) in a four-year study and Lapinskas (2000) in a five-year trial. Positive effects of inoculation on dry matter yield of alfalfa in the first years of growth were highlighted by Stout et al (1997) and Jarak et al (2002). According to Careli et al. (2000), the *R. meliloti*-alfalfa symbiosis is general rather than specific, suggesting that the selection of *Rhizobium* strains should be aimed at attaining tolerance of stress conditions, primarily soil acidity as well as at enhancing nitrogen-fixation capacity in the soils having optimal pH for alfalfa. Zhao-Hai et al. (2007) stress the justifiability of selecting *R. meliloti* strains for a particular cultivar.

The absence of the positive effect of inoculation on the dry matter yield in the tested alfalfa cultivars in the third year resulted from the low persistence of the inoculant in the very acid soil i.e. from its competition with indigenous strains. This conforms to the suggestion made by Toro (1996), Del Papa et al (2003) and Soto et al (2004) that the ability of inoculants to compete with acid-resistant indigenous strains is a frequent limiting factor for the successful use of inoculants.

The dry matter yield obtained in all years was found to be substantially higher in cv. K-28 than in most other cultivars (Tab. 1). The substantially higher dry matter yield of cv. K-28 in the third year, as compared to the yield of the other cultivars, where inoculation had no positive effects, indicates its tolerance of lower soil pH.
Pan et al. (2008) also report differences in the degree of alfalfa cultivar susceptibility to soil acidity i.e. toxic effects of Al. The obtained results are inconsistent with those of Humphries and Auricht (2001) indicating very poor tolerance of low soil pH, the degree of tolerance being within a very narrow range.

### Table 1. Average dry matter yield (t ha\(^{-1}\)) of alfalfa cultivars in the control (Ø) and under inoculation (R) treatments over a three-year period of observation

<table>
<thead>
<tr>
<th>Year</th>
<th>Cultivar</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ø</td>
<td>R</td>
<td>Ø</td>
<td>R</td>
</tr>
<tr>
<td>2006</td>
<td>1.89</td>
<td>4.07</td>
<td>2.98a</td>
<td>7.63</td>
</tr>
<tr>
<td>2007</td>
<td>2.12</td>
<td>4.13</td>
<td>3.13a</td>
<td>8.39</td>
</tr>
<tr>
<td>2008</td>
<td>1.09</td>
<td>3.98</td>
<td>2.54b</td>
<td>10.26</td>
</tr>
<tr>
<td>Average</td>
<td>1.23</td>
<td>3.62</td>
<td>2.43b</td>
<td>5.88</td>
</tr>
</tbody>
</table>

Values followed by different small letters within columns are significantly different (P<0.05) and those with different small letters in brackets within rows are also significantly different (P<0.05), according to the Lsd test.

Higher protein contents in all cultivars were recorded in the treatments without inoculation (Tab. 2). The obtained results are incompatible with those of Stout et al. (1997) and Lapinskas (2000) who observed the protein content to be significantly higher under inoculation, as well as with the results given in Segundo et al. (1999) reporting the absence of the effect of inoculation on protein content. The retarded shoot growth in the control and the related smaller plant habitus and a higher leaf proportion are a possible explanation for the higher protein content in the control.

### Table 2. Chemical composition (g kg\(^{-1}\)) of dry matter in alfalfa cultivars in the control (Ø) and under inoculation treatments (R)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Inocul.</th>
<th>CP</th>
<th>CF</th>
<th>CA</th>
<th>CFs</th>
<th>NFE</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Ø</td>
<td>14.27</td>
<td>35.16</td>
<td>12.30</td>
<td>2.34</td>
<td>35.93</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>13.41</td>
<td>27.08</td>
<td>11.38</td>
<td>3.64</td>
<td>44.50</td>
</tr>
<tr>
<td></td>
<td>Ø</td>
<td>15.25</td>
<td>27.71</td>
<td>11.20</td>
<td>1.23</td>
<td>44.62</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12.42</td>
<td>31.93</td>
<td>10.62</td>
<td>3.21</td>
<td>41.83</td>
</tr>
<tr>
<td></td>
<td>Ø</td>
<td>14.02</td>
<td>31.31</td>
<td>10.80</td>
<td>2.08</td>
<td>41.79</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12.02</td>
<td>27.94</td>
<td>10.83</td>
<td>3.71</td>
<td>45.50</td>
</tr>
<tr>
<td></td>
<td>Ø</td>
<td>13.91</td>
<td>25.75</td>
<td>11.34</td>
<td>2.64</td>
<td>46.35</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12.40</td>
<td>27.80</td>
<td>10.83</td>
<td>3.80</td>
<td>45.17</td>
</tr>
<tr>
<td></td>
<td>Ø</td>
<td>15.80</td>
<td>29.79</td>
<td>11.76</td>
<td>2.31</td>
<td>40.34</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>14.04</td>
<td>30.68</td>
<td>9.97</td>
<td>2.55</td>
<td>42.76</td>
</tr>
<tr>
<td>Average</td>
<td>Ø</td>
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<td>29.94</td>
<td>11.48</td>
<td>2.12</td>
<td>41.81</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12.86</td>
<td>29.09</td>
<td>10.73</td>
<td>3.38</td>
<td>43.95</td>
</tr>
</tbody>
</table>

CP-crude proteins; CF-crude fibre; CA-crude ash; CFs-crude fats; NFE- nitrogen-free extract

### Conclusions

The inoculation of alfalfa seeds with a pH-resistant *Rhizobium meliloti* strain in a very acid pseudogley soil resulted in a substantial increase in dry matter yield in all cultivars during the first two years.
The absence of the positive effect of inoculation in the third year was induced by low persistence of the strain in a very acidic environment i.e. by its competition with indigenous strains present in the soil.

Dry matter yield was significantly higher in cv. K-28 in all years than in most other cultivars. The considerably higher dry matter yield of cv. K-28 in the third year as compared to the other cultivars suggested its tolerance of lower soil pH.

Given the poor persistence of the above strain in an acidic environment, studies should focus not only on the further selection of acid-resistant strains of *R. meliloti* but also on the selection of alfalfa cultivars tolerant of low soil pH.

References


