Post-Emergent Inoculation and Fertilization of Alfalfa Stands

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Introduction

Typically, alfalfa fields produce maximum yields for the first few years after establishment, until fertility and moisture levels decrease and/or weed populations out compete the introduced crop. Seed alfalfa is much less nutrient demanding than alfalfa grown for hay, and stands tend to remain vigorous for longer periods of time. Although it is a recommended practice, many producers do not inoculate the seed with a commercial *Rhizobium* inoculant, believing that indigenous or previously introduced populations of rhizobia are sufficient to nodulate the plants. Scientific studies show alfalfa to be an excellent N-fixing crop, capable of fixing up to 200kg N ha^{-1}. It is assumed that the high productivity of this crop in producer's fields is due to N fixation, however, alfalfa also is extremely efficient at extracting inorganic N from soil solution (Blumenthal and Russelle, 1996) and when it is intercropped it is a strong competitor for soil N (Tomm et al.,1995). Strains of alfalfa have been selected for their efficient nitrate uptake characteristics, and have been used to "clean-up" nitrate fertilizer spills on agricultural land (Russelle et al., 2001). It also has been demonstrated that biological N fixation continues when extremely high levels of N fertilizer is applied (Lamb et al., 1995). When 840 kg N ha^{-1} was applied, a first production year stand of alfalfa obtained 20-25% of its N from biological N fixation. It is not clear if alfalfa can benefit through both biological N fixation and N fertilization.

Studies performed in the 1980's in northern Alberta, looked at post-emergent inoculation of alfalfa (Rice and Olsen, 1991; Rice, 1987). Granular rhizobial inoculants were placed beside the seed row the year after establishment. Other treatments included powdered seed applied inoculants, granular inoculants placed with the seed at seeding, granular inoculants placed below the seed at seeding, and liquid formulations placed both at seeding and post-emergently. None of the treatments showed any difference in yield over a three year period. Nodule numbers were higher for inoculated plants than controls, but all nodule numbers per plant were low and declined substantially with each year of the study. By the third year an average range of 0 to 2.4 nodules per plant was recovered from any of the treatments. Although not a conclusion of the authors the lack of yield response may have been because some or all of the plants were extracting soil N rather than fixing atmospheric N. Inoculation may have only been effective in the first year or two of establishment. Plant N contents were not assessed in the study. Furthermore, the lack of response with post-emergent inoculation may be due to damage caused by the post-emergent application itself rather than ineffective inoculation. There was no description in the report of the equipment used to place inoculant post-emergently. Low disturbance disc seeders may improve the probability of successful post-emergent inoculation. Annual applications of fertilizer to forages is recommended in the *Forage Crop Production Guide 2003 (SAF, 2003),* but in practice very few forage producers follow this advice. More commonly fertilization is utilized as a strategy for rejuvenation of forage grasses but is rarely performed on alfalfa. Alfalfa tends to be terminated once stand condition deteriorates. Nitrogen fertilization is not recommended for forage legumes. Inorganic soil N reduces the effectiveness of N fixation in legumes. It is assumed that because alfalfa fixes N biologically, N supply to the crop is not limiting. However, direct evidence of this is lacking. Little is known about how soil fertility changes under an aging stand. Furthermore, nodules are extremely difficult to find on the roots of an aging stand. Whether this is because they don't persist long term or is simply because they are difficult to harvest because of the depth of rooting is uncertain. In addition, we know little about the long-term survival and the long-term effectiveness of introduced rhizobia that may survive in the soils. The assumption that a reduction in N fertility is not the cause of stand degeneration may not be substantiated. Even if the nodules do persist, the addition of N fertilizer may invigorate the stand, enabling it to tiller more and reestablish in the stand. Nitrogen fertilizer does not permanently impair biological N fixation but simply delays it until low N conditions return. Another reason for not applying N fertilizers to alfalfa is that N fertilizers may increase the competitiveness of grasses in the stand. This is a concern in mixed stands. However, due to the extremely competitive nature of alfalfa for extracting inorganic N from the soil, this may not be as much of a problem as was initially thought. In pure stands, a combination of a herbicide to control grassy weeds and an N-fertilizer application might prove to be the most effective strategy for rejuvenating an aging stand.

Phosphorus fertilization is recommended for alfalfa stands which are more than 4 years old, and K and S are recommended for coarse textured soils and gray-wooded soils. Levels of plant available P can be extremely low in Saskatchewan soils and hence the potential for P deficiency to develop in forage crops is high. Phosphorus is a difficult nutrient to apply as an amendment because of its extremely low solubility. Strategies and/or products that increase the solubilization of soil P or fertilizer P may prove effective in alfalfa.
Our study examined the effectiveness of microbial inoculants and fertilizers applied to mature stands, where productivity is declining. In addition, rhizobial inoculants and *P. bilaiae* inoculants were applied post-emergently to newly established alfalfa stands.

Field Sites:

Rejuvenation Study:
Three fields with aging alfalfa stands were chosen for study. The fields are located near Star City, Smeaton and Tisdale, SK. The fields were selected on the basis of soil tests; all fields had P test results in the marginal to deficient range and N test results in the marginal range. All of the fields were showing decreased productivity (as reported by the farmer) that was suspected to be the result of fertility decline rather than weed invasion.

Treatments were applied in early May, 2004 and are outlined in Table 1. The granular inoculant manufactured by Philom Bios is composed primarily of $CaSO_4$. Applying the granule at 10 times the recommended rate results in an application of 40 kg/ha, which corresponds to just under 10 kg S/ha. To avoid the confounding affect of the added sulphur, uninoculated granules were applied to each of the treatment plots (except one of the control treatments), in quantities that resulted in all treatments receiving the same amount of sulphur.

The inoculants were applied using a modified small-plot seeder supplied by Philom Bios. The seeder was equipped with narrow-row disc openers with on-row press wheel packing (Flexi-

Coil). The seeder had five rows spaced 30 cm apart. Inoculants were placed 4 cm deep. Soil and residue disturbance was minimal although some damage was evident.

Table 1. Fertilizers and microbial inoculants applied to aging alfalfa stands.

Treatment	**Application method**
Nitrogen :	
Ammonium nitrate (100 kg N ha^{-1})	Broadcast
Urea (100 kg N ha^{-1})	Broadcast
Urea & Agrotain (100 kg N ha^{-1})	Broadcast
Slow release urea[1](100 kg N ha^{-1})	Broadcast
Rhizobia (1X)[2]	Banded
Rhizobia (10X)[2]	Banded
Phosphorus :	
Triple superphosphate (40 kg P ha^{-1})	Banded
P. bilaiae (10X)[2]	Banded
P. bilaiae (10X)[2] & triple superphosphate (20 kg P ha^{-1})	Banded
Nitrogen and Phosphorus:	
Rhizobia and *P. bilaia*e (10X)[2]	Banded
Sulphur:	
Ammonium sulphate	Banded
Controls:	
Undisturbed	
Mechanically disturbed	Banded
Gypsum	Banded

[1]Agrium, slow release urea
[2]Applied at the recommended (1X) or ten times the recommended (10X) application rate – recommended application rate is 4.0 kg ha^{-1}.

Delayed inoculation Study:

Field sites were established near Aberdeen, SK and near Arborfield, SK. All sites had deficient P values and marginal N values. All of the plots received a blanket treatment of potassium sulphate fertilizer. Spring treatments were seeded in May, 2004. Delayed treatments were applied in May 2005 (Table 2)

Table 2. Description of treatments. Year of seeding treatments had inoculants applied at the time of seeding. Delayed inoculation treatments were banded into a 1 year old standing crop.

Year of seeding treatments	**Delayed treatments**
Control: Uninoculated seed, no fertilizer	Control: mechanical disturbance
Seed inoculated with rhizobia[3]	Rhizobia banded
Seed inoculated with *P. bilaiae*[4]	*P. bilaiae* banded
Seed inoculated with rhizobia and *P. bilaiae*	Rhizobia and P. bilaiae banded
Seed inoculated with *P. bilaiae* on soil fertilized with ½ rate of P.	*P. bilaiae* and ½ rate P banded

[1]20 kg N h^{a-1} side-banded
[2]20 kg P ha^{-1} seed-placed
[3]4 hg ha^{-1}
[4]4 kg ha-[1]

Results and Discussion:

<u>*Rejuventation Study:*</u>
Mechanical Disturbance:
Damage from the plot seeder used to apply the inoculants was assessed by counting numbers of alfalfa plants in undisturbed and disturbed plots. Only at Crooked River were plant numbers reduced by the damage from the seeder, but even then plant numbers were comparable to the other sites.

Table 3. Mean alfalfa stand counts in May 2004 from mechanically disturbed plots and control plots at the three field sites.

	Star City	**Crooked River**	**Smeaton**
	------------------- *plants* m^{-2} -------------------		
M.D. ‡	†30 (20)	32 (15)	33 (14)
Control	26 (4)	36 (14)	28 (16)
	------------------------- *P-value*§ ---------------------------		
	0.61	0.03	0.39

† Mean of 6 replicates with standard deviation in parenthesis.
‡ Mechanical Disturbance
§ Level of probability that means are not significantly different according to the two-tailed, paired sample Student's *t*-test.

Sulphur Fertility
Ammonium sulphate fertilizer broadcast on the treatment plots lead to significant increases in nutrient uptake and biomass accumulation. Improving S fertility increased uptake of N and P as

well as S. Banding the gypsum inoculant carrier, supplying 10 kgS/ha, also positively affected fertility and productivity of the stands. The response to the S applications was strongest at the Star City site.

Nitrogen Fertility

Both N fertilizer applications and *Rhizobial* inoculation were ineffective at improving fertility and productivity in the mature alfalfa fields, indicating that the stands were still actively fixing N from the atmosphere.   Quantification of rhizobia capable of nodulating alfalfa indicated that numbers of rhizobia in the soil were high (Table 4).

Table 4 Mean *Rhizobium* population ratings (± standard error) of soils from three sites sampled in June 2005 (n=3).

Treatment†		**Star City**	**Crooked River**	**Smeaton**
		---------------- *Rating‡*	------------------	
4-kg ha^{-1} *Rhizobium*		4.7 (0.3)	4.7 (0.3)	4.0 (0.6)
40-kg ha^{-1}*Rhizobium*		5.0 (0.0)	5.0 (0.0)	5.0 (0.0)
Control		4.7 (0.3)	4.7 (0.3)	4.0 (0.6)
ANOVA				
Source of Variation	*df*	------------------- *P-value¶*	--------------------	
Treatment	2	0.69	0.69	0.25

† Refer to Table 3.1 for a complete description of treatments and application methods.
‡ Soil samples rated from 1-5 by number *Rhizobium* cells present (rhizobia g^{-1}).
¶ Probability level of analysis of variance.

Although applying the inoculants at ten times the recommended rate (40 kg/ha) did increase the populations of *rhizoid* in the soil one year after the inoculants were applied, the higher numbers did not increase the amount of biomass produced compared to uninoculated controls.

Phosphorus Fertility

Somewhat surprisingly, there was similarly no response to P fertilization nor *P. bilaiae* inoculation, indicating that the stands were not limited by P availability. Alfalfa, like other legumes, has root systems that are very effective at extracting P from the soil. Acids produced by and excreted from the roots serve to solubilize P that is normally insoluble under Saskatchewan soil pH conditions.   It appears that the mature alfalfa stands are effective at extracting the P needed for production.

<u>Delayed Inoculation</u>

Overall, delaying the application of the *Rhizobium* and the *P. bilaiae* inoculants by one year had little affect on biomass production and N and P uptake (Table 5) compared to the time of seeding applications at the Aberdeen site.   However, at the Arborfield site, applying the *Rhizobium* at the time of seeding was superior to the delayed application. In contrast delaying the *P. bilaiae* application at the Arborfield site resulted in higher productivity and nutrient uptake by the alfalfa.   Unlike many annual crops, where it is recommended to apply *P. bilaiae* with a reduced rate of P fertilizer, this combination was generally among the poorest treatments in terms of biomass produced at both sites, especially in the delayed application.

Both of these sites benefited from the alfalfa being inoculated with *Rhizobium*, indicating that the populations of *Rhizobia* already existing in the soil were not sufficient to achieve optimal N fixation.

Table 5. Biomass production and nutrient uptake for alfalfa at Aberdeen and Arborfield, SK sampled in 2005 inoculated or fertilized at the time of seeding (2004) or post-emergently, one year after seeding (2005). Values are means of six replicates with standard deviation in parentheses. Details of the treatments are outlined in Table 2.

		Aberdeen			**Arborfield**		
Treatment	**Treatment Year**	**Biomass** (kg ha^{-1})	**N uptake** (kg ha^{-1})	**P uptake** (kg ha^{-1})	**Biomass** (kg ha^{-1})	**N uptake** (kg ha^{-1})	**P uptake** (kg ha^{-1})
Control	2004	5367	80.3	7.7	4725	115.3	12.6
Rhizobium	2004	5578	89.2	8.3	5470	95.0	10.9
P. bilaiae	2004	6277	114.6	11.3	5183	101.8	12.2
Rhiz..+ P.b.	2004	5197	93.2	8.9	5040	100.0	11.9
P.b+1/2P	2004	6067	99.7	10.5	4831	96.7	11.4
Rhizobium	2005	5613	101.9	9.7	4723	92.3	10.5
P. bilaiae	2005	6255	103.0	10.3	5473	115.8	13.6
Rhiz. + P.b	2005	5840	88.1	9.0	5057	122.3	13.8
P.b+1/2P	2005	5552	80.1	8.2	4760	107.4	12.3

Acknowledgements:
This project was funded by SAF ADF. Inoculants and in kind assistance with seeding a plot maintenance was provided by Philom Bios Inc. (Saskatoon, SK). None of our work can be accomplished without the kind assistance of our producer cooperators.

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