THE SURVIVAL OF THE COMMERCIAL INOCULANT IN WHITE CLOVER AND LUCERNE

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Introduction

For maximum N_2 fixation to occur, legumes must be nodulated by efficient strains of rhizobia. When resident naturalised populations of rhizobia are high, the need for commercial inoculant has been questioned (Lowther & Kerr, 2011). This research investigated the survival of the commercial inoculant for white clover and lucerne in the field in New Zealand. The survival of the commercial inoculants RRI128 for lucerne (*Medicago sativa* L.) and TA1 for white clover (*Trifolium repens* L.) was investigated at two Lincoln University dryland sites. The aim was to quantify the duration of survival of the commercial inoculants for lucerne and white clover in the presence of naturalized strains.

Materials and Methods

Lucerne and white clover were gown at Lincoln University Field Research Centre as pure swards in neighbouring paddocks that had been previously been sown to lucerne and white clover, respectively. The lucerne paddock was sown in November 2010 and the white clover in December 2014. The lucerne paddock was inoculated with RII128 and the white clover with TA1. The lucerne paddock had two inoculant preparations , peat slurry and a seed coat. TA1 was applied to the white clover in a seed coat. At each sampling, 10 – 20 lucerne plants were extracted from both the peat and coated seed treatments. Samplings occured in January 2011, January 2014 and January 2015. For white clover 10 plants were dug up every 8 weeks after sowing for 8 months. Both the lucerne and white clover all plants showed nodulation of the roots, so 1-10 nodules were selected and sterilized. In total, ~50 nodules were collected for each treatment, at each sampling. Rhizobia were recovered from the nodules. DNA extraction and ERIC PCR of bacterial DNA was also carried out as described in Wigley *et al*. (2015). The presence of the commercial inoculant was confirmed by nodules that had the same ERIC banding pattern as the commercial inoculant. This was confirmed by 16S rRNA gene sequencing of the bacteria in the nodules with the same banding pattern as the commercial inoculant.

Results and Discussion

The percentage of nodules containing the commercial inouclant for lucerne and white clover changed over time (Table 1). In 2011, two months after sowing, RRI128 was dominant and recovered from 40% of the nodules plants grown from coated seed (n=20 nodules). In 2014, 3 years after sowing, RRI128 was also most common in the nodules from peat and coated seed plants at 64% (n=34) and 48% (n=29). Overall the occupancy of RRI128 in the nodules had increased in both the peat (22%; n=11 *vs* 64%; n=34) and coated (40%; n=20 *vs* 48%; n=29) seed treatments from 2011 to 2014. In 2015, four years after sowing, the commercial inoculant for lucerne was still the most common genotype found in the peat seed plants at 61% (n=31). In the nodules of coated seed plants the commercial inoculant was only found in 13% (n=5) of the nodules. The commercial inoculant for lucerne has also been found to be effective at increasing plant growth. Black & Moot (2013) and Berenji *et al*. (2015) found that when lucerne was sown in soil that had not previously been sown in lucerne the application of a peat inoculant doubled first or second year yield compared with a bare seed control. This suggests it had competed with the naturalised strains in the soil for nodule occupancy. In contrast, for white clover, 2 months after sowing TA1 was found in only 8% of the nodules collected (n=4). At 4 months after sowing TA1 was found in 24% of the nodules and eight months after sowing TA1 was found in 8% of the nodules. Brockwell *et al.* (1975) also found that when seed inoculated with TA1 at a rate of 135 rhizobia per seed was sown into a soil containing 10,000 naturalised rhizobia per gram, less than 10% of nodules were

Table 1. Percentage of the commercial inoculant observed in isolates recovered from the nodules of lucerne plants treated with lime coat and peat inoculant and white clover at Lincoln University, New Zealand

Lucerne	**2011**	**2014**	**2015**
Coated Seed	40	48	13
Peat Seed	22	64	61
White Clover	**Feb 2015**	**April 2015**	**August 2015**
	8	24	8

occupied by TA1. It appears that TA1 was not competitive or persistent in its ability to nodulate white clover plants, and that it is easily out-competed by naturalised strains in the soil. This contrasts with the commercial strain for lucerne which survived and persisted for over four years.

Conclusions

The commercial inoculant for lucerne was able to persist four years after sowing when applied as a peat slurry, whereas the commercial inoculant for white clover was present in only 8% of nodules 8 months after sowing.

References

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