

# Two annual legume species and their selection of nodule occupants



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## Introduction

Despite New Zealand's reliance on legume and ryegrass dominated pastures, little is known about naturalised rhizobia strains in New Zealand soils. This work sets out to determine whether naturalised rhizobia resident in a Canterbury dryland pasture soil will nodulate two different annual legume species. ERIC (enterobacterial repetitive intergenic consensus) PCR is a method used to determine bacterial strain diversity<sup>1</sup>. It can be used to distinguish even closely related rhizobia strains and has been used in numerous studies to determine nodule occupancy<sup>2,3</sup>.



Figure 1. Haresfoot trefoil (left) and striated clover (right) in flower (photos courtesy of Keith Pollock).

## Methods

**Pot trial** – Haresfoot trefoil (*Trifolium arvense*) and striated clover (*T. striatum*). Duration: 4-5 months. Eleven replicates, randomised block design.

**Nodule isolation** – Bacteria isolated from 45 nodules per treatment.

**Genotyping** – Using ERIC PCR<sup>1</sup>. Agarose gel photographs analysed using Phoretix 1D Pro. UPGMA dendrograms constructed using Jaccard similarity.



Figure 2. An example of ERIC-PCR products run on an agarose gel. A selection of rhizobial genotypes are visible.

## References

<sup>1</sup>Versalovic *et al* (1991) Distribution of repetitive DNA sequences in eubacteria and applications to fingerprinting of bacterial genomes. *Nucleic Acids Research* 19, 6823 – 6831.

<sup>2</sup>Nangul *et al* (2013) Nodule occupancy by *Rhizobium leguminosarum* strain WSM1325 following inoculation of four annual *Trifolium* species in Canterbury, New Zealand. *NZ Journal of Agricultural Research* 56, 215–223.

<sup>3</sup>Youseif *et al* (2014) Phenotypic characteristics and genetic diversity of rhizobia nodulating soybean in Egyptian soils. *European Journal of Soil Biology* 60, 34-43.

## Results

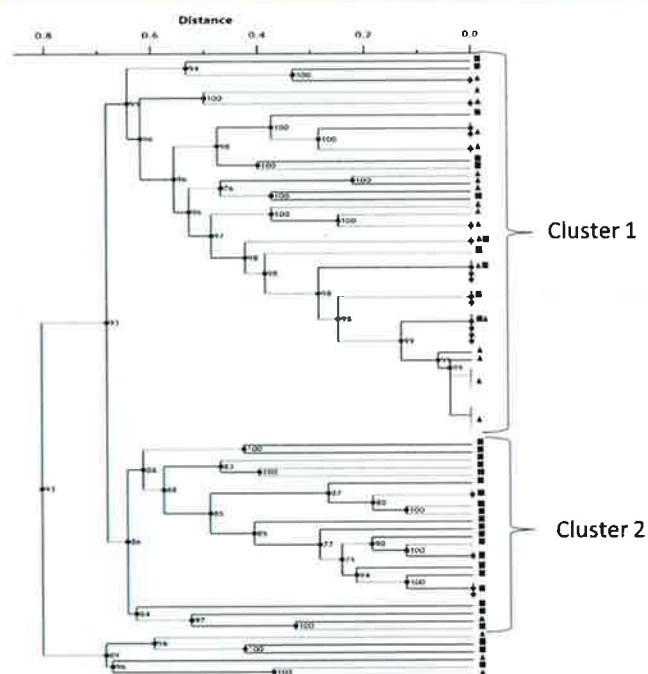


Figure 3 . Dendrogram (UPGMA) showing clustering of the 81 rhizobial isolates genotyped. Haresfoot trefoil (▲) and striated clover (■) annotated. Branches with both ▲ and ■ indicate rhizobial genotypes found in both legume species.

### Clusters:

- Cluster 1 – 68% are strains specific to striated clover.
- Cluster 2 – 96% are strains specific to haresfoot trefoil.

### Genotypes:

- Striated clover (n=22), haresfoot trefoil (n=34).
- Neither species colonised by a single dominant strain.
- Three genotypes common to both annual legumes.

## Conclusions

- Diverse genotypes of rhizobia in NZ pastures are capable of nodulating haresfoot trefoil and striated clover.
- These annual legumes select for different genotypes.
- The rhizobial populations are a natural resource that should be explored in future work to rank their N fixing abilities.