

Increased dry matter yield of alfalfa following inoculation with plant growth promoting rhizobacteria

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Introduction

Biological nitrogen fixation accounts for approximately 140 million metric tons of nitrogen fixed annually and this represents the largest input of nitrogen to the land surfaces (Jenkinson, 1990). Legumes in rotation have been recognized by Soil Conservation Canada as critical to economic diversification, disease control and to enhancing soil quality.

Over the last decade the knowledge of rhizosphere biology has advanced with the discovery of a specific group of microorganisms, called PGPR, that colonize the plant root and promote plant growth (Kloepper *et al.*, 1980). The beneficial effects of the PGPR are direct plant growth promotion and disease suppression. Direct plant growth promotion by the rhizobacteria is thought to be due to the production of plant growth regulators. Rhizobacteria are capable of disease suppression by virtue of certain metabolic activities they possess. For example, (1) competition for iron in iron-poor soils by the siderophore-producing strains of rhizobacteria may reduce the population of iron-requiring plant pathogenic microorganisms (Kloepper *et al.*, 1980, Loper and Buyer, 1991). (2) production of antibiotics, many rhizobacteria produce antibiotics (Bull *et al.*, 1991, Dahiya *et al.*, 1988) or cyanide (HCN) (Voisard *et al.*, 1989) *in vitro* and these compounds may participate in disease suppression in the field. An additional mechanism of disease suppression by the rhizobacteria which has just begun to be addressed is their ability to induce the plants defense mechanisms (van Peer, Niemann and Schippers 1991, Wei, Kloepper and Tuzun, 1991).

NPR are classified as PGPR which will increase nodulation as well as plant growth. Co-inoculation studies with NPR and Rhizobium have demonstrated improved root and shoot weight, plant vigour, improved nodulation and yield. The legumes studied include common bean (Grimes and Mount, 1983), soybean (Singh and Subba

Rao, 1979, Polonenko et al., 1987, Li and Alexander, 1988), alfalfa, clover (Harris, 1953, Yahalom et al., 1987), lentil and pea (Chanway, Hynes and Nelson, 1987).

The purpose of this research was to investigate the potential of NPR to improve the growth of alfalfa when co-inoculated with R. meliloti.

Materials and Methods

Strains

NPR 1-102 and 2-68 was identified as Serratia sp and all other strains used in this study as Pseudomonas sp.

Greenhouse and Field Alfalfa Trials

The NPR were seed applied in an algenate formulation to the alfalfa seed. In the greenhouse trials the treated seed was sown into top soil at a rate of 21 seeds per pot. An RCBD with 8 reps per treatment was used.

For the alfalfa field trials (Beaverlodge, Ab.), 0-46-0 was incorporated into the plots prior to seeding. Alfalfa, variety Peace was seeded at a rate of 8 lb/acre and the NPR used in this trial were formulated in algenate. This trial were planted on the 15th of June, 1988, and the 1st cut was obtained in the establishment year and 2 cuts per year were obtained in subsequent years (1989 and 1990).

At the Wisconsin site the variety of alfalfa used was Pro Cut II. Seeding rate and formulation for the NPR was as above.

At the Outlook site cultivar Beaver was seeded at the same rate as above, however, the NPR and Rhizobium meliloti were formulated in Nitragin Gold which had been kindly supplied by Dr. R.S. Smith, Lipha Tech, Milwaukee. Stand counts of alfalfa and nodule rating was done at the Outlook site. The mean number of plants per metre per plot was estimated 3 and 4 weeks after planting. Nodule rating, based on a scale from 0 (no nodules) to 5 (excellent nodulation) was done 2.5 months after planting. Weeding was done by hand.

Results

1. Alfalfa - Greenhouse Trials

Final stand of alfalfa, variety Saranac, was significantly improved ($p=0.05$) over the control by all of the NPR tested and with variety Iroquois, NPR 31-12 significantly improved ($p=0.05$) final stand (Tables 1 and 2). Dry matter yield and nodule number of alfalfa, variety Iroquois, were increased significantly ($p=0.10$) by 4 of the 7 NPR tested (Table 1). The strains of NPR that promoted the greatest yield increases were 31-12, G11-32 and 1-102 (29, 23, and 17% change in the dry matter yield, respectively).

Table 1 Effect of NPR on final stand dry matter yield and nodule number of alfalfa, variety Iroquois, in the greenhouse.

Treatment	Final Stand	DWT/Pot ¹	Nodule #/Pot ²
G2-8	13	6.23	62.5
G20-18	15	6.12	75.0*
G11-31	13	7.29*	82.9*
1-102	13	6.81*	79.1*
G2-26	13	6.49	50.9
31-12	17**	7.93*	85.0*
61-9A	11	6.98*	61.6
Control	13	5.64	51.1

1. Cumulative yield (g/pot) 3 cuts.

2. * represents a significant difference from the respective control using the LSD test at the 10% level, CV=23;

** represents a significant difference from the respective control using the LSD test at the 5% level, CV=21.

Table 2 Effect of NPR on final stand of alfalfa, variety Saranac, in the greenhouse

Treatment	Final Stand
G2-8	12**
G20-18	11**
G11-32	14**
1-102	11**
G2-26	11**
31-12	11**
61-9A	12**
Control	9

1. ** represents a significant difference from the respective control using the LSD test at the 5% level, CV=17.

2. Alfalfa - Field Trials

Beaverlodge, Alberta

Dry matter yield of alfalfa was increased (non-significant) by all of the NPR at the Agriculture Canada site (Figure 1). At the Toews farm site (Figure 2), NPR's 31-12, 61-9A and G2-8 improved the dry matter yield (non-significant) over the uninoculated treatment. Addition of Rhizobium increased the dry matter yield by 2854 kg/ha (non-significant). The strain of NPR that performed best in these trials was 31-12, promoting a yield increase of 6%.

Figure 1: 3 Year Cumulative (1988-90) Dry Matter Yield of Alfalfa, variety Peace, Agriculture Canada, Beaverlodge, Alberta

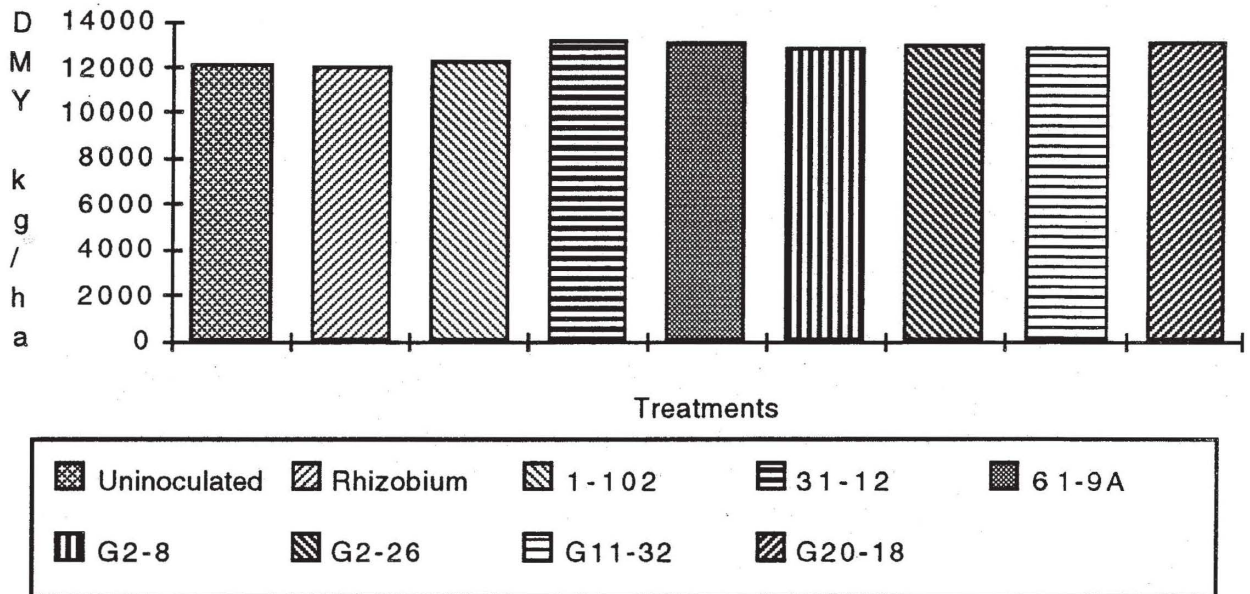
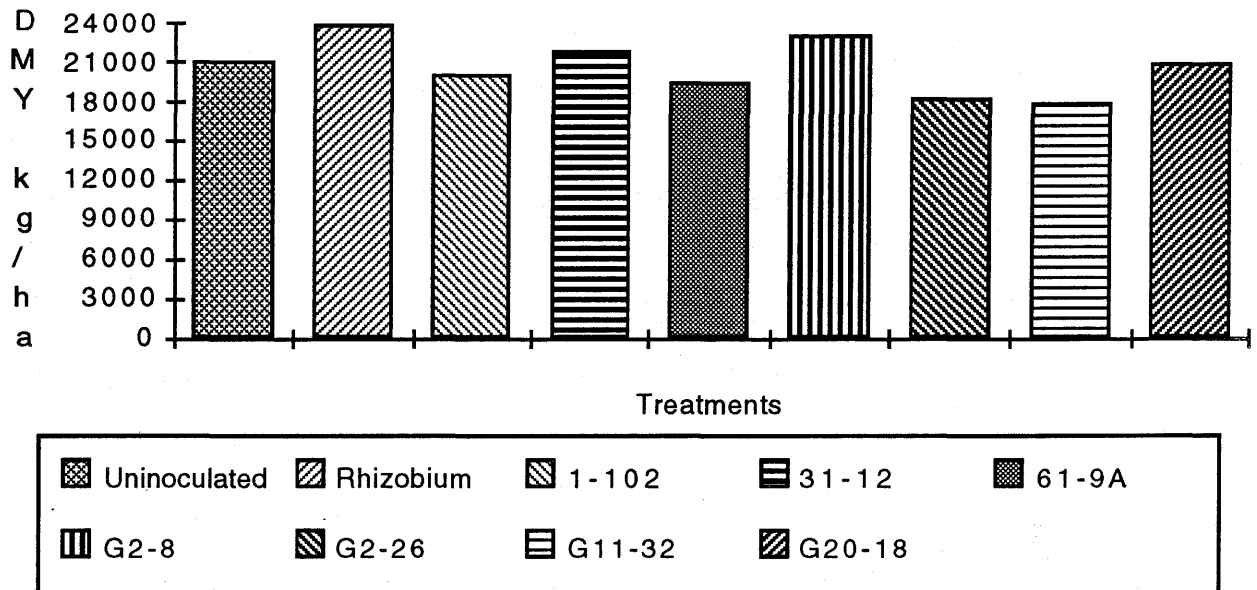


Figure 2: 3 Year Cumulative (1988-90) Dry Matter Yield of Alfalfa, variety Peace, Toews Land

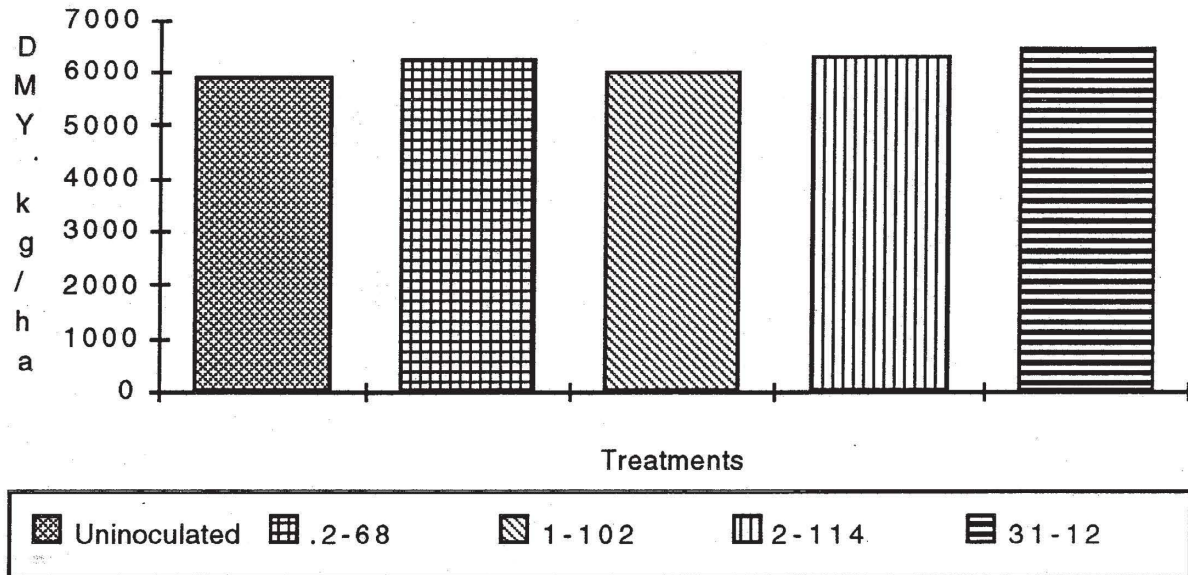


2. Wisconsin

Three of the four NPR tested significantly increased ($p=0.10$) the dry matter yield of alfalfa, variety ProCut II, at the Wisconsin sites (Fig. 3).

The strain of NPR that performed best at these trials was 31-12 promoting a yield increase of 8%.

Figure 3: Three site, Cumulative Dry Matter Yield of Alfalfa, variety ProCut II, Wisconsin, U.S.



3. Outlook

The levels of N, P, K and S in the Bradwell loamy fine sand and the amount of rainfall for the 1991 season at the Outlook site is shown in Table 3. Moisture levels were excellent for the establishment of the alfalfa.

Table 3: Rainfall and soil nutrient levels (1991) at the Outlook, Sk. alfalfa site.

<u>Nitrate</u>	<u>Phosphorus</u>	<u>Potassium</u>	<u>Sulfur</u>
<u>kg/ha</u>			
28	9.8	326	13.8

Moisture accumulation (May to October 1991)

<u>Month</u>	<u>Rain Guage (mm)</u>
May	82.6
June	175.2
July	55.4
August	4.8
September	13.4

The number of NPR per g of Nitragin Gold is reported in Table 4. The microorganisms survived very well in this formulation.

Table 4: Numbers of nodule promoting rhizobacteria per g of Nitragin Gold

<u>Rhizobacteria</u>	<u>Microorganisms/g N. Gold</u>
31-12	4.7×10^8
2-68	1.8×10^9
G2-26	3.3×10^8
61-9A	2.6×10^8

Two stand counts were done 3 and 4 weeks after planting and the results are shown in Table 5. One of the NPR's, G2-26 co-inoculated with Rhizobium, significantly ($p=0.1$) increased the stand of alfalfa over that of Rhizobium alone treatment (Count 1), however, this trend was not observed in the second count.

Table 5: Alfalfa stand

Treatment	Count 1	Count 2
G2-26	329.0 A	222.7 A
61-9A	289.0 AB	231.8 A
Untrt.	283.3 AB	220.3 A
31-12	277.2 AB	206.2AB
Rhiz. NGA-3	238.5 BC	214.8 A
2-68	184.7 C	160.7 B

Note: Numbers followed by a common letter are not significantly different at the 90% level. Counts were obtained from 1 m².

Nodule rating was carried out 2.5 months after planting and the results are shown in Table 6. Tap and lateral root nodulation was improved by NPR's 61-9A, 31-12 and 2-68.

Table 6: Nodule rating

Treatment	Nodule Rating
61-9A	4.0
31-12	3.5
2-68	3.0
G2-26	2.0
Rhiz. NGA-3	2.0
Untrt.	2.0

Note: 10 plants per treatment harvested.

Rating: 0, no nodules to 5, excellent nodulation.

Two cuts of alfalfa were taken and the dry matter yields are reported in Figure 4. The average moisture content of the alfalfa for both cuts was 75%. Treatment with NPR 31-12 increased the dry matter yield 7% in both cuts taken over the Rhizobium alone treatment. Also the total nitrogen in the straw from the second cut was increased 7% with treatment of 31-12.

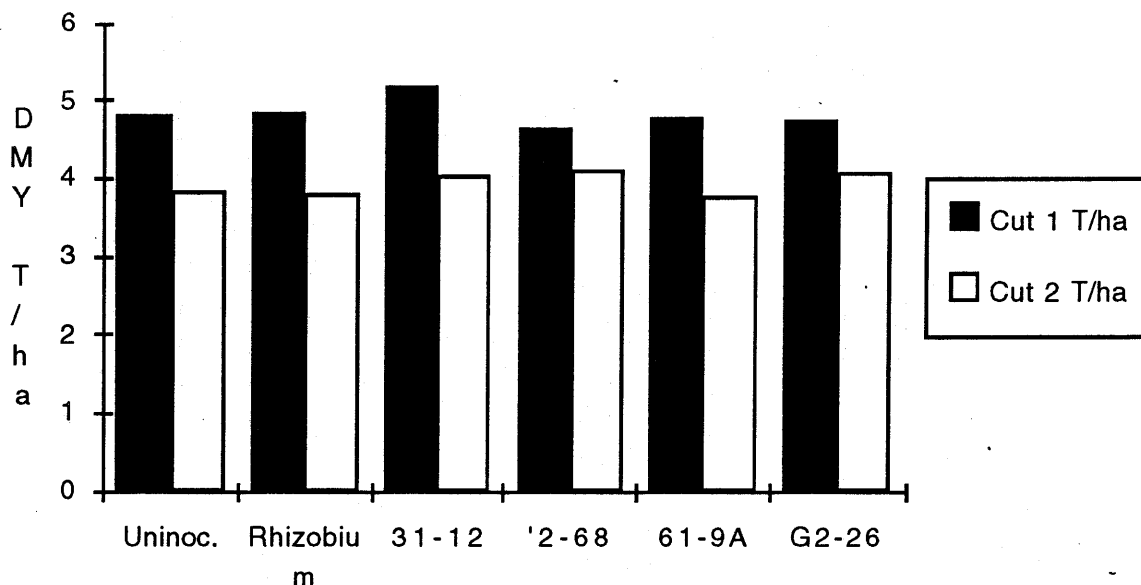


Figure 4. Dry matter yield of alfalfa, Outlook, Sk.

Conclusion

Nodule promoting rhizobacteria improved final stand, nodule number and, at some field sites, the dry matter yield of alfalfa. Other legumes co-inoculated with NPR and Rhizobium have demonstrated similar responses (Grimes and Mount, 1983; Singh and Subba Rao, 1979; Polonenko *et al.*, 1987; Li and Alexander, 1988; Harris 1953; Yahalom *et al.*, 1987 and Chanway, Hynes and Nelson, 1983) The most consistent performer from all of the trials was Pseudomonas 31-12 since it significantly improved final stand in the greenhouse trials and dry matter yield at the Wisconsin sites and promoted non-significant increases in dry matter yield at the Beaverlodge and the Outlook trials. At the Outlook trial the total nitrogen content of the alfalfa was also increased by co-inoculation with 31-12 and Rhizobium. Nodule rating suggested that 31-12 improved nodulation of alfalfa and this have contributed to the increase in dry matter yield. Interestingly, NPR 31-12 was capable of promoting alfalfa growth when formulated in either algenate or Nitragin Gold.

These results demonstrate that NPR may be of value as a co-inoculant with Rhizobium meloliti on alfalfa.

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