Granular Inoculation of Alfalfa

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Introduction

Rhizobium inoculants for alfalfa production typically are delivered as coatings on pre-inoculated seeds or as a peat-based powder formulation. Pre-inoculated seed is very convenient, requiring no additional operation at the farm level, but is highly susceptible to loss of viability of the applied rhizobia. Peat-based inoculant applied with a sticker can be very messy and time-consuming when the producer's time is at a premium. In recent years, manufacturers have begun to develop and formulate *Rhizobium* inoculants as compressed peat or clay-based granules. In the granular form, the *Rhizobium* inoculant is applied to the soil instead of the seed, in the same manner as fertilizer, and can be applied before, during or after the seeding operation. Granular formulations are increasing in popularity as inoculants for pulse crops but are not yet commercially available for forage crops. In several studies with pulses, granular inoculants lead to improved nodulation and higher seed yields compared to traditional formulations (Bezdicek et al., 1978; Muldoon et al., 1980). Granular inoculant formulations applied in the seed row have been shown to encourage better nitrogen fixation and higher yields in chickpea and bean compared to the peat-based or liquid counterparts (F. Walley, person. communic.).

Inoculation of alfalfa (and clovers) with granular formulations may prove beneficial for a number of reasons. Because of its extremely small seed size, alfalfa must be planted very shallowly in the soil (maximum depth of ¾ inch, depending on soil conditions). Placement of the inoculant below the seed row should protect rhizobia from temperature and moisture fluctuations (Smith, 1992). In addition, the small seed size limits the number of rhizobia that can be attached to the seed coat (Clayton et al., 1996). While this may be overcome by the large seed numbers that are planted, placement of granules should prove effective in increasing numbers of viable rhizobia in contact with the root system because the rate of granular placement can be regulated. Placement of rhizobia below the seed ensures that the rhizobia are favorably placed in relation to the roots.

Alfalfa typically is seeded along with a companion crop, like oat or canola, in the establishment year. Companion crops compete with forage seedlings for moisture, light and nutrients, but can also provide some protection for alfalfa seedlings from heat, wind and water and suppress weeds. They are used because they provide some production

from the field in the year of establishment but usually reduce subsequent yields of the forage (SAF 1998). In the appropriate management system, an alternative for delivering rhizobia to the soil may be to inoculate the seed of the companion crop, thereby using the companion crop as the delivery system for the inoculant. This only would apply to large seeded companion crops (like oat) that are seeded deeper in the soil than the alfalfa. Inoculation of the oat seed rather than the alfalfa seed would place the *Rhizobium* deep in the soil, providing protection from climatic fluctuations. In addition, the larger seed size of oat compared to alfalfa, would enable more *Rhizobia* to be attached to the seed coat. However, companion seed inoculation would still have the potential disadvantage of being messy and inconvenient to use.

Little information is available about the effective use and application of granular inoculants in forage legumes. To my knowledge no one has ever investigated the possibility of using the companion crop seed as a delivery system for inoculant. Information is needed about the efficacy of granular inoculants as compared to more traditional inoculant formulations. Ease of application, effectiveness of nodulation and cost of inoculation are all factors that must be considered.

Materials and Methods

Three field sites were established in the spring of 1999. One site was established at the Seager Wheeler farm near Rosthern, a second site at the Conservation Learning Centre (CLC) south of Prince Albert, and a third site on producer's land near Tisdale. The three field sites provide a range of soils and topography typical of alfalfa production.

The Tisdale site was established as a processed "dehy" production site using canola (46A73; Pioneer Hi-Bred Production Ltd.) as the cover crop and was seeded May 25, 1999. The site had been seeded to flax in 1998 and represented a conventionally tilled site. Individual treatment plots were 2.6-m x 11-m. The experimental design was a randomized complete block design with four replications. Treatments consisted of: 1, uninoculated *alfalfa (Medicago sativa* cv. Beaver; this variety was used for all of the inoculated treatments) – control; 2, pre-inoculated alfalfa; 3, on-site inoculated alfalfa (peat-based inoculant applied at seeding); 4, alfalfa + granular inoculant banded (ca. 2.5 cm below the seed row); 5, granular inoculant placed with the alfalfa seed; and 6, on-site inoculated alfalfa hand-broadcast and raked in. Plots were seeded using a disc seeder with 8-inch row spacing. The seeder was equipped with two boxes and a set of cones. Alfalfa was seeded at a rate of 7 lb acre^{-1}; canola at 5 lb acre^{-1}; and the granular inoculant applied at a rate of 7 lb acre^{-1}. Plots were seeded in two passes. The canola and phosphate fertilizer (20 lb acre^{-1}, 12-54-0) were placed in the first pass. Canola was placed approximately ¼ inch deep and phosphate 1 inch to the side and below the seed. Alfalfa seed and the inoculant were placed in the second pass. Alfalfa was seeded approximately ¼ inch deep. Depending on the treatment, granular inoculant was placed with the alfalfa seed or banded at the depth of the fertilizer. Plots were sprayed post-emergently with Pursuit according to manufacturer's recommendations.

The Seager Wheeler site was seeded May 26th, 1999. This site was established as a typical hay production site, using oat (*Avena sativa*) as the cover crop. The site had been seeded to a triticale/pea mix in 1998 and represented a conventionally tilled site. The plot size and seeding equipment were the same as those used at the Tisdale site. The same treatments as the Tisdale site were seeded except that oat was seeded instead of canola as the cover crop. In addition, instead of the broadcast treatment of on-site inoculated alfalfa (treatment 6) the oat seed was on-site peat inoculated and placed approximately 1 inch deep. Oat was seeded at a rate of 40 lb $acre^{-1}$. Plots were seeded in two passes. In the first pass the oat and phosphate fertilizer (20 lb $acre^{-1}$, 12-54-0) were placed. Oat was placed approximately 1 inch deep and the phosphate fertilizer 1 inch to the side of the seed. Alfalfa seed and the inoculant were placed in the second pass. Alfalfa was seeded approximately ¼ inch deep. Depending on the treatment, granular inoculant was placed with the alfalfa seed or side-banded at the depth of the fertilizer/oat. Plots were sprayed post-emergently with Pardner according to the manufacturer's recommendations.

The CLC site was seeded June 2nd, 1999. Like the Seager Wheeler site, this site was established as a typical hay production site, using oat as the cover crop. The site had been seeded to barley in 1998 and represented a minimum till site. Several weeks before seeding the field was sprayed with Roundup transorb. Alfalfa and oat were seeded directly into the barley stubble. Plots were seeded using an Edwards hoe drill with 8 inch row spacing. The size of individual treatment plots was 4-m x 1-m. The seeder was equipped with two split boxes. The treatments including seeding rates, and seed and fertilizer placements were the same as those used at the Seager Wheeler site. Plots were seeded in two passes. The oat seed and phosphate fertilizer (20 lb $acre^{-1}$,12-54-0) were placed in the first pass. Alfalfa seed and inoculant were placed in the second pass. Depending on the treatment, granular inoculant was placed with the alfalfa seed or side-banded at the depth of the fertilizer/oat. No post-emergent herbicide was deemed necessary.

All of the inoculant formulations were prepared from the same batch of *Rhizobium meloliti* by MicroBioRhizogen Corporation, Saskatoon.

Measurement variables considered in this paper are nodulation, stand establishment and above-ground biomass production. The system for scoring nodulation is outlined in Table 1 and was adapted from a similar nodulation scoring system for field pea obtained from Agriculture Canada. Stand establishment was determined by counting the number of emerged alfalfa plants in a 1 m^2 area. The m^2 frame was positioned to span three crop rows. Stand establishment was determined the third week of July at all of the sites. Biomass was measured by harvesting plants in a 1 m^2 area. Plants were harvested the last week of August.

Table 1. Nodulation Scoring for Alfalfa. Assessment Based on 5 Plants/Sample.

No. plants with pink nodules	Total no. of nodules	Score
>3 plants	>30	5
>3 plants	10-30	4
>3 plants	5-10	3
>3 plants	<5	2
1-3 plants	<5	1
0 plants		0

Results and Discussion

The Conservation Learning Centre had the poorest nodulation for all of the inoculant formulations and placements of all of the sites (Table 2). All of the inoculant treatments resulted in more nodulation compared to the check plots and the two granular inoculant treatments and the inoculated oat seed showed the best nodulation at this site. Nodulation at Tisdale and Seager Wheeler was good in all treatments including the check plots. At both of these sites the poorest nodulation occurred in the granular inoculant placed with the alfalfa seed. It appears that seed placed inoculants are more successful in terms of nodulation if they are in direct contact with the seed.

Table 2. Median Nodulation Scores for Field Samples at the Three Sites.

Treatment	Tisdale	Seager Wheeler	CLC
	---------- Nodulation ----------		
Control	2.5	4.0	0.0
Pre-inoculated	3.0	5.0	2.0
On-site inoculated	3.0	4.0	1.0
Granular side-banded	3.0	3.0	3.0
Granular seed-placed	2.0	1.5	2.5
On-site inoculated oat[1]/broadcast alfalfa[2]	4.0	3.0	2.5

[1]Treatment at CLC and Rosthern where oat was the cover crop
[2]Treatment at Tisdale where canola was the cover crop

Only at the CLC site were there any differences in alfalfa stand establishment (Table 3). Establishment was extremely poor in all of the treatments where the inoculant was seed placed. Establishment in the banded granular inoculant treatment was comparable to the check plots. Stand establishment was the highest in the on-site inoculated oat seed. It appears that at this site, placing the inoculant below the seed row was beneficial to establishment. For reasons unknown, placing the inoculant with the seed interfered with alfalfa stand establishment.

There were no statistical differences between treatments for stand counts at the other Tisdale and the Seager Wheeler site (Table 3). However, at the Seager Wheeler site there was a tendency for higher stand counts associated with both of the granular inoculant treatments. At Tisdale, the pre-inoculated seed showed a tendency for better establishment. Unlike the CLC site, seed placing the inoculant had no detrimental effect on alfalfa establishment.

Table 3. Mean Alfalfa Stand Establishment for Three Field Sites.

Treatment	Tisdale	Seager Wheeler	CLC
	----------Alfalfa stand (No. m^{-2}) ----------		
Control	22	28	21
Pre-inoculated	31	27	2
On-site inoculated	23	23	3
Granular banded	16	34	25
Granular seed-placed	23	42	2
On-site inoculated oat[1]/broadcast alfalfa[2]	17	28	36
ANOVA		*Probability*	
Block	0.461	0.632	0.174
Treatment	0.332	0.362	0.000

[1]Treatment at CLC and Rosthern where oat was the cover crop
[2]Treatment at Tisdale where canola was the cover crop

Table 4. Mean Above-Ground Biomass (g m^{-2}) of Alfalfa from the Three Field Sites.

Treatment	Tisdale	Seager Wheeler	CLC
	---------- Biomass (g m^{-2}) ----------		
Control	35	33	28
Pre-inoculated	58	31	3
On-site inoculated	37	45	7
Granular banded	55	48	46
Granular seed-placed	44	38	4
On-site inoculated oat[1]/broadcast alfalfa[2]	17	28	58
ANOVA	*Probability*		
Block	0.024	0.224	0.174
Treatment	0.046	0.016	0.000

[1]Treatment at CLC and Rosthern where oat was the cover crop
[2]Treatment at Tisdale where canola was the cover crop

The poor stand counts associated with the seed placed inoculant treatments at the CLC site translated into lower biomass production in these same treatments (Table 4). The CLC site was the only site that was a minimum till site. It appears that the site may not have been a good choice in terms of forage production, however, the control and granular banded treatments were quite successful. Some factor at this site was unsuitable for inoculant placement directly with the seed and interfered with nodulation, stand establishment and ultimately productivity in treatments where the inoculant was applied to or placed with the alfalfa seed.

At all three of the sites the banded granular inoculant treatment was among the most productive (Table 4). At Seager Wheeler all of the treatments showed excellent productivity. Except for the pre-inoculated seed and the on-site inoculated oat seed, all of the inoculation treatments were superior to the check, but even these treatments along with the check were very productive.

At Tisdale, the on-site inoculated alfalfa that was broadcast placed resulted in the poorest biomass production of all the treatments (Table 4). The pre-inoculated and granular banded treatments were the most productive. Except for the broadcast treatment, all of the treatments were at least as good as the uninoculated control.

Conclusion

In the establishment year the granular inoculants showed promise as alternative formulations for delivering *Rhizobium* to the soil in a legume forage production system. At all three of the study sites, banding the inoculant below the seed row resulted in alfalfa stands and biomass productivity that was at least as good or superior to the traditional on-site inoculated treatments. At the CLC site the advantage to the granular banded treatment appears to be the depth of placement because the inoculated oat seed produced comparable results. At the Seager Wheeler site the both of the granular treatments worked well in terms of nodulation, stand establishment and productivity but were not superior to the traditional on-site peat inoculation treatment. At the Tisdale site, both of the granular placements (banded or seed-placed) resulted in biomass production that was superior to the on-site peat inoculation, but at this site the pre-inoculated treatment performed equally as well as the granular placements. Although the granular formulations show promise as formulations for delivering *Rhizobium* to legume forages they are not consistently superior to existing formulations.

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Key Topic Words

Alfalfa, Forages, Granular inoculants, *Rhizobium meliloti*