Identification and Specificity of Rhizobia in New Zealand

Bevan Weir

Supervised by: Susan Turner (Auckland University),
Warwick Silvester (Waikato University)
John Young (Landcare Research)

NZ Native Legumes

- Kowhai (*Sophora* spp.)
- Kaka beak (*Clianthus* sp.)
- NZ Broom (*Carmichaelia* spp.)
- Scree Pea (*Montigena* sp.)

Carmichaelia

Sophora

Clianthus

Montigena

Questions - Natives

- What rhizobia are present in NZ?
  - NZ split 80 mya, became isolated
  - Co-evolution between native legumes and rhizobia since that time
- How specific are the relationships that have formed since that time?
  - Does each legume species/genus have its own rhizobia or are the relationships more general

Woody Legume Weeds in NZ

1-2 million hectares, tens of millions $ pa.

- Gorse (*Ulex europaeus*)
- Broom (*Cytisus scoparius*)
- Wattles (*Acacia* spp.)

Gorse (*Ulex europaeus*)

Broom in Porters Pass, South Island

Acacia longifolia

Questions - Exotics

- Weed legumes were introduced
  - Present for less than 200 years
- How are the weeds able to nodulate?
- Three possibilities:
  - They have adapted to use native rhizobial populations
  - Effective rhizobia were co-introduced with the exotic legumes
  - Effective cosmopolitan rhizobial populations are present in New Zealand

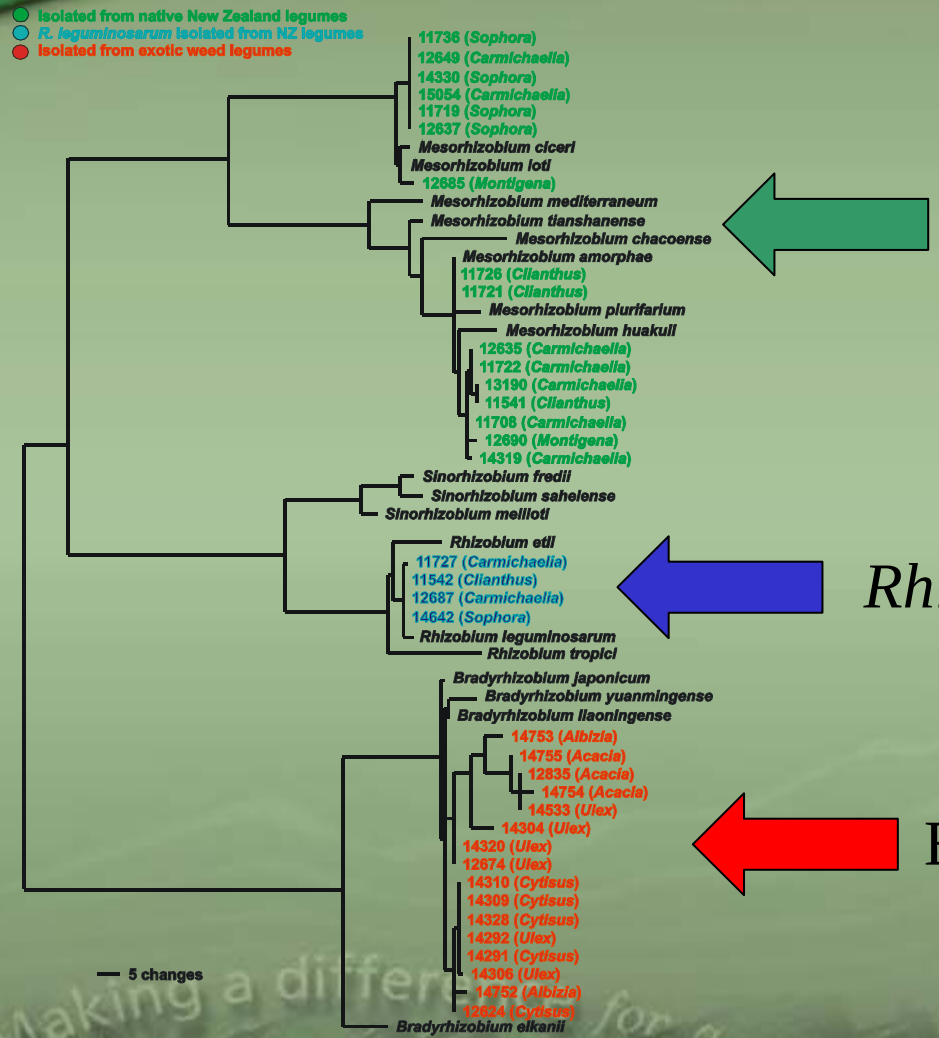
Research Strategy

- **Identification**
  - Multiple gene sequencing, phylogenetics
  - Biolog
  - Fatty acids
- **Specificity**
  - Nodulation gene
  - Host-range: Inoculate native and exotic legumes with rhizobial strains, acetylene reduction, nodule counting.

Phylogenetics

- Isolated 40 strains from root nodules
- Sequenced 5 genes
  - 16S, *recA*, *atpD*, *glnII*, *nodA*
- Built phylogenetic trees
  - NJ, ML, Bayesian
  - ProtTest, Modeltest

16S Phylogeny of NZ Rhizobia



Native legumes - *Mesorhizobium*

Rhizobium leguminosarum

Exotic legumes - *Bradyrhizobium*

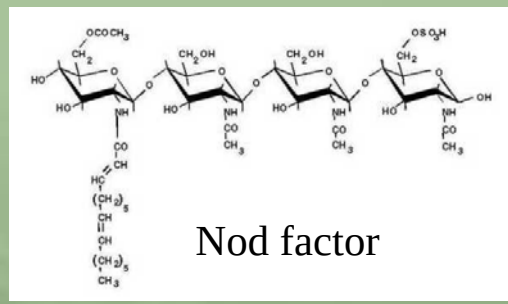
Making a difference for a truly clean green New Zealand

Manaaki Whenua
Landcare Research

Nodulation genes

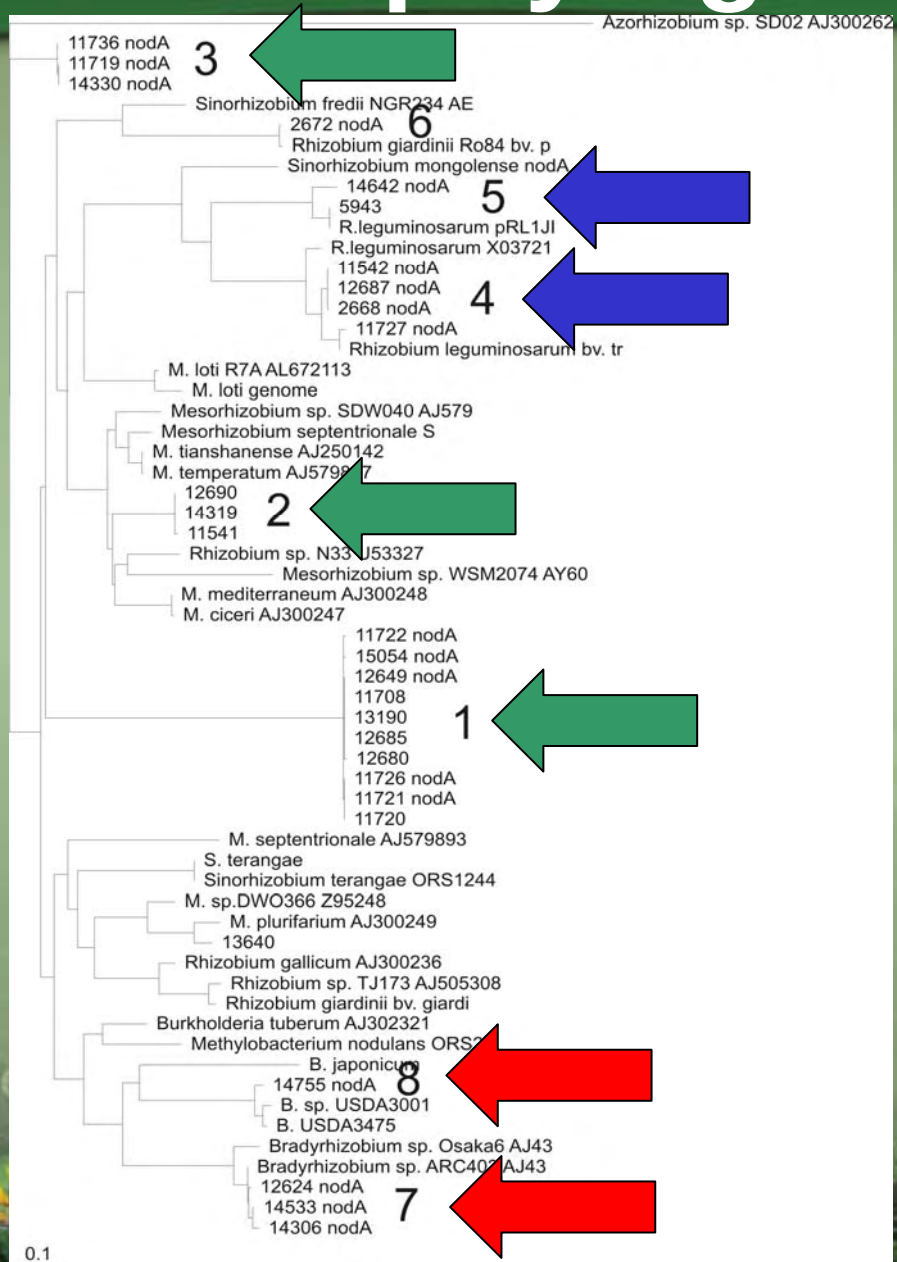
- Nod factor is a bacterial signal molecule that initiates nodule formation

- Nod factor "core" created by three genes:
  - ***nodA***, *nodB*, *nodC*
  - Some degree of relation to host range


Nod factor

- Sequenced *nodA:*
  - reveal evolutionary history of the gene, and
  - relation to specificity in NZ legumes

nodA phylogeny



nodA phylogeny

- Three novel *nodA* types in NZ *Mesorhizobium*
  - No correlation with house-keeping genes
    - Carried on transposable symbiosis island
  - No correlation with host legume
- *R. leguminosarum* strains have typical *nodA*
  - Haven't picked up *Mesorhizobium* symbiosis island
- *Bradyrhizobium nodA* similar to others found
  - Possibly host specific

Research Strategy

- **Identification**
  - Multiple gene sequencing, phylogeneti
  - Biolog (not shown)
  - Fatty acids (not shown)
- **Specificity**
  - Nodulation gene
  - Host-range: Inoculate plants with strains, acetylene reduction, nodule counting.

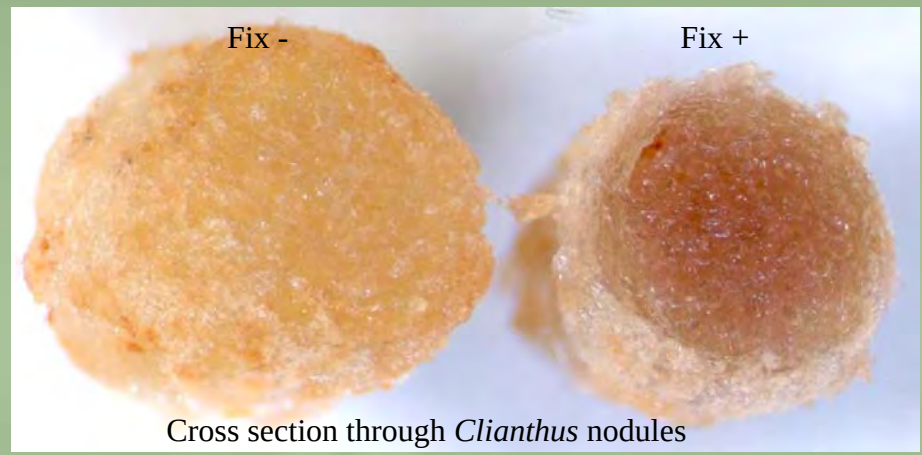
Research Objectives for Specificity

- Investigate symbiotic promiscuity of *Mesorhizobium* strains nodulating native NZ legumes.
- Investigate symbiotic promiscuity of *Bradyrhizobium* strains nodulating exotic weed legumes.
- Investigate interactions between *R. leguminosarum* and native NZ legumes.
- Verify the previously established division of *Mesorhizobium* nodulating natives, and *Bradyrhizobium* nodulation exotics.

Mesorhizobium – Native Legumes

- 10 *Mesorhizobium* strains

- 5 Native legumes
  - Two *Sophora*
  - Two *Carmichaelia*
  - *Clianthus*



Cross section through *Clianthus* nodules

- Five replicates, uninoculated controls, Full cross inoculation, vermiculite, acetylene reduction

Mesorhizobium – Native Legumes

Mesorhizobium strain	Genomic group	*nodA* type	nod/fix response on inoculated native legume				
			Sophora microphylla	*Sophora tetraptera*	*Carmichaelia australis*	*Carmichaelia stevensonii*	*Clianthus puniceus*
14330 (*Sophora*)	A	3	nod^+ fix^+	nod^+ fix^+	nod^+ fix^-	nod^+ fix^-	nod^+ fix^+
11719 (*Sophora*)	A	3	nod^+ fix^+	nod^+ fix^+	nod^+ fix^-	nod^+ fix^-	nod^+ fix^+
15054 (*Carmichaelia*)	A	1	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
12685 (*Montigena*)	B	1	nod^- fix^-	nod^- fix^-	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
11721 (*Clianthus*)	C	1	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
11726 (*Clianthus*)	C	1	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
11541 (*Clianthus*)	D	2	nod^+ fix^-	nod^+ fix^-	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
12680 (*Clianthus*)	D	1	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
12690 (*Montigena*)	D	2	nod^- fix^-	nod^- fix^-	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+
13190 (*Carmichaelia*)	D	1	nod^- fix^-	nod^- fix^-	nod^+ fix^+	nod^+ fix^+	nod^+ fix^+

Mesorhizobium – Native Legumes

- Generally broad nodulation
  - All nodulated *Clianthus* effectively
- Exceptions: *Sophora*
  - 3 strains unable to form nodules, 1 ineffective
  - 2 strains isolated from *Sophora* ineffective on *Carmichaelia*
- No clear correlation to genomic or *nodA* groups
- Just how broad is the symbiosis? …

Mesorhizobium – Exotic Legumes

- Broad nodulation in natives
  - Can they nodulate other legumes?
- Best possible targets:
- Chick Pea (*Cicer*), Milk Vetch (*Astralagus*), *Lotus*, other *Sophora*

- Inoculated four exotic legumes with seven *Mesorhizobium* strains

Mesorhizobium – Exotic Legumes

Mesorhizobium strain	Genomic group	*nodA* type	nod/fix response on inoculated legume species			
			Astragalus membranaceus	*Lotus tetragonolobus*	*Cicer arietinum*	*Styphnolobium japonicum*
14330 (*Sophora*)	A	3	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-
15054 (*Carmichaelia*)	A	1	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-
11719 (*Sophora*)	A	3	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-
12685 (*Montigena*)	B	1	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-
11726 (*Clianthus*)	C	1	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-
11541 (*Clianthus*)	D	2	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-
13190 (*Carmichaelia*)	D	1	nod^+ fix^+	nod^- fix^-	nod^- fix^-	nod^- fix^-

Mesorhizobium – Exotic Legumes

- Only *Astragalus* nodulated
  - No correlation to genomic or *nodA* type

- Wagstaff et al. (1999): *Astragalus*, *Clianthus*, *Carmichaelia* are closely related.
  - *Lessertia*, *Sutherlandia*, *Swainsona*, *Colutea* [Galegeae]
  - NZ *Mesorhizobium* nodulate the Galegeae tribe and related

- Interesting that some strains nodulated exotic *Astragalus*, but not some native legumes

Rhizobium leguminosarum

- Four isolates identified as *R. leguminosarum*
  - Thought to nodulate peas, bean, clover
  - Host range extended through GM
- Hypothesis is that they are contaminants
  - Clover innoc. Widely used
- Tested the four isolates, plus one each of the three biovars: *trifolii*, *phaseoli*, *viceae*
- Inoculated 3 native legumes, and three typical hosts (pea, bean, clover)

Rhizobium leguminosarum

Rhizobium leguminosarum strain	*nodA* type	nod/fix response on inoculated legume species					
		Sophora microphylla	*Carmichaelia australis*	*Clianthus puniceus*	*Trifolium repens*	*Phaseolus vulgaris*	*Pisum sativum*
11542 (*Clianthus*)	4	nod^- fix^-	nod^+ fix^-	nod^+ fix^-	nod^- fix^-	nod^+ fix^+	nod^+ $fix^\pm$
11727 (*Carmichaelia*)	4	nod^- fix^-	nod^+ fix^-	nod^+ fix^-	nod^+ fix^+	nod^+ fix^+	nod^+ $fix^\pm$
12687 (*Carmichaelia*)	4	nod^- fix^-	nod^+ fix^-	nod^+ fix^-	nod^- fix^-	nod^+ fix^+	nod^+ $fix^\pm$
14642 (*Sophora*)	5	nod^+ fix^-	$nod^\pm$ fix^-	nod^+ fix^-	nod^- fix^-	nod^+ fix^-	nod^+ $fix^\pm$
2668 (*trifolii*)	4	nod^- fix^-	nod^+ fix^-	nod^+ fix^-	nod^+ fix^+	nod^+ fix^-	nod^+ fix^+
2672 (*phaseoli*)	6	nod^- fix^-	nod^- fix^-	nod^- fix^-	nod^- fix^-	nod^+ fix^+	nod^+ $fix^\pm$
5943 (*viceae*)	5	nod^- fix^-	nod^- fix^-	$nod^\pm$ fix^-	nod^+ fix^-	nod^- fix^-	nod^+ fix^+

Rhizobium leguminosarum

- *R. leguminosarum* strains nodulate NZ legumes but ineffectively
- *R. leguminosarum* from natives nodulated pea, bean and clover effectively
- Probably typical of most *R. leguminosarum* strains
  - Novel host range expansion


Nodulation in Pea

Bradyrhizobium - Exotic Legumes

- 4 *Bradyrhizobium* strains, isolated from *Acacia*, gorse, and broom

- 3 exotic legumes:
  - *Acacia*, gorse, and broom

- Full cross inoculation

Nodulation in Broom

Bradyrhizobium - Exotic Legumes

Bradyrhizobium strain	Genomic group	*nodA* type	nod/fix response		
			Cytisus scoparius	*Ulex europaeus*	*Acacia longifolia*
12674 (*Ulex*)	I	7	nod^+ fix^+	nod^+ fix^+	nod^+ fix^-
14291 (*Cytisus*)	J	7	nod^+ fix^+	nod^+ fix^+	nod^+ fix^-
14533 (*Ulex*)	G	7	nod^+ fix^+	nod^+ fix^+	nod^+ fix^-
14755 (*Acacia*)	G	8	nod^+ fix^+	nod^+ $fix^{\pm}$	nod^+ fix^+

Bradyrhizobium - Exotic Legumes

- Broad nodulation
  - All strains nodulated all legumes
- Gorse and Broom isolates ineffective on *Acacia*
  - Correlation to *nodA* gene

Conclusions

- The native legumes are effectively nodulated by diverse *Mesorhizobium* populations that have a host range encompassing all of the native legumes (and other members of the Galegeae).

- The native legumes are also nodulated — albeit ineffectively — by some strains of *Rhizobium leguminosarum.*

Conclusions

- The exotic weed legumes: Broom, Gorse, *Acacia* are effectively nodulated by diverse Bradyrhizobium species, and can cross nodulate.

- Confirmed that *Bradyrhizobium* strains do not nodulate the native legumes, and *Mesorhizobium* do not nodulate exotic weed legumes

Origins of Bradyrhizobia?

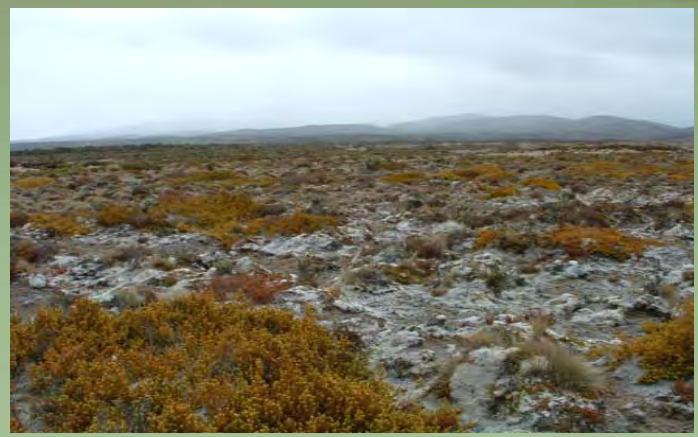
- Three possibilities of nodulating exotic weeds:
  - They have adapted to use native rhizobial populations
  - Effective rhizobia were co-introduced with the exotic legumes
  - Effective cosmopolitan rhizobial populations are present in New Zealand

- If introduced: would expect a different distribution in pristine soils than disturbed soils

- Investigated by baiting NZ soils with trap hosts…

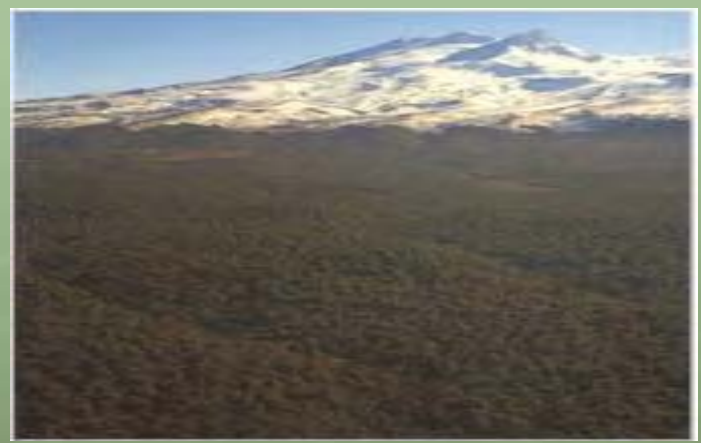
Sample Regions

Urewera



Rangipo Desert - Ruapehu

Tussock land - Tongariro

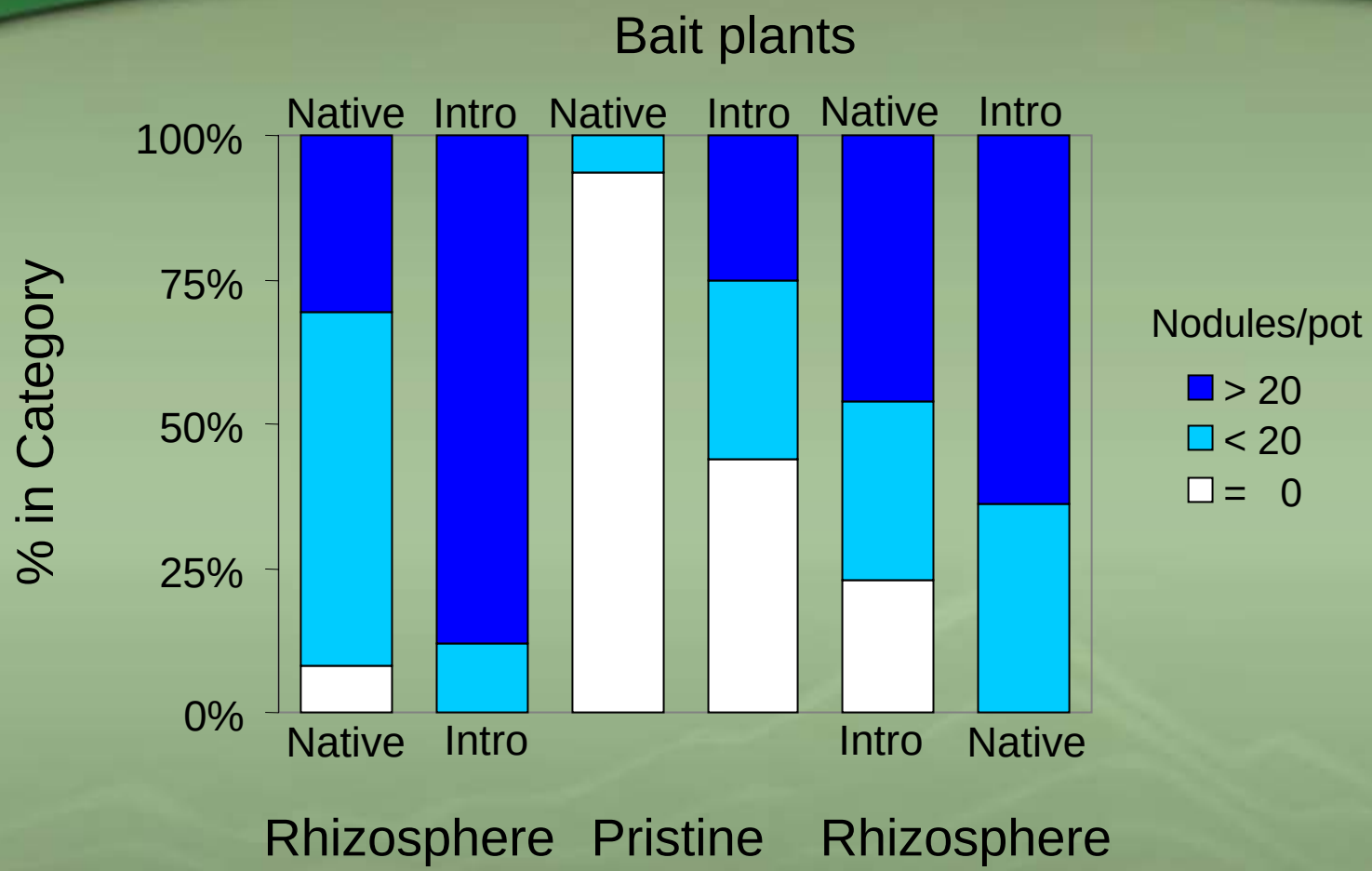


Forest - Ruapehu

Soil Investigations

- Soil samples were collected from:
  - *Carmichaelia* and *Sophora* rhizospheres
  - Broom and gorse rhizospheres
  - pristine soils (legumes not in sight)
- Soils into pots, then planted with native and introduced legumes
- Examined for nodulation after 16 weeks
- Rhizobia isolated and identified (sequencing)

Results


Bait plants
Native
Intro
Native
Intro
Native
Intro
100%
75%
50%
25%
0%
% in Category
Native
Intro
Intro
Native
Rhizosphere
Pristine
Rhizosphere
Nodules/pot
> 20
< 20
= 0

Summary

- Little or no nodulation occurs when native legumes are sown in pristine soils

  - consistent with earlier reports (Greenwood 1978; Sullivan & Ronson 1997); Mesorhizobia are present in soil but do not nodulate - perhaps because they lack the symbiosis island

- Nodulation of introduced legumes readily occurs when sown in pristine soils

  - consistent with a concept of cospolitain Bradyrhizobia nodulating introduced legumes

More Information

- Web: **http://www.rhizobia.co.nz**
- Email: info@rhizobia.co.nz
- Weir *et. al*. (2004) AEM 70(10): 5980-5987

Biosecurity / Restoration

- Endangered native *Clianthus* forms effective symbiosis with all *Mesorhizobium* spp.
- But… also forms ineffective nodules with *R. leguminosarum*, could compete.
- Need to think about “contaminating” national parks with foreign rhizobia