

A New Zealand Research Update



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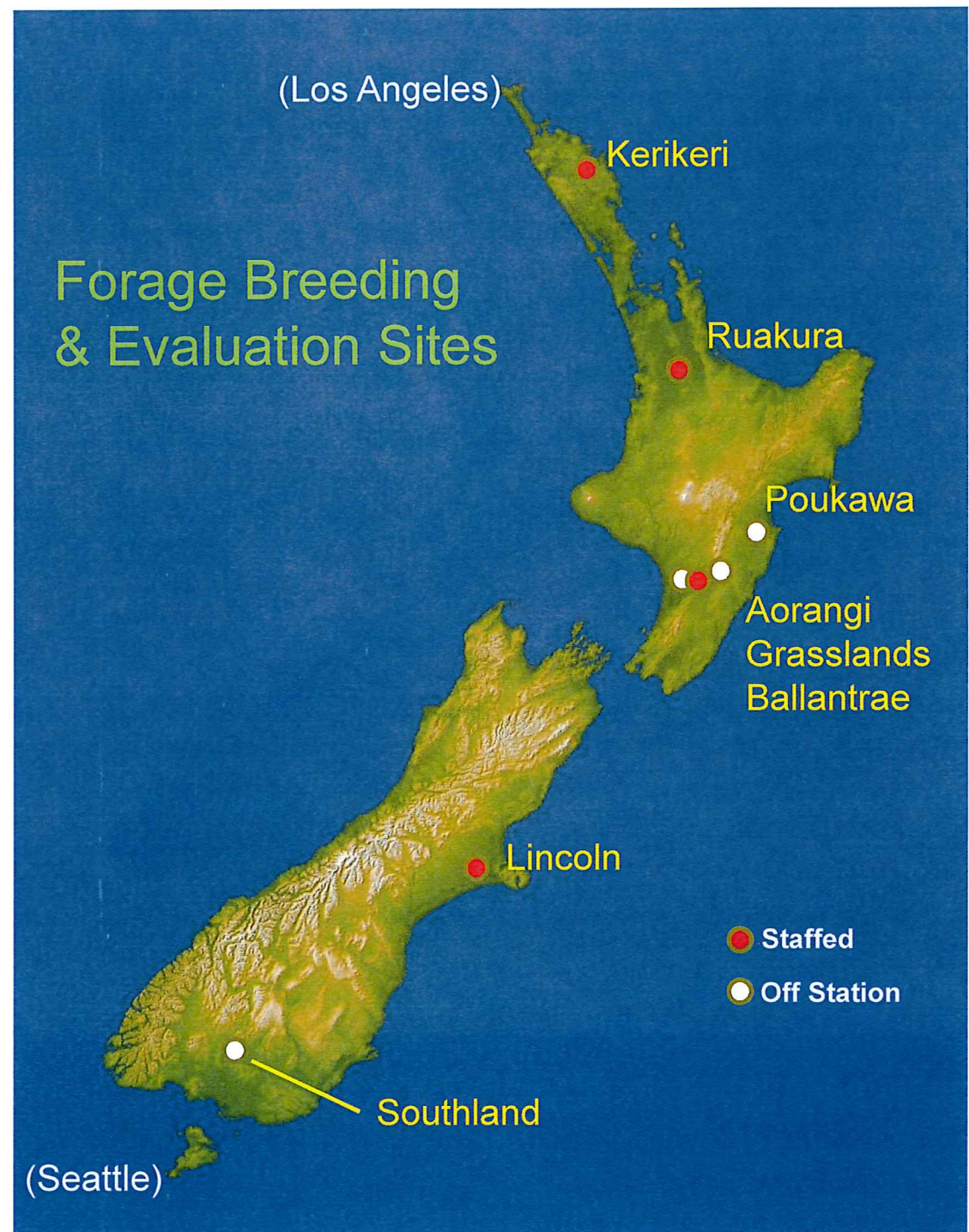
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New Zealand Pastoral Agriculture

- 50% of land area in pastoral farming
- 50% of export \$s
- Grasses, Legumes, Herbs
- Growth in dairy, plantain, alfalfa, endophytes
- Grazing!

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Forage Agronomy



Decision Support For Farmers & Breeders

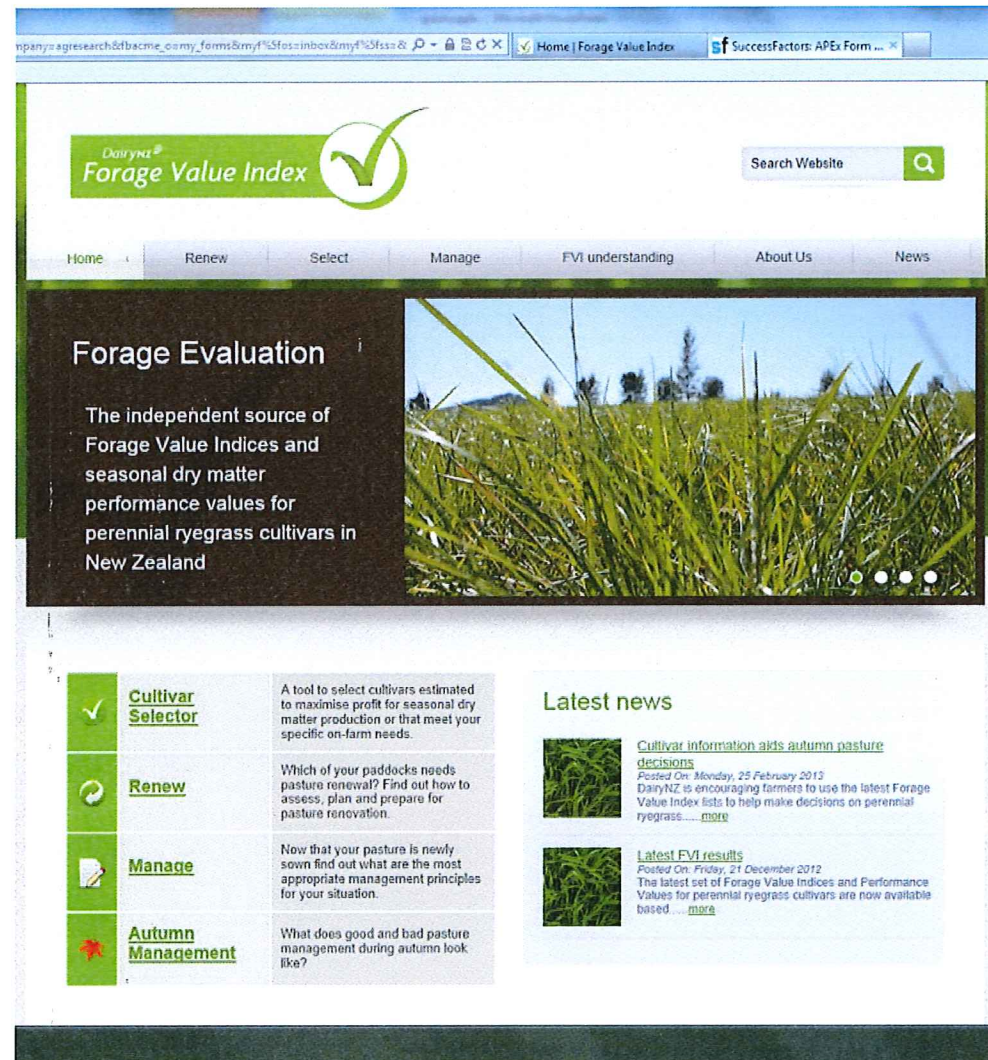
Forage Value Index

Standards & Benchmarking

Focus on Farm Profitability

Underpinning Research

Information to Farmers





Forage Value: Research Scope

- Can we build a comprehensive, scientifically robust FVI that inspires farmer confidence and adoption?
 - That incorporates productivity and environmental traits
- Science stretch
 - Pasture ecophysiology to whole farm system including economics
 - Multiple traits and interactions
 - Fundamental knowledge gaps regarding phenotypic variation and its control



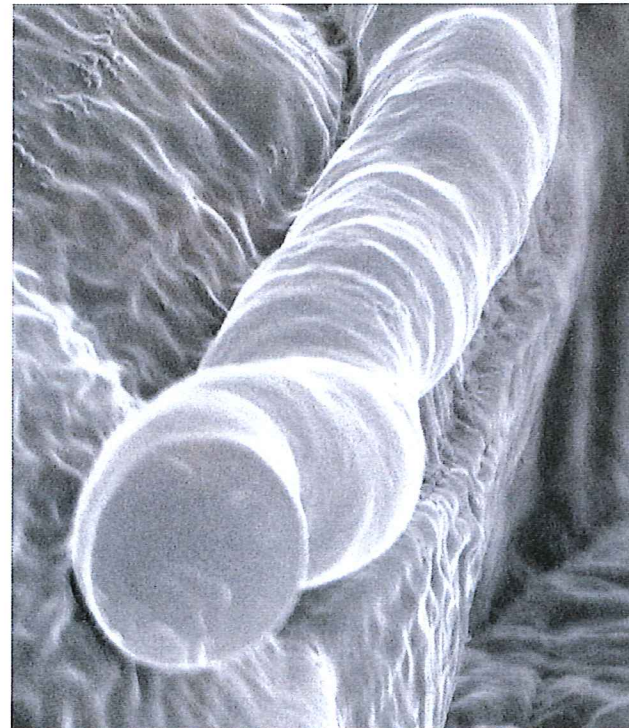
Forage Value: Research Activities

- How?
 - Accurately define key productivity traits and how to measure them
 - Quantify variability among cultivars for these traits
 - Account for interactions between
 - Genotype and environment
 - Pasture species (grass/clover)
 - Plants and animals
 - Conduct a rigorous 'strain' trial (actually, a systems test of the FVI)
 - Establish an on-farm cultivar proving system (long-term resource)
 - Incorporate other species (beyond ryegrass)
 - Derive economic (EV) and performance (PV) values for environmentally-related traits e.g. potential N emissions

FORAGE VALUE INDEX – ISSUES & OPPORTUNITIES

1. Trait Definition, Priority & Measurement
2. Breeding Targets & Evaluation Systems
3. Rate of Genetic Gain Realised at *Pasture* Level
4. Cultivar Performance Information for Farmers
5. Verification & Maintenance of the Forage Value Index
6. Potential Game Changer for Australasian Pastoral Agriculture & Forage Research

Ryegrass::Endophyte Research

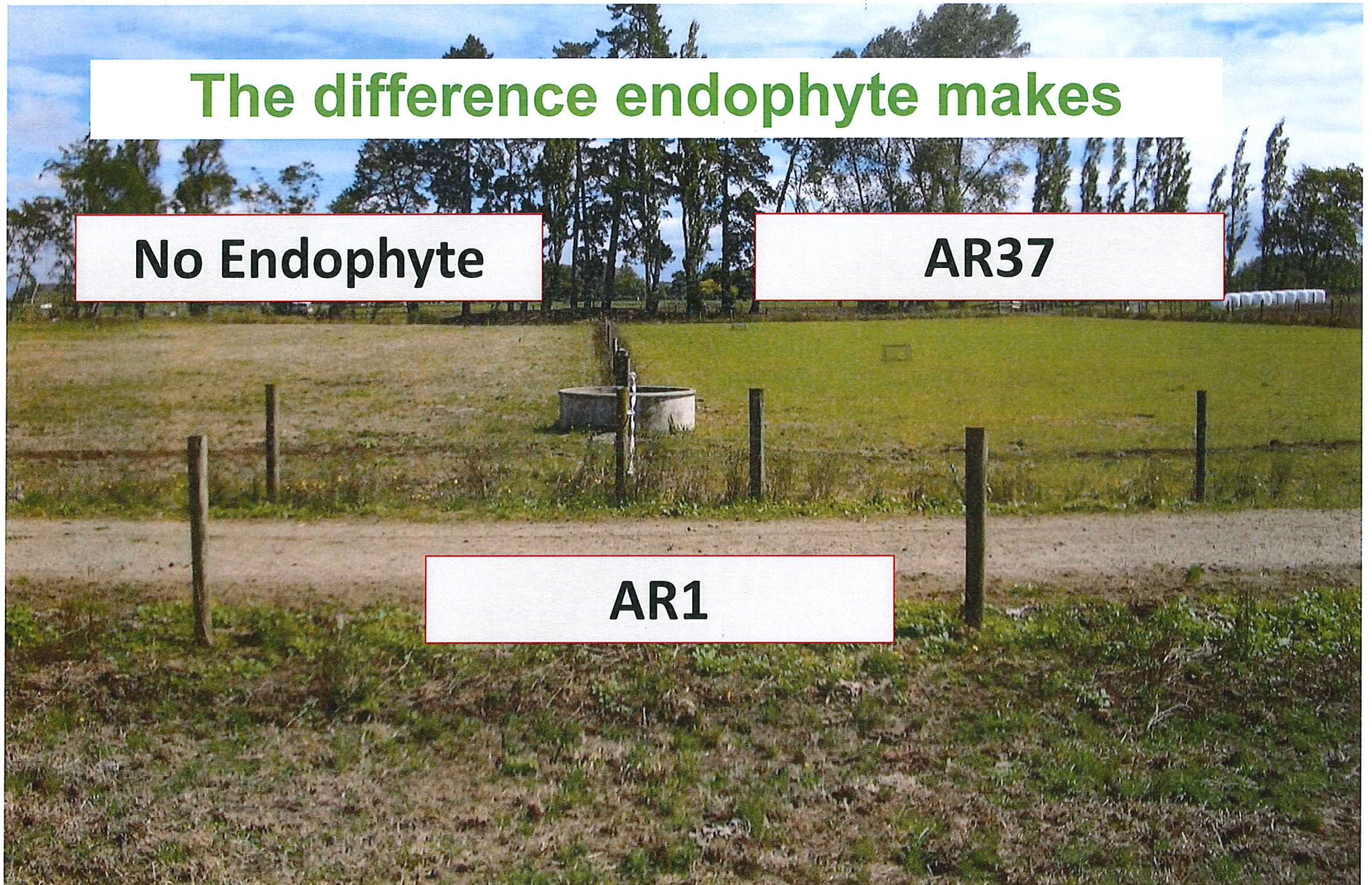


The difference endophyte makes

No Endophyte

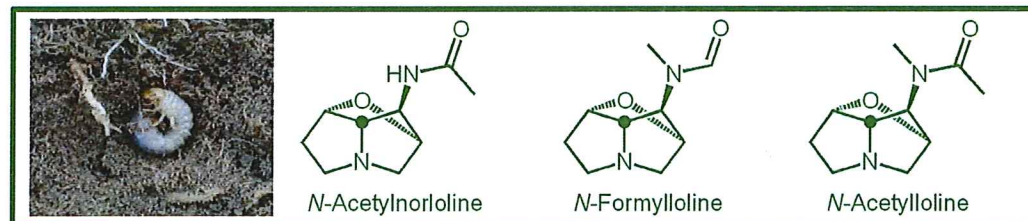
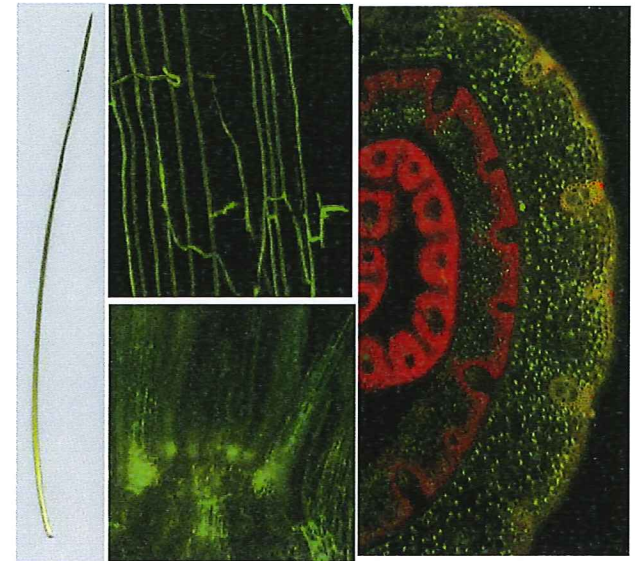
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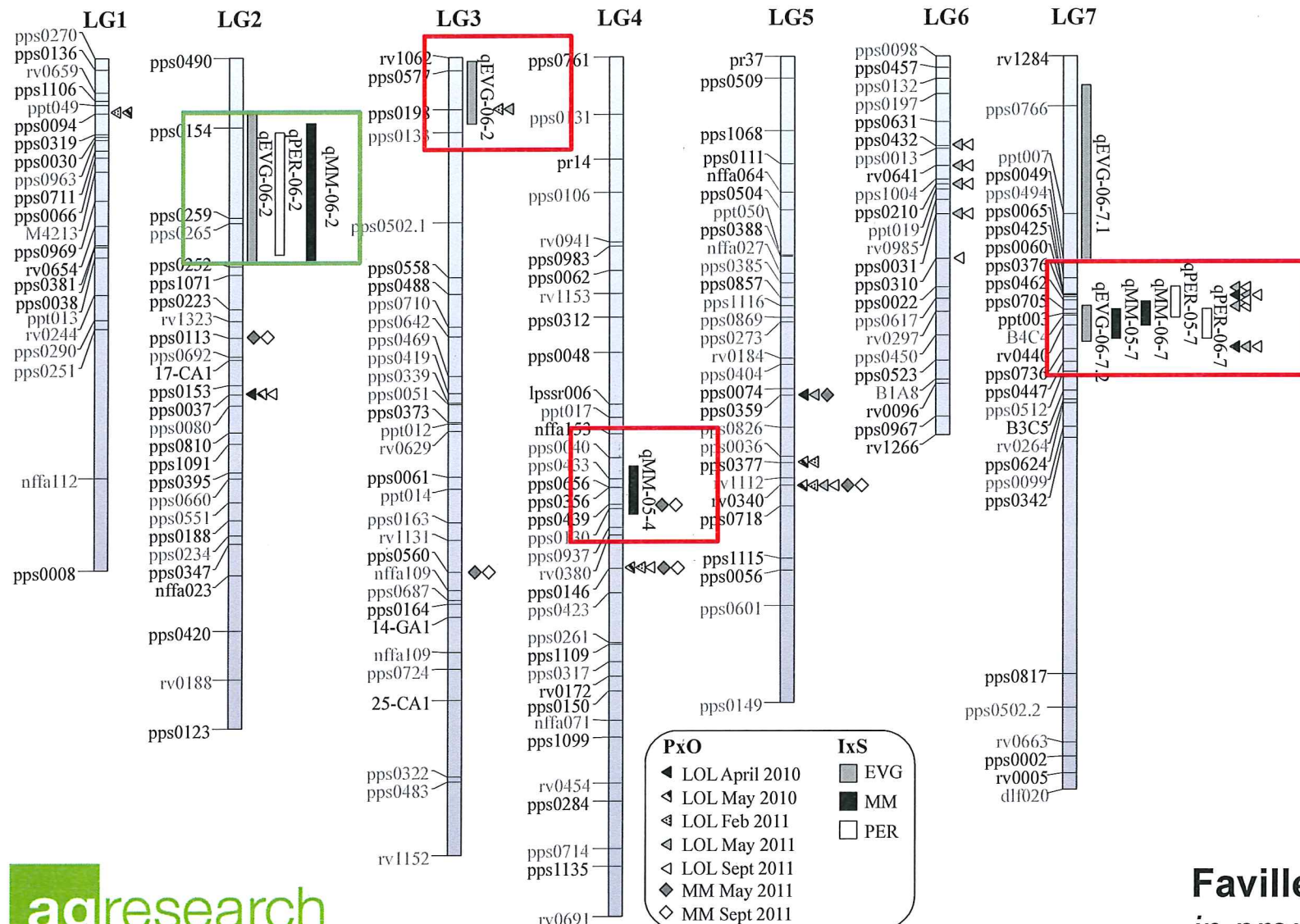


ENDOPHYTE SCIENCE PROGRAMMES

- Discovery of new naturally occurring endophytes
- Non-pastoral use of endophytes
- Understanding of marketed novel endophytes
- Food safety
- Seed transmission and storage
- Discovery of new bioactives
- Loline research
- Regulation of secondary metabolism
- Compatibility of host-endophyte interaction
- Colonisation & endophyte growth in host

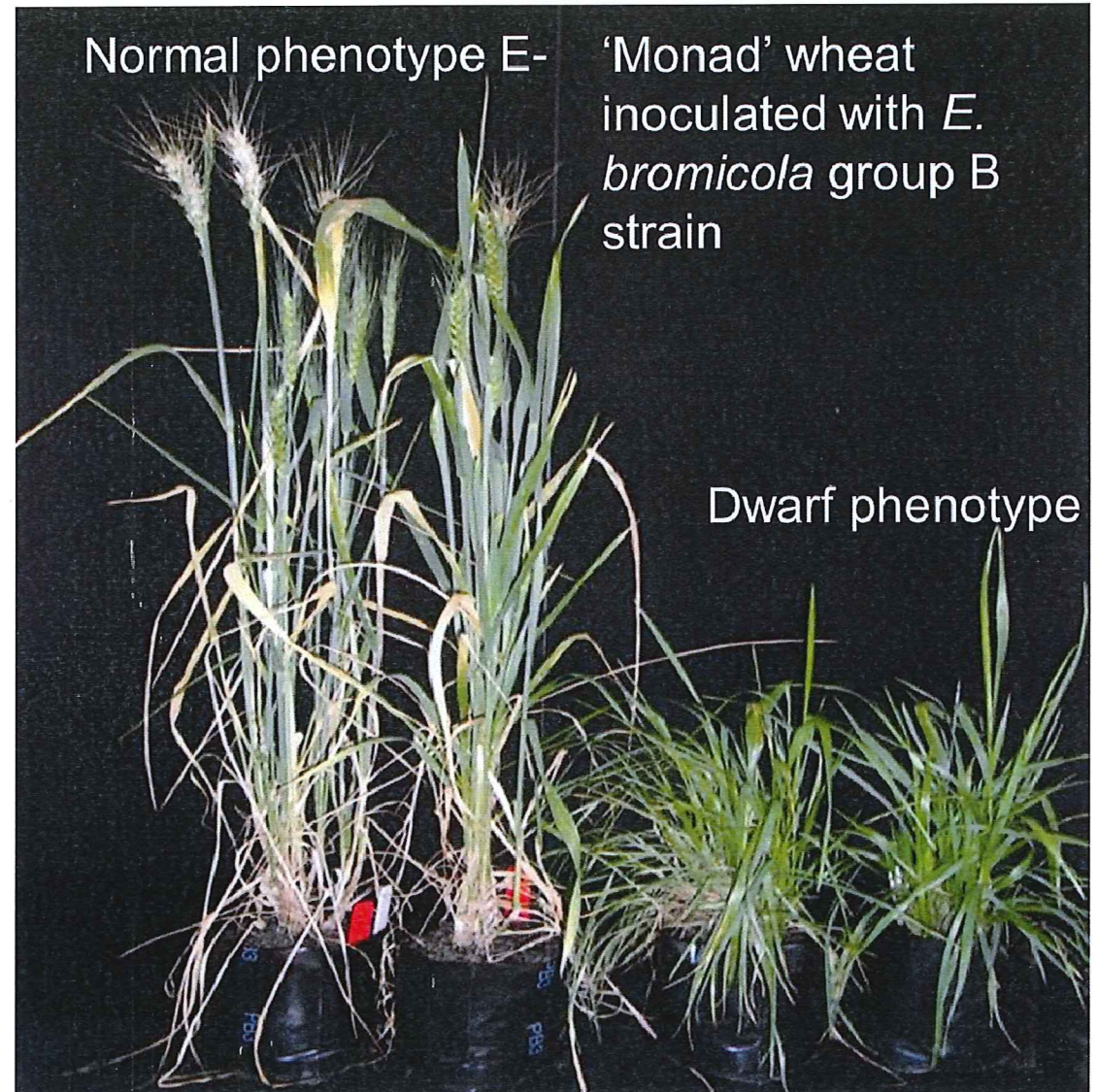


HOST GENETIC INFLUENCE ON ENDOPHYTE TRAITS



CEREAL ENDOPHYTE

- Insect Resistance
- Abiotic Stress
- Food Safety
- Challenging Science!

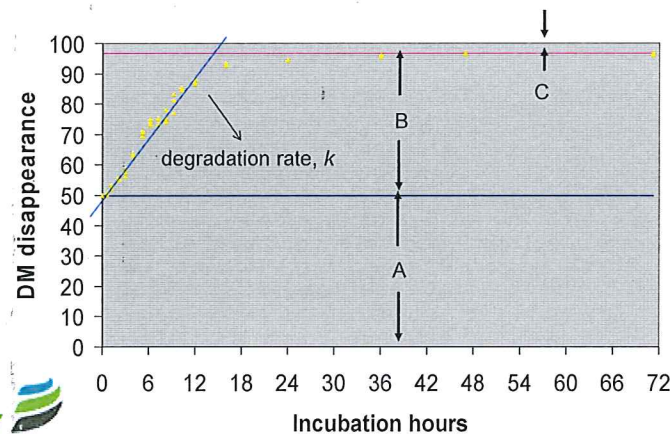


IMPROVING RYEGRASS DEGRADATION RATES

Degradation rate of fibre in the rumen limits passage through the gastrointestinal tract, constraining voluntary feed intake

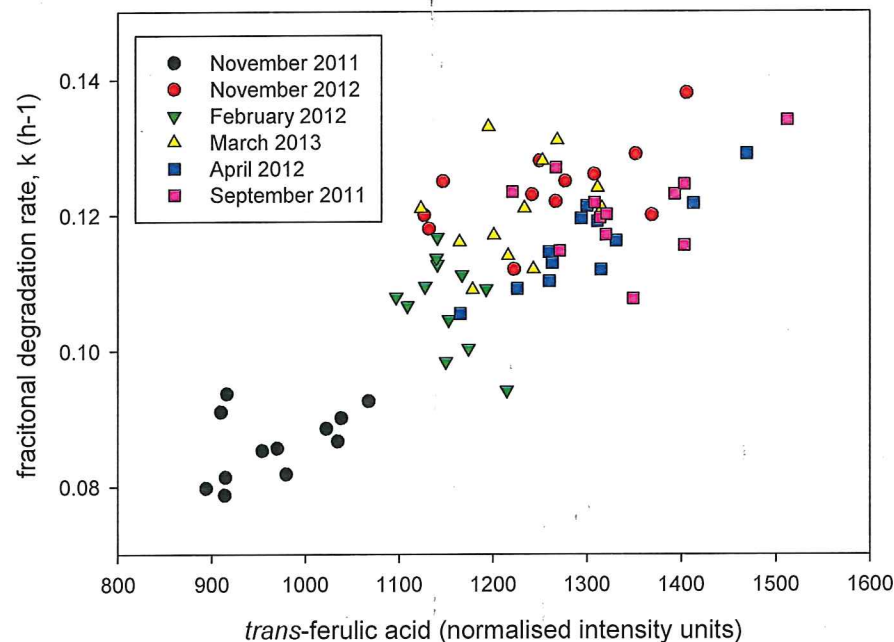
In sacco incubation technique used to accurately estimate ruminal degradation rate (time course of dry matter disappearance in rumen)

Too expensive and labour intensive for routine breeding application



IMPROVING RATE OF RUMINAL DEGRADATION

Selecting for fibre composition (hydroxycinnamic acids) to improve *in sacco* ruminal degradation rate in perennial ryegrass



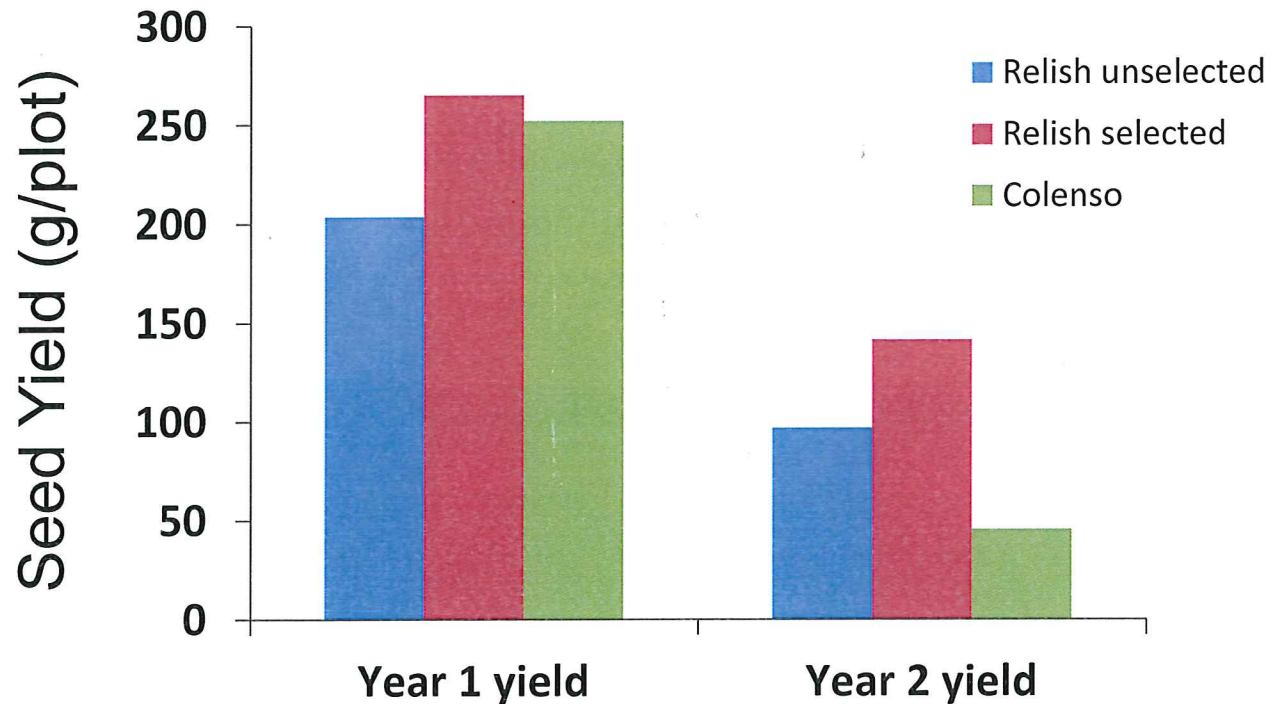
Trans-ferulic acid released by mild alkaline treatment positively correlated with fractional degradation rate of ryegrass leaves

Clover Research

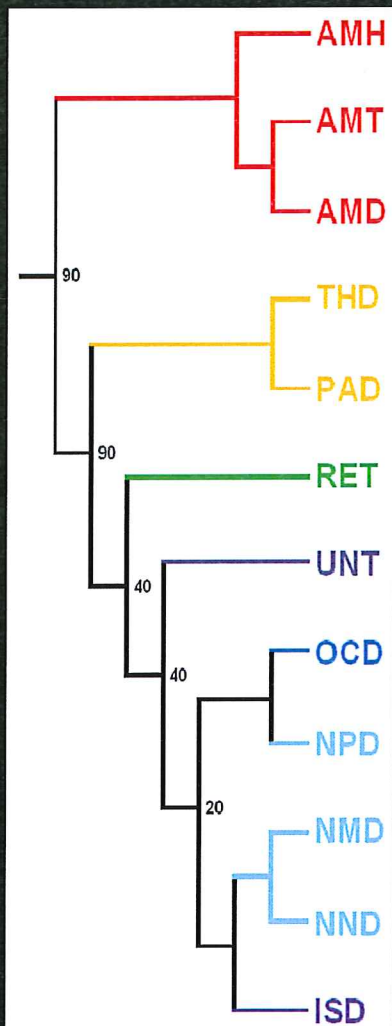


RED CLOVER SEED YIELD IMPROVEMENT

Single Cycle of Selection, c. 25% Improvement in Seed Yield

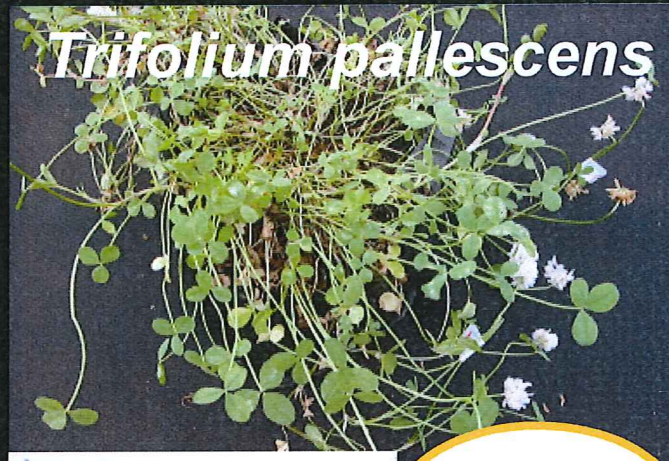


WHITE CLOVER'S RELATIVES



	AAAAAA		
<i>T. ambiguum</i>	AAAA	rhizomes	altitudinal series root depth
	AA		
<i>T. thalii</i>	TT		seedling vigour
<i>T. pallescens</i>	PP		prolific flowering
<i>T. repens</i>	RRR'R' PrPr O'O'	stolons	most productive
<i>T. uniflorum</i>	UUUU		taproot, thick leaves
<i>T. occidentale</i>	OO		salt and drought
<i>T. nigrescens petrisavii</i>	N ^p N ^p		long flowering
<i>T. nig.meneghinianum</i>	N ^m N ^m		upright (2 m)
<i>T. nigrescens nigrescens</i>	N ⁿ N ⁿ	annuals	prolific flowering
<i>T. isthmocarpum</i>	II		Salt tolerant

Trifolium repens is a natural allopolloid $R R R'R'$ or $P^r P^r O^r O^r$



$pp\ pp$



X



$P^r P^r$
 $O^r O^r$

Trifolium occidentale



$O^o O^o$



Trifolium uniflorum

Small

Little known

Wild clover

Greece

Rocky soils



T. repens × *T. uniflorum* hybrid drought response

Effects on growth and senescence



Hybrid plant

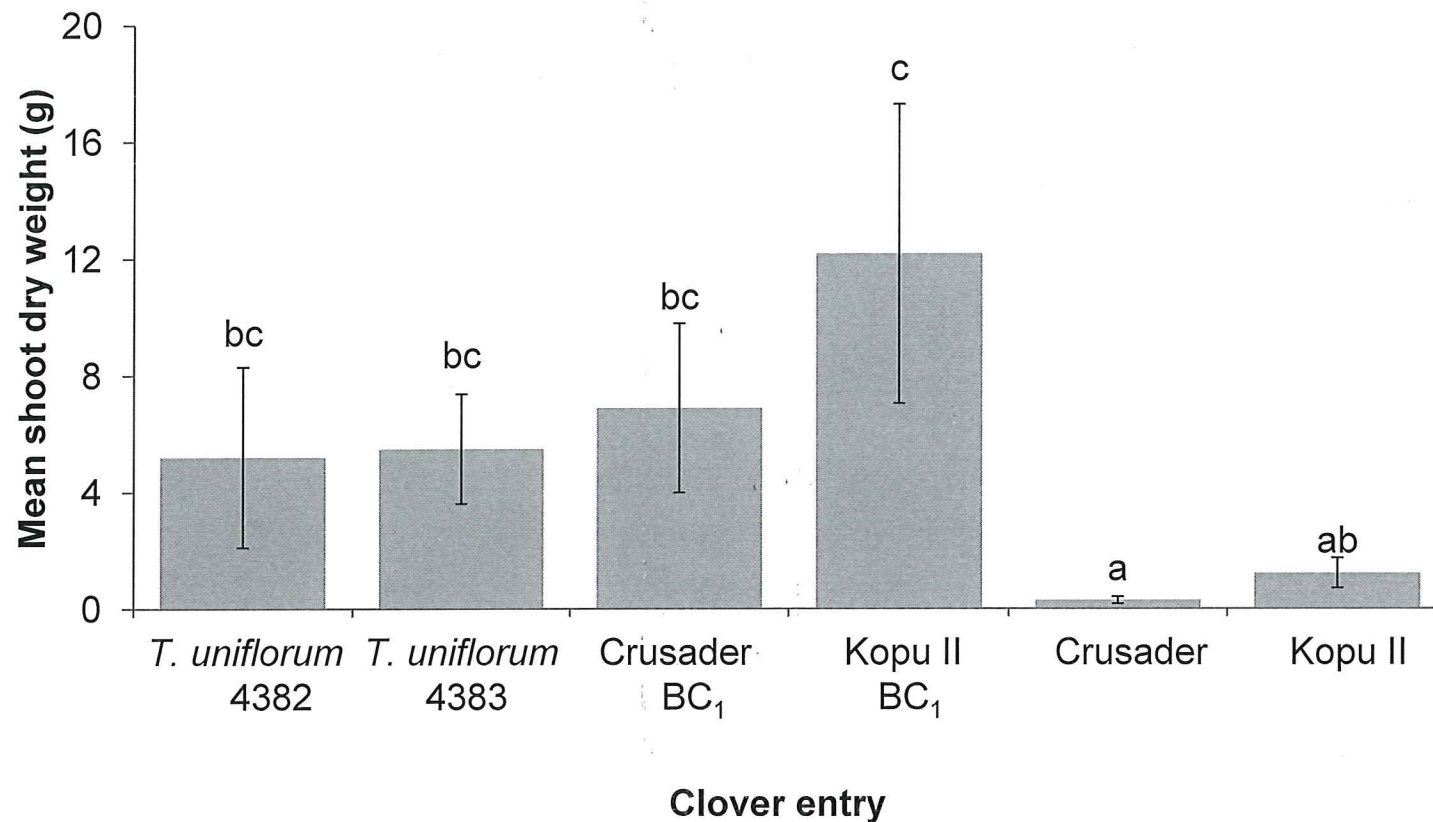


White clover parent

Maintenance of photosynthesis & water uptake

T. repens x *T. uniflorum* response to low external P

8 months growth in sand culture



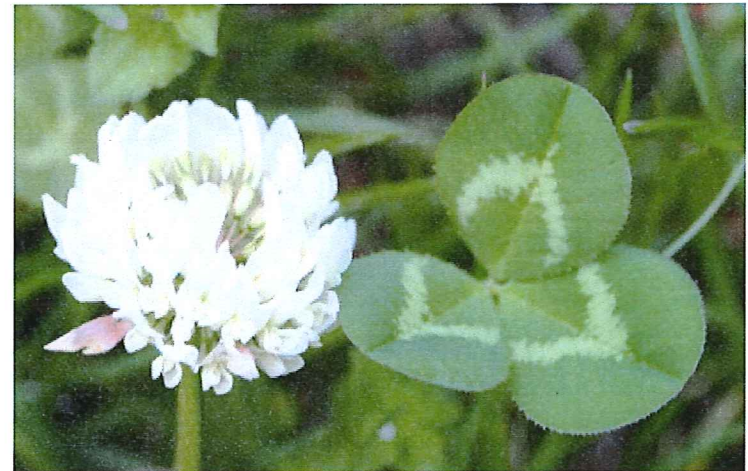
TEASING APART WHITE CLOVER: *DE NOVO* GENOME SEQUENCING

ANDREW GRIFFITHS

ROGER MORAGA

STIG ANDERSEN

ANAR KHAN



WHITE CLOVER – ALLOTETRAPLOID – AN ISSUE

Trifolium repens

- Allotetraploid ($2n=4x=32$)
- Functional diploid (disomic)
- Allogamous



Issues

- Polyploidy and Homoeologue Similarity (impedes assignment of sequence to contigs and contigs to correct homoeologue)
- Heterozygosity (impedes assembly)

Solutions

- Polyploidy and Homoeologue Similarity
 - Sequence progenitors (*T. occidentale*; *T. pallescens*) to provide a filter to assign sequence to contigs and contigs to homoeologues
- Heterozygosity
 - sequence inbred white clover genotype
 - (S₉ 99.8% inbred, Crau-derived)



SEQUENCING PROGRESS

Data Sources

- 454, Illumina, Illumina TruSeq Synthetic Long-Read (Moleculo)
- RNA sequencing from Floral, Root, Leaf and Stem/Stolon for genome annotation

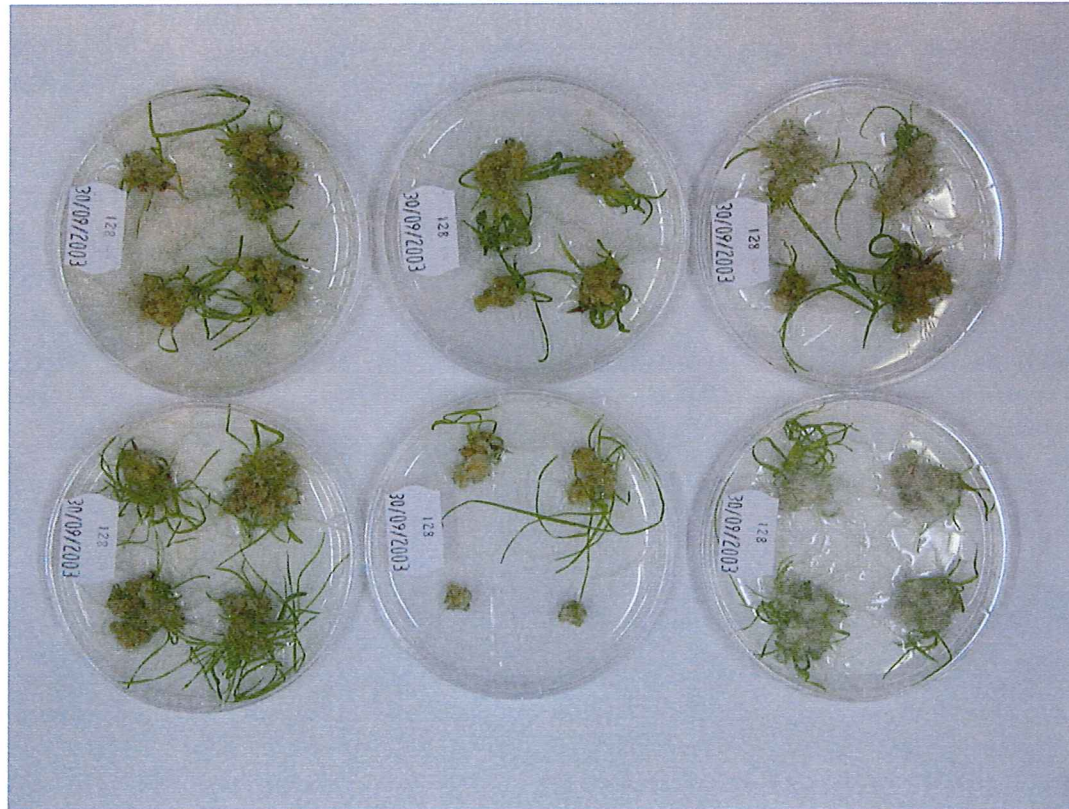
Current Assemblies

	T. repens		T. occidentale		T. pallescens	
N-Value	Contig Size (bp)	No. contigs	Contig Size (bp)	No. contigs	Contig Size (bp)	No. contigs
N10	403,668	114	604,483	49	631,290	36
N50	139,620	1,394	97,122	624	81,662	562
Total		13,957		7,231		6,925
Assembly Size	698.6 Mb (70%)		443.1 Mb (89%)		391.5 Mb (78%)	

Annotation

- *T. occidentale* genome locked and annotated (Aarhus University)
- *T. pallescens* genome locked and annotation underway (Aarhus University)
- *T. repens* assembly and homoeologue assignment under way prior to locking

Forage Biotechnology



Condensed Tannins → White Clover & Alfalfa

T. arvense

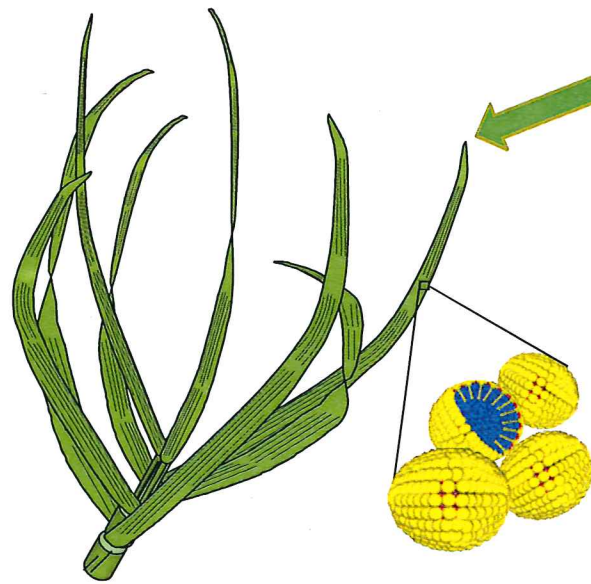


T. repens



HIGH METABOLISABLE ENERGY RYEGRASS

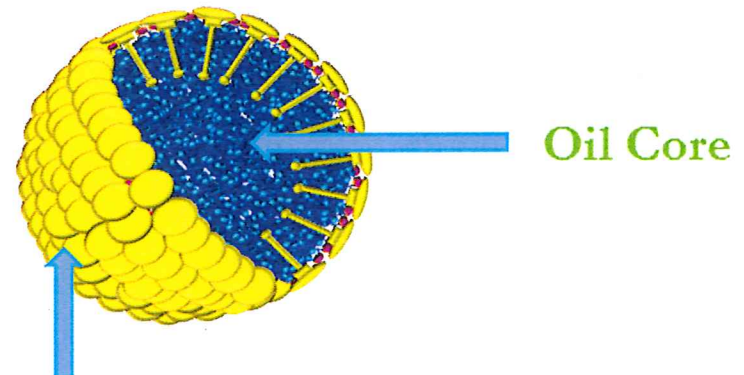
Cysteine-Oleosin & DGAT1 coexpressed in leaves



Perennial Ryegrass

Target Species - Forages

- In model species (*Arabidopsis thaliana*)
1. Accumulate oil in leaves (40-fold increase)
 2. Elevate photosynthesis (24% increase)
 3. Higher biomass (up to 50%)



Outer Cysteine Oleosin Layer

LOOKING AHEAD

Research Directions

- Less: plant functional genomics
- More: breeding strategies, wide crosses, genetic resources, endophytes, GMOs; substantive collaborative approaches
- Recently Funded Projects: GBS, Clover:Rhizobia, Seed Science
- Proposed Projects: Genomic Selection, *Trifolium* Introgression, *Lolium* Association Genetics & Allele Mining

New Zealand Forage Science Capability Needs

- Quantitative Genetics
- Field Breeding & Field-Based High Throughput Phenotyping
- Pasture & Plant Eco-Physiology
- Species Experts



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Forage Value Index

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