Rhizobial requirements for successful establishment of birdsfoot trefoil (*Lotus corniculatus* L.)

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Abstract

Nodulation problems, due to poor survival of rhizobia (*Mesorhizobium loti*) on the seed, can limit the establishment of birdsfoot trefoil (*Lotus corniculatus* L.). High rates of inoculation (approximately 250,000 rhizobia per seed) and sowing within one day have been shown to be necessary to ensure successful nodulation of birdsfoot trefoil under oversown conditions. However, this scenario is not practical for farmers. Trials were conducted to determine whether nodulation could be improved by the selection of rhizobia with high rates of survival on the seed. Seven strains of *M. loti* were incorporated into peat inoculants and used to inoculate birdsfoot trefoil seed in a commercial pelleting process. Seed was pelleted two or four weeks before being oversown onto two Central Otago tussock grassland sites. Rhizobia per seed and percentage of seedlings nodulated for each strain were determined. After two weeks storage, seed inoculated with the currently recommended strain had 16,000 rhizobia per seed and 28% of seedlings were nodulated. Two other strains maintained populations greater than 23,000 rhizobia per seed, and 45% and 48% of seedlings were nodulated. After four weeks storage, all seed had less than 5,000 rhizobia and resulting nodulation was low (<12%). These results confirm the importance of high numbers of rhizobia on the seed at sowing for successful nodulation of birdsfoot trefoil. Establishment of birdsfoot trefoil can be improved by selection of rhizobia for enhanced survival on seed; however, pre-inoculated seed must have high populations of rhizobia at sowing.

Additional key words: Mesorhizobium loti, *nodulation, oversowing, tussock grasslands*

Introduction

Successful establishment of birdsfoot trefoil (*Lotus corniculatus* L.) depends on seedling nodulation by the introduced inoculant rhizobia (*Mesorhizobium loti*) except in a few areas of New Zealand where *L. tenuis* or *L. corniculatus* have become naturalised (Greenwood and Pankhurst, 1977). Successful nodulation following oversowng can only be guaranteed if very high numbers of rhizobia are present on the seed at sowing (Patrick and Lowther, 1992).

Survival of rhizobia on the seed, both before and after sowing, is recognised as an important factor in the success of inoculation (Date, 1976). Lowther and Patrick (1995) proposed that the survival of rhizobia on birdsfoot trefoil seed in the first 24 hours after inoculation could be used as a rapid method of initially screening strains of inoculant rhizobia. They identified that the then recommended New Zealand inoculant strain (ICMP9005) (Manaaki Whenua Landcare Research) had poor survival (1%), while another strain (USDA3471) had very high survival (89%), but cautioned that final strain evaluation should be under field conditions.

The use of commercially pre-inoculated, pelleted seed is widespread in New Zealand for both oversowing and drilling (Scott *et al.*, 1995), and therefore development of effective commercial products with a reasonable shelf life is a priority. Patrick and Lowther (1992) found that commercially inoculated and pelleted seed stored for three weeks at 15°C, had 300 rhizobia per seed at sowing and resulted in only 6% seedling nodulation. These are typical storage conditions for commercially pelleted seed. To obtain satisfactory establishment of commercially pelleted birdsfoot trefoil seed, higher numbers of rhizobia must be present on the seed at sowing; this may be achieved by using a better adapted strain of rhizobia in the inoculant. This experiment investigated the survival and subsequent performance of seven strains of *M. loti*, which were incorporated into experimental pellets produced by a commercial process, then stored for two or four weeks before being oversown onto two tussock grassland sites, to simulate general commercial and farming practice.

Materials and Methods

Rhizobia

Six strains of *M. loti* were compared with the current Australian recommended inoculant strain (SU343) used in Australian inoculants imported into New Zealand. Strains USDA3471 and NZP2235 had shown consistently good results over two years in field trials when incorporated into laboratory experimental peat (Pryor unpublished data). Naturalized strains of rhizobia, better adapted to their environment (Dowling and Broughton, 1986) could be superior as inoculant strains; therefore, four strains of *M. loti* collected from a 7 year old birdsfoot trefoil stand were included (ICC262, ICC263, ICC264 and ICC265). These differed in their genomic DNA fingerprints from the strain (ICMP9005) used originally to inoculate the birdsfoot trefoil seed (Sullivan *et al.*, 1995).

Rhizobial strains were grown on yeast mannitol agar slopes (Vincent, 1970) at AgResearch Invermay and supplied to Nitrobug® for incorporation into experimental, commercial peat inoculants. These were used by Coated Seed Ltd to Prillcote® seed of birdsfoot trefoil cv. Grasslands Goldie in their experimental processing plant on 18 and 30 August 1994. Prillcote® seed was stored in normal factory conditions for two and four weeks then sent to Invermay for field-testing. A control of seed inoculated with Nitrobug® commercial inoculant (containing strain SU343) and lime, coated at Invermay one day before sowing, was included.

Populations of rhizobia in the peat inoculants, and on the seed one day after inoculation were counted by duplicate plate counts (Vincent, 1970). Populations at the time of oversowing were estimated by the plant infection method (Brockwell, 1963), with duplicate counts and 10-fold dilutions.

Seedling nodulation

The seven treatments were oversown on 14 September onto two field sites in undeveloped snow tussock environments on high country yellow-brown earth soils. These were at Naseby (pH 5.2, 730 m.a.s.l.), and Hawkdun (pH 5.3, 760 m.a.s.l.). A basal dressing of 250 kg/ha sulphur-molybdic-superphosphate (28% S; 0.01% Mo; 7% P) was applied before oversowing. Seed was oversown at the rate of 5 kg/ha bare seed onto the undisturbed soil on 14 September 1994. Mitral (50 g/kg isazophos) was applied at 20 kg/ha at sowing and two months later to reduce damage from broad-nosed weevils (Barratt and Johnstone, 1984). Treatments were randomised in four replicate blocks with plot size of 3 x 2 m.

When seedlings reached the cotyledon/unifoliate leaf stage at each site, 20 seedlings in each plot were randomly selected and identified with relocatable wire markers. The percentage nodulated seedlings was determined by recording the number of healthy seedlings still present in April 1995 (Patrick *et al.*, 1994). This technique has proved a good means of assessing the effectiveness of treatments because non-nodulated seedlings fail to grow where there are low levels of available soil-N, and the number of seedlings established at the end of the first season has been shown to be directly related to the percentage of nodulated seedlings (Patrick *et al.*, 1994). An analysis of variance was carried out on the percentage of nodulated seedlings.

Results

Peat inoculants prepared with the different strains all had acceptable populations of rhizobia, ranging from 1.4 - 5.3 x 109 per g peat (Table 1). The number of rhizobia on the seed one day after inoculation, varied from 5,000 - 230,000 per seed (Table 1). There was no consistent relationship between the number of rhizobia in the peat inoculant and the number on the seed one day after inoculation.

There was no significant interaction between sites and strains and hence results from the two sites have been combined in Table 2. Seed inoculated with strains USDA3471 and ICC265 and sown two weeks after processing resulted in 49% and 45% of seedlings nodulated, respectively. These were significantly higher than the 28% seedling nodulation obtained from

Table 1. Number of rhizobia in the peat inoculant and on the seed one day after inoculation

Strain	Rhizobia/g peat ($x10^9$)	Rhizobia/seed at one day after inoculation (x 1000): After 2 weeks storage[2]	After 4 weeks storage[3]
NZP2235	4.75	26	25
SU343[1]	4.4	190	40
USDA3471	5.3	230	100
ICC262	2.35	45	19
ICC263	1.4	35	5
ICC264	3.1	56	14
ICC265	3.1	110	50

[1] Commercial inoculant strain
[2] Inoculated on 30-8-94
[3] Inoculated on 18-8-94

inoculation with the commercial strain SU343 (Table 2). Although all three strains had similar populations of rhizobia in their peat inoculant, seed inoculated with USDA3471 and ICC265 had populations in excess of 23,000 rhizobia per seed at sowing, compared with 16,000 for SU343.

Seed inoculated and stored for four weeks had consistently low nodulation with all strains of rhizobia, even with populations as high as 4,800 rhizobia per seed at sowing.

The seed inoculated and pelleted in the laboratory one day before sowing had 5,700 rhizobia per seed at sowing and 28% of seedlings had nodulated, which was the same as that from the Prillcote coated seed using this strain which had been stored for two weeks.

Discussion

The results confirm that high numbers of rhizobia must be present on the seed at sowing for successful nodulation of oversown birdsfoot trefoil (Chapman *et al.*, 1990). The populations of rhizobia maintained for two weeks on the Prillcote seed in this experiment were well in excess of those present after storage for only one week reported by Patrick and Lowther (1992). It is therefore recommended that rhizobial populations should be counted on commercially pelleted seed used in experiments to enable results to be extrapolated to practical farming conditions.

After two weeks storage, nodulation from commercially pelleted seed inoculated with the recommended strain (SU343) was similar to laboratory inoculated and pelleted seed sown one day after inoculation. Higher seedling nodulation from the laboratory treatment could have been expected if high inoculation rates had been used (Patrick and Lowther, 1992).

The high seedling nodulation after two weeks storage following inoculation with strains USDA3471 and ICC265 was associated with the highest number of rhizobia per seed at sowing. This appears to be due to the superior ability of these strains to survive on the seed for the first two weeks after inoculation. However, appraisal of strain evaluation experiments is difficult due to the various factors that can affect the result. An important factor is to ensure that equal numbers of rhizobia are applied to the seed at inoculation. The inoculants for the best two strains had similar populations of rhizobia in the peat as the recommended strain, and hence similar populations would be expected on the seed at inoculation. This cannot be confirmed, as initial counts of rhizobia on seed at inoculation were not carried out. Strain USDA3471 was included as it had previously been shown to have very high survival in the first 24 hours after inoculation (Lowther and Patrick, 1995). The magnitude of the increase in nodulation from these two strains over the present recommended strain justifies further experimentation to confirm their advantage.

Table 2. Populations of rhizobia on the seed at sowing and percentage of nodulated seedlings from 2 sites (see text).

Strain	Pelleted seed stored for 2 weeks		Pelleted seed stored for 4 weeks	
	Rhizobia/seed[2]	% nodulated seedlings[3]	Rhizobia/seed	% nodulated seedlings
NZP2235	2,300	21	260	7
SU343[1]	16,100	28	4,240	9
USDA3471	>23,000	49**	4,800	8
ICC262	2,300	28	290	11
ICC263	950	14	670	5
ICC264	4,670	13	70	10
ICC265	>23,000	45*	3,270	5
SED		6.4		6.4
Laboratory inoculated and pelleted - one day storage				
SU343[1]	5,700	28		

[1] Commercial inoculant strain

[2] Factor for 95% confidence limits, approx. 3.5

[3] * or ** denote significant difference from the commercial strain (SU343) at P = 5% or 1% level respectively.

The virtual nodulation failure of the seedlings in the 4-week storage treatments was associated with low bacterial populations on the seed at sowing. These low rhizobial numbers may have been due to either increased mortality on the seed, or lower initial numbers applied to the seed. All 4-week storage treatments, except NZP2235, had lower 1-day populations compared to each of their corresponding 2-week storage treatments. It is also possible that the increased storage time could have affected the nodulating potential of the strains through physiological changes (Pinochet *et al.*, 1993).

Conclusions

The use of a superior strain of *M. loti* to inoculate birdsfoot trefoil should improve seedling nodulation and hence establishment of birdsfoot trefoil. However, pre-inoculated seed should only be stored for a limited time before oversowing to ensure sufficient numbers of rhizobia on the seed at sowing to maximise nodulation.

Acknowledgements

Thanks to Coated Seed Ltd, Christchurch for supply and commercial coating of seed, Bill Lowther, Invermay for continual help and advice and Kevin Trainor for fieldwork.

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