Issues affecting the competitiveness of white clover rhizobia in New Zealand pastures

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Abstract

Research into improving symbiotic nitrogen fixation of white clover in New Zealand pastures through the introduction of effective rhizobia is reviewed. Naturalised populations of rhizobia are usually highly diverse and of reduced effectiveness compared to inoculant strains, and large increases in nitrogen fixed have been found in situations where high nodule occupancy by an inoculant strain was obtained. The likelihood of an inoculant strain initially forming a high proportion of nodules is dependent on the size of the naturalised and inoculant populations, and the strain of rhizobia. Lack of persistence of the inoculant strain in competition with naturalised rhizobia also limits improvement of symbiotic nitrogen fixation in pasture through inoculation. Recent studies suggest that genetic instability of inoculant strains and exchange of symbiotic plasmids contribute to the diversity of naturalised populations and lack of inoculant persistence. Therefore, it is necessary to understand the ecology of naturalised populations, including their genetic interactions with inoculant strains, in order to develop strategies to improve the competitiveness and persistence of inoculant strains. Alternatively it may be possible to increase the effectiveness of indigenous populations through gene transfer from the inoculant strain. The possibility of breeding specific host cultivar/rhizobial strain combinations also merits further research.

Keywords: competition, genetic stability, inoculation, nitrogen fixation, rhizobia, white clover

Introduction

New Zealand's pastoral agriculture is largely dependent on symbiotic nitrogen fixation between rhizobia (*Rhizobium leguminosarum* biovar *trifolii*) and white clover (*Trifolium repens* L.). Although genetic diversity within the host has been widely utilised to improve productivity, little of the diversity within rhizobia has been exploited. To enhance symbiotic nitrogen fixation, introduced rhizobia must persist and compete with naturalised rhizobia to nodulate the host legume. In this paper we review research into improving symbiotic nitrogen fixation of white clover through introduction of effective rhizobia and discuss future options for overcoming the limitations that have been identified.

Populations of rhizobia in soil

Rhizobia capable of nodulating white clover were originally introduced to New Zealand by chance during early days of settlement of the country (Greenwood 1964). They are now widespread but are still absent from some areas (Greenwood 1965; Gaur & Lowther 1980). Populations under white clover/ryegrass pastures range from 10^4 to more than 10^6 per gram of soil (Hale 1980; Rys & Bonish 1981; Gaur & Lowther 1982a). Lower numbers may occur in unimproved hill country (Macfarlane & Bonish 1986) and following cropping (Bonish & Steele 1985).

Rhizobia vary widely in their symbiotic effectiveness and are commonly ranked by comparison with an effective inoculant strain under controlled environmental conditions. In Taranaki, Rys and Bonish (1981) sampled rhizobia from nodules of white clover growing on 30 sites ranging from low to high producing dairy pastures. The average effectiveness of the isolates ranged from 51% to 102% of the effectiveness of the standard strain. No isolates were completely ineffective. The mean effectiveness of isolates from 5 white clover/ryegrass pastures on the Central Plateau ranged from 68% to 80% (Hale 1980). In Otago the effectiveness of individual isolates ranged from 8% to 117% (mean 85%) from a cultivated paddock (Gaur & Lowther 1982a), and from 2% to 118% (mean 81%) in tussock grassland sites (Gaur & Lowther 1980). The overall effectiveness of rhizobial populations may be correlated to the fertility of the soil, with acid soils containing less effective strains (Holding & King 1963).

The above results were obtained using Huia white clover as a test host. Nodule isolates from indigenous white clover growing in wet, infertile soil in Ireland were more effective with the indigenous clover from which they were isolated than with a commercial cultivar (Sherwood & Masterson 1974). Commercial clover sown in such a soil became nodulated initially with a relatively ineffective population of rhizobia, but the effectiveness of the nodulating population apparently improved with time. Bonish & Rys (1982) found no evidence of such an adaptation on 12 of the 14 sites they sampled in

Taranaki. More recent results also suggest that white clover cultivars or ecotypes do not show a strain preference (Harrison et al. 1989; Leung et al. 1994), in contrast to a strong strain preference shown by annual clovers (Leung et al. 1994).

Introduction of effective rhizobia

The importance of inoculating clover seed in areas lacking naturalised rhizobia is well recognised in New Zealand (Greenwood 1965). Several studies have also indicated the potential of improving symbiotic nitrogen fixation in white clover through the introduction of efficient rhizobia at sowing. In a pot trial with soil from under pasture, more than a 50% increase in clover foliage weight was obtained where the inoculant strain formed 49% to 56% of nodules (Hale 1980). Gaur & Lowther (1982a) reported a 22% increase in total N content of white clover in a cultivated paddock when the inoculant strain formed more than 50% of nodules. Inoculation had no effect when the inoculant strain formed <20% of nodules. Under oversowing conditions, increases in clover dry matter and total N content of up to 47% were reported when inoculant strains formed more than 50% of nodules (Gaur & Lowther 1982b).

The likelihood of an inoculant strain forming a high proportion of nodules is dependent on the size of the naturalised population, the size of the inoculant population (Ireland & Vincent 1968), the strain of rhizobia (Gaur & Lowther 1982a; 1982b) and the inoculation method. Hale (1980) reported low occupancy (<20%) with conventional seed inoculation (10^3 rhizobia per seed) and concluded that either soil or liquid inoculation was necessary to introduce effective rhizobia. However, the strain used by Hale is a poor competitor compared to other clover-nodulating strains (Gaur & Lowther 1982b).

Lack of persistence of the inoculant strain in competition with naturalised rhizobia may be a major factor limiting improvement of symbiotic nitrogen fixation in pasture through inoculation. Hale (1980) found that the percentage of nodules occupied by the inoculant strain declined rapidly over the first 12 months and concluded that although the introduction of rhizobia showed some promise, improvement in the persistence of introduced strains was required for long-term benefit. However, Gaur & Lowther (1982a; 1982b) recorded over 50% nodule occupancy 15 months after oversowing and 18 months after sowing into a cultivated paddock with some strains and stressed the importance of strain selection.

The above results suggest that the successful introduction of highly effective rhizobia can improve symbiotic nitrogen fixation in white clover based pastures. However, the introduced rhizobia must be able to persist and compete with indigenous rhizobia for nodule formation.

Genetic structure of rhizobial populations

Most of the above studies relied on serology to differentiate strains as accurate methods of identifying strains were not available. However more recently methods of multilocus enzyme electrophoresis and DNA fingerprinting that identify individual strains have been used in studies of rhizobial population ecology. Limited studies of rhizobia from white clover have shown that naturalised populations are usually highly diverse, even within small areas (Schofield et al. 1987; Harrison et al. 1989; Leung et al. 1994). The genetic basis of strain diversity is not clear; however different symbiotic plasmids have been found in the same chromosomal background and the same plasmid in different backgrounds (Schofield et al. 1987). This suggests that transfer and recombination of symbiotic plasmids may occur under field conditions and contribute to the variation in effectiveness observed in naturalised populations.

As is the case elsewhere in the world, the fact that naturalised populations under New Zealand pasture have been long established makes it difficult to determine their origin. However, areas of the Te Anau basin are devoid of naturalised clover rhizobia. We recently isolated rhizobia from nodules on clover plants from plots in Te Anau established twenty years ago with inoculation. The area surrounding the plots is still devoid of clover rhizobia. All 14 isolates examined had an identical chromosomal type to the original inoculant, suggesting all are descended from it. However, 10 different plasmid profiles were found amongst the 14 strains, with only one being identical to the inoculant strain. DNA fingerprinting using nodulation gene probes revealed three major patterns, with again one strain like the inoculant. The others fell into two groups, one of six and the other of seven strains. When tested on red clover all strains were fully effective; however when tested on white clover one group formed ineffective to poorly effective nodules, while the other group formed effective nodules (unpublished data). These results suggest that the population has diversified through plasmid exchange with soil bacteria. Recombination of the symbiotic plasmid in the nodulation gene region is likely to have contributed to the altered symbiotic properties of some of the strains and to the apparent lack of persistence of the inoculant strain.

Not all clover inoculant strains are necessarily unstable. Caucasian clover has highly specific rhizobial requirements and must be inoculated, as rhizobia able to nodulate it are absent from New Zealand soils. Only two strains that differ by plasmid content were found nodulating Caucasian clover in the field, even in stands established for 20 years (McIntyre & Ronson unpublished data). Thus the association between Caucasian clover and its effective inoculant strains is very stable. This is despite the fact that Caucasian clover rhizobia are genetically similar to white clover rhizobia and are able to nodulate white clover rapidly, albeit ineffectively.

Overcoming the competition problem

It is apparent that to overcome the competition problem, it is first necessary to understand the ecology of naturalised populations including their genetic interactions with inoculant strains. The genetic stability of an inoculant strain, in particular the stability of its symbiotic plasmid and its ability both to transfer plasmids and to act as a recipient for plasmid transfer, are likely to contribute to its persistence. There is also the intriguing possibility of manipulating the effectiveness of the naturalised population through plasmid transfer, even in the absence of inoculant strain persistence. It may therefore be possible to identify or develop inoculant strains that are genetically stable or to construct strains that raise the effectiveness of naturalised populations through aggressive transfer of a highly effective and stable symbiotic plasmid.

Another strategy to overcome the competition problem is to exclude the naturalised population from nodulating by breeding specific clover cultivar/*Rhizobium* strain combinations. Caucasian clover is an example of such an association, being nodulated by only two highly effective strains after 20 years. Caucasian clover is closely related to white clover and fertile hybrids of various ploidies between the two species have been produced (W. Williams, AgResearch, *pers. comm*.). It may therefore be possible to breed a specific white clover/*Rhizobium* combination using the plant hybrids as starting material for the plant specificity gene and a *Rhizobium* strain that is effective with white clover germplasm but contains the host specificity gene(s) from Caucasian clover rhizobia.

Conclusions

Substantial increases in symbiotic nitrogen fixation have been obtained in situations where high nodule occupancy by an inoculant strain was achieved, indicating that naturalised rhizobial populations of lower symbiotic effectiveness reduce the ability of modern white clover cultivars to achieve their symbiotic potential. Our recent results suggest that strains of rhizobia used for inoculant purposes are not genetically stable and plasmid transfer can result in the development of populations with different levels of effectiveness. Understanding the origin of these diverse stains of rhizobia may allow development of strategies to improve the competitiveness and persistence of inoculant strains, or to manipulate the effectiveness of indigenous populations. The possibility of breeding specific host cultivar/rhizobial strain combinations also merits further research.

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