Legume Inoculation Principles & Developments

Floyd Sullivan, Business Development

ALOSCA Agronomy

Introduction

- Various Rhizobia Carrier systems
- Mechanics of legume inoculation
- Common factors influencing Rhizobium populations & efficacy
  - pH plus **aluminium toxicity**
  - Desiccation
  - Herbicides
- Developments
  - New strains
  - New methods

Company profile

- Privately owned, WA based developer, manufacturer and marketer of temperate legume inoculants.
- Focused on the development of inoculant carrier technologies.
- ALOSCA clay carrier system discovered 2000-01 company started trading in 2004.
- Supplier of granular, freeze dried and peat based legume inoculants to reseller networks.
- Current market: Domestic and export Mediterranean and temperate cropping and pasture zones.

Rhizobia are bacteria that live in the soil, on plant roots and in legume nodules.

- **Also known as *root nodule bacteria:***
- **Form root nodules on legume plants:**
- **Rhizobia absorb carbohydrate from the plant & in return fix atmospheric nitrogen for use by the plant:**

Focus on inoculant carrier systems

Peat based	Freeze dried	Granular
		
Older technology, slurry on to seed budget product. Wet soil product.	In-furrow liquid inject or slurry on to seed. Wet soil product, vulnerable in drying conditions.	Highly protective, versatile. In-Furrow moist or dry seeding. Mix with seed or fertiliser.

Focus on inoculant carrier systems - peat

Peat based

Older technology 60 years old, slurry on to seed budget product. **Wet soil product**.

Key usage points.

- Ensure adhesive solutions are cool before adding the inoculant.
- Best sown on the day of inoculation to maximize live rhizobia delivered with the seed to the soil.
- Highly effective when sowing seed into moist soil.
- Aerial or dry sowing peat-inoculated seed should be avoided.
- Use clean, potable water and clean containers for mixing.
- Consider doubling rates of application if drying seedbed conditions are expected or the furrow environment exposes the inoculant to armful pesticides.

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Focus on inoculant carrier systems - FD

Freeze dried

In-furrow liquid inject or slurry on to seed.
Wet soil product, vulnerable in drying conditions.

Key usage points.

- Compact 2-part product, 30mL vial (treats 500kg coarse grain) + 100g sachet of Protecting Agent.
- Fully soluble, will not block mesh or orifice plates in liquid kit setups.
- Only minimal agitation required to avoid settling.
- Deliver injection streams onto or very close to seed.
- Minimum water rate 50L/ha, higher rates will deliver a better result particularly if there is some separation from the seed.
- Best sown **within 5 hours** if slurried on to the seed.
- Avoid mixing with pesticides and fertilisers.
- Do not freeze the vials.

Focus on inoculant carrier systems - FD

Freeze dried

In-furrow liquid inject or slurry on to seed.
Wet soil product, vulnerable in drying conditions.

If mixing… Read Instructions:

- Avoid low pH solutions.
- Avoid TEs that are toxic to Rhizobium. Zn, Cu, Mn and some forms of Mo.
- Avoid starter N in the solution.
- If mixing use the mixture as soon as possible, do not leave to stand for any length of time. Do not store overnight.
- Consider doubling rate of application when tank mixing.
- Mix order :
  1. Water (2-3L) for concentrate.
  2. Contents of vial (allow to stand)
  3. **<u>Protection agent. Leave it in the water for 4 hours</u>**

  Slurry apply to seed or…

  4. Dilute down in tank for in-furrow injection
  5. Additives last just before injection.

Focus on inoculant carrier systems - Granular

Granular

Highly protective, versatile.
In-Furrow moist or dry seeding.
Mix with seed or fertiliser.

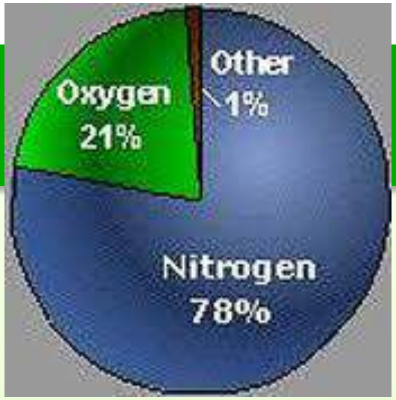
Key usage points in-furrow

- In-furrow application rate 10kg/ha mixed with seed or fertiliser or standalone.
- Can be sown in to dry seed beds well ahead of rain if need be.
- Physically buffers rhizobia against the harmful effects of pesticide seed dressings.
- Keep dry during delivery, storage, handling and application.
- Avoid use when seeding equipment is damp or where the product will come into contact with moisture prior to reaching the seed bed.
- **Avoid over handling.**

The ALOSCA range, full application flexibility.

Carrier type	Seed moist	Mix with seed	Mix with fertiliser	IF Liquid inject	Seed Dry	Approx $/ha
ALOSCA Dry Granule	YES	YES	YES	NO	YES	$15-$19
EasyRhiz (Freeze Dried)	YES	YES (slurry)	NO	YES	NO	$6-$7
Nodule N (Peat)	YES	YES (slurry)	NO	YES (blockage risk)	NO	$5-$6

Biologically captured Nitrogen



<u>Rhizobia:</u>

Reduces atmospheric nitrogen (N_2) into ammonia (NH_4)

↑

Fuel for bacterial metabolism

Pulling Nitrogen into your system

<u>Legume:</u>

Use (NH_4) to grow in nitrogen deficient soils

↓

Produce Carbon compounds (Sugars)

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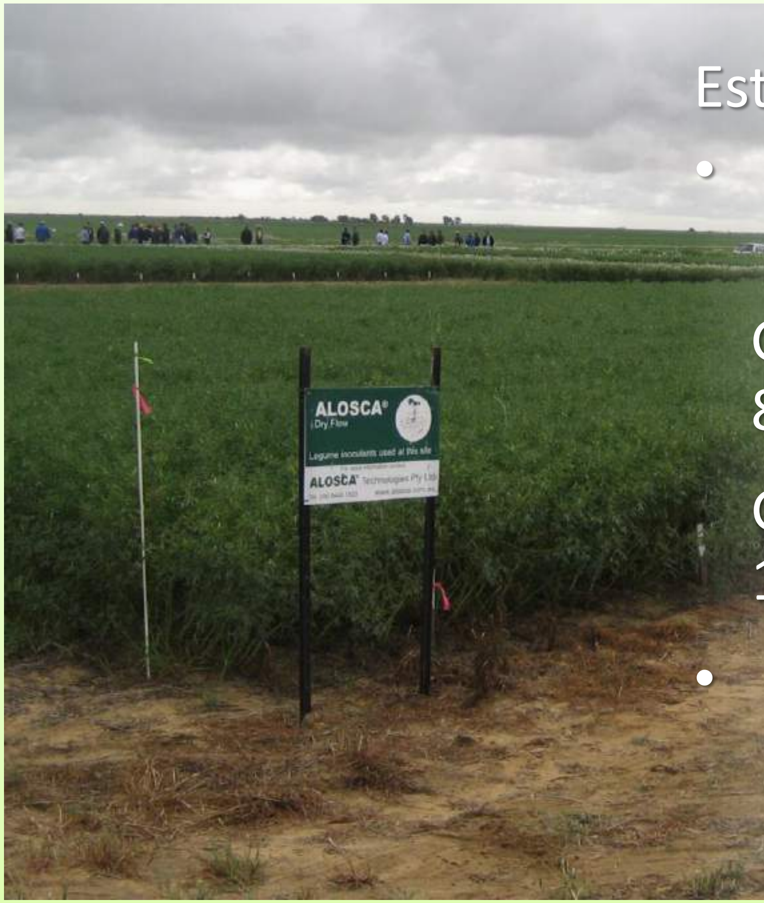
Current situation with grain cropping and legume N_2 fixation in Australia

Crop or forage type	Estimated N fixed (million t)	Area (million ha)
Cereals		19.3
Canola		2.3
Crop legumes	**0.17 (7-9%)**	**1.8 (4%)**
All grains		23.4
Legume-based pastures rotated with crops	**1.6-2.3 (93-91%)**	**23.0 (50%)**
Total area used for cropping	1.8-2.5	46.4

- Estimated 25Mill HA legume-based pastures & crop legumes rotated with about 20Mill HA cereals and oilseeds.
- Uncertainty about exactly how much N fixed annually by crop and pasture legumes in Australia.
- Likely to be in the vicinity of 1.8-2.5 Mt annually, valued at $3-4 billion annually .

Dry Matter drives N fixation benefits



Estimating N benefit

- Up to 3.5% of above ground DM is N as kg/ha (and up to 20% of roots).

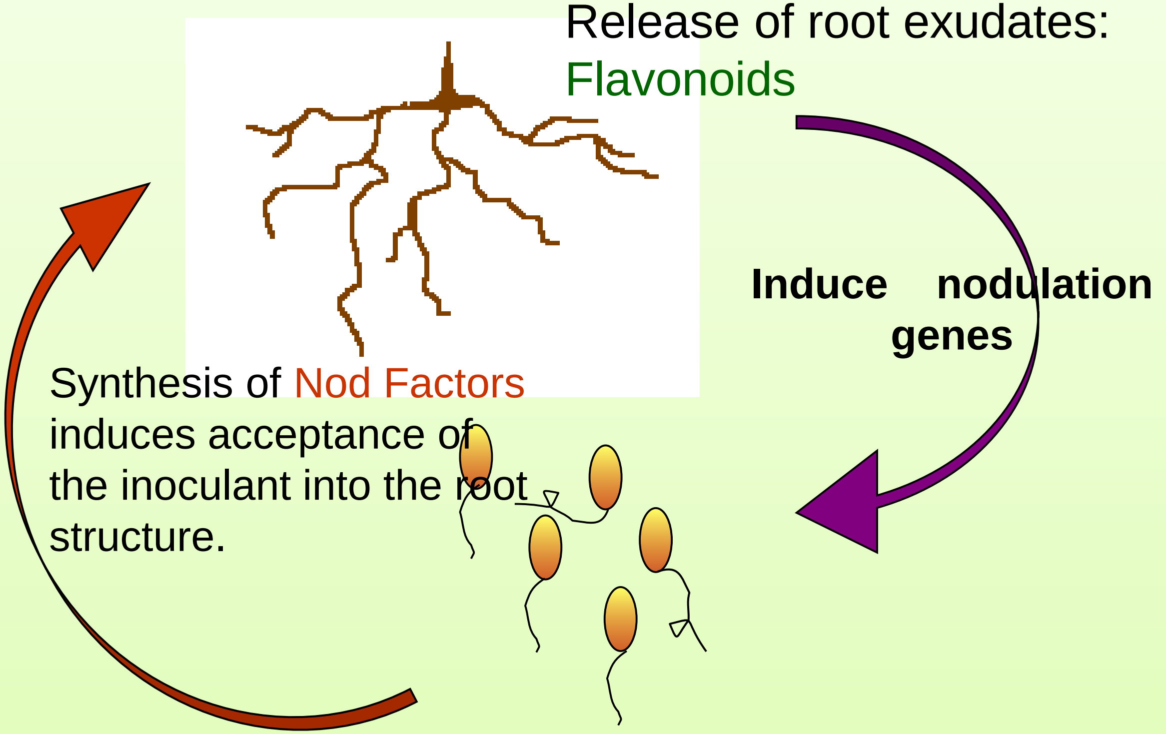
Good pasture (5t top & 3t roots)
8t = 280kg N/ha

Good lupin (8t top & 4t roots)
12t = 360kg N/ha

- Legume captured nitrogen then becomes available to the plant in the rotation over the following 3 years.

No purchase, storage or application cost. Less prone to leaching.

Nodulation initiation, exchange of molecular signals

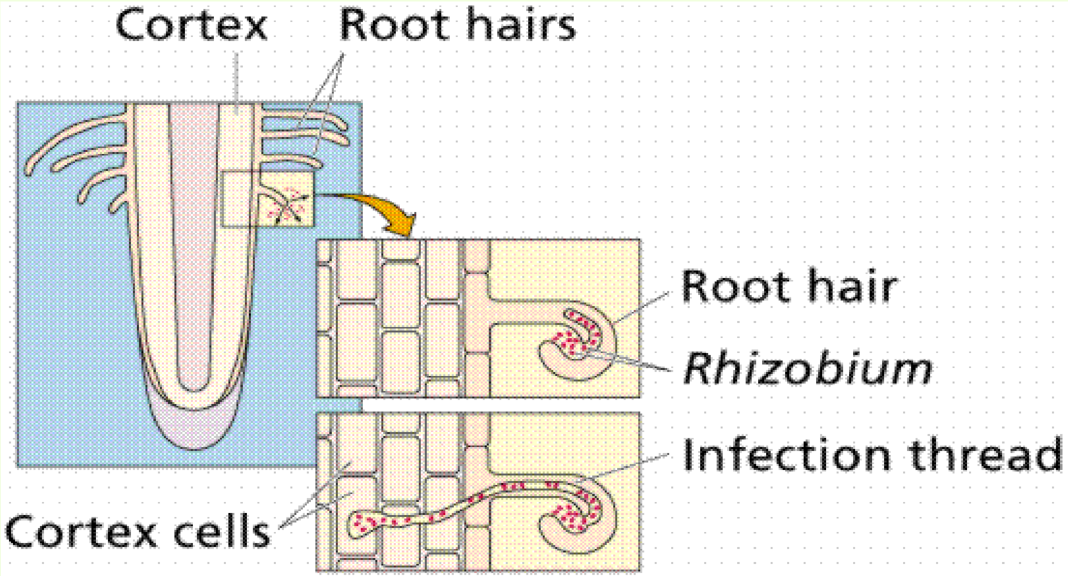


The host symbiont excretes Flavonoids to attract the Rhizobium from the soil. These chemicals induce the formation of NodD, which in turn activates other genes involved in the expression of nod factors and their secretion into the soil.

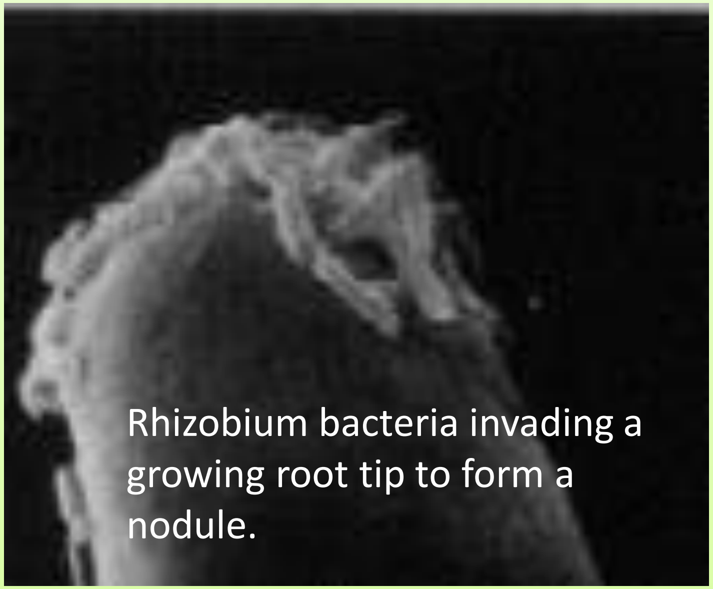
Nodulation initiation through the root hairs

Delicate process, cells need protecting to maintain high numbers

Symbiosis between root nodule bacteria and legumes is a highly specific interaction. **The correct group must be used.**



Nod factors induce root-hair curling enveloping the bacterium. This is followed by the localized breakdown of the cell wall and entry into plant cell membrane, allowing the bacterium to form an infection thread and enter the root hair. The end result is the nodule, the structure in which nitrogen is fixed.


Rhizobium bacteria invading a growing root tip to form a nodule.

Strains, what’s ideal, what are we after?

- Sufficient numbers (CFU/g soil) at time and post germination to establish early nodulation, set the plant up for winter.
- Robust strains

1. Able to survive/persist through the rotation (unfavourable pH, aridity, low clay/niche)
2. Good communicators/initiators of nodulation
3. Efficient N fixers, an energy expensive process for the plant

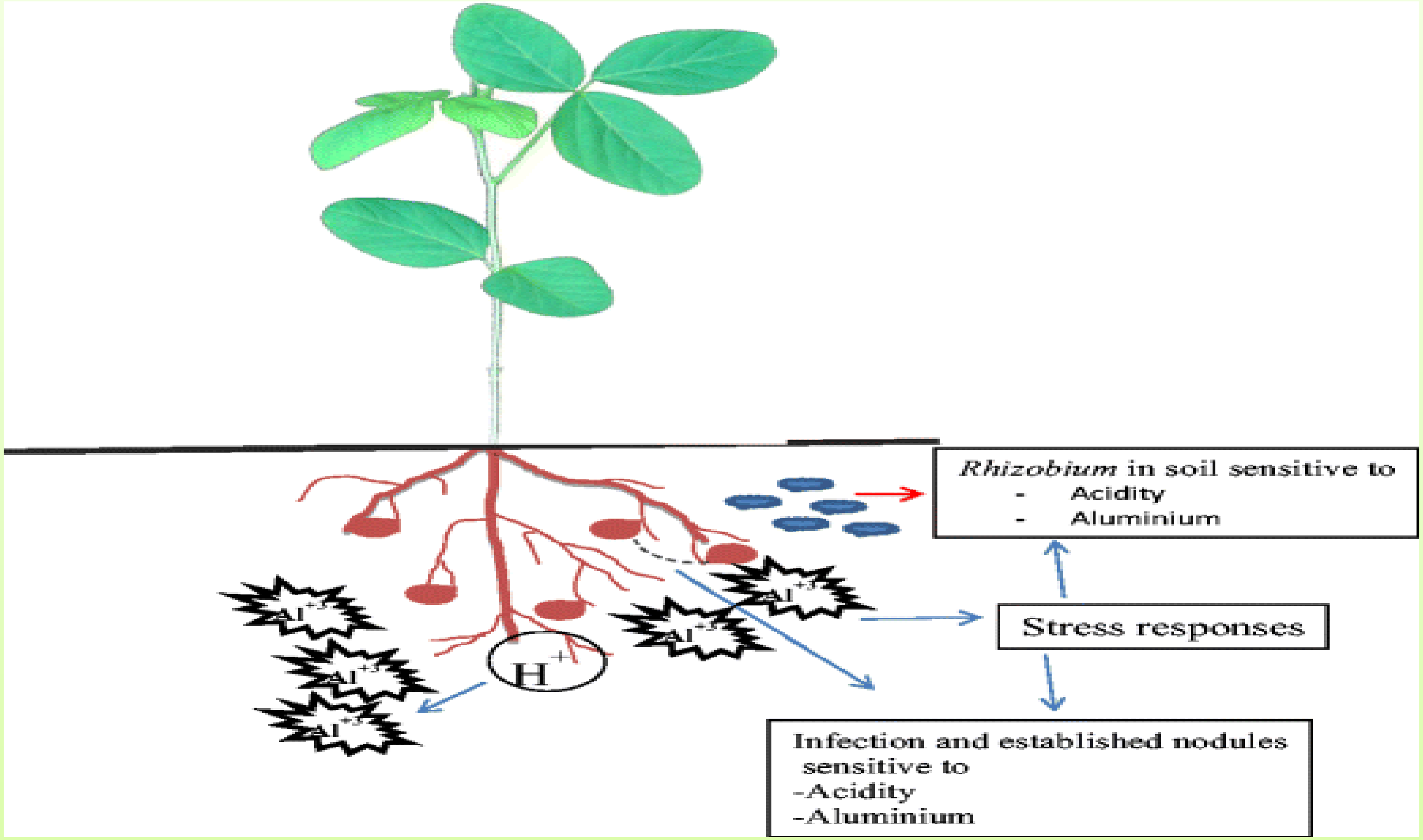
Common factors influencing Rhizobium populations & efficacy

- Soil acidity/alkalinity – toxicity root development
- Aridity, drying out of the soil profile
- Soil texture - low clay reduced niche
- Long rotational breaks from host legume
- Pesticide impact on microfauna and root development
- Hybridisation of paddock strains - poor N fixers
- Poor delivery/inoculation methods....bacteria not surviving the journey to the root zone.

Hybridisation of genetic purity

After 6 years in the field, 8 % of Biserrula nodule occupants were chromosomally different to the inoculant strain
The new genotypes were ineffective for N fixation.

Effect of aluminium on legume nodulation under acidic conditions



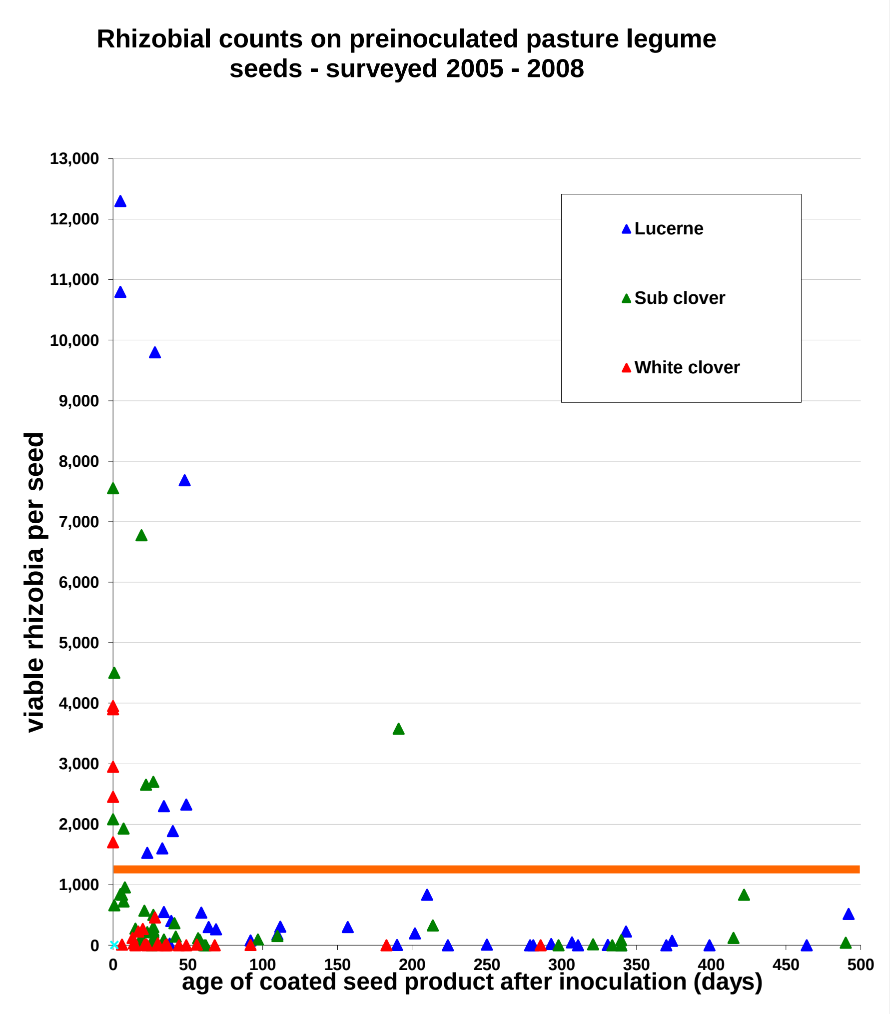
Different RNB Groups have different tolerances and habits

Genus	Plant Group (example)	Typical pH range	Tolerance to desiccation
Rhizobium	C & F (Trifoliate clovers & Pea/Vetch)	broad	comparatively poor
Bradyrhizobium	G/S (lupin/serradella)	favours acid	comparatively poor
Sinorhizobium	AL & AM (Lucerne & Medics)	favours alkaline	comparatively good
Mesorhizobium	N & BS (Chickpea & Biserrula)	broad	intermediate

Sensitivity of key rhizobia to pH, where red is sensitive and green is optimal.

Rhizobium Genus & species	Host legume	pH 4.5	pH 5.0	pH 5.5	pH 6	pH 7	pH 8
Bradyrhizobium spp. lupinus.	Lupin, Serradella	Orange	Green	Green	Green	Orange	Red
Rhizobium leguminosarum bv. *trifolii*	Clovers	Orange	Green	Green	Green	Green	Green
Rhizobium leguminosarum bv. *viciae*	Pea, Faba bean, Lentil, Vetch (watch this space)	Red	Orange	Green	Green	Green	Green
Mesorhizobium ciceri	Chickpea	Red	Red	Orange	Green	Green	Green
Mesorhizobium spp	Biserrula	Red	Orange	Green	Green	Green	Green
Sinorhizobium spp.	Lucerne, Medics, Messina.	Red	Red	Orange	Green	Green	Green

Sensitivity of key rhizobia to desiccation, seed coating technology requires more research to improve Rhizobium shelf life



More recent retail surveys show that overall, irrespective of brand, the quality of preinoculated seed in terms of rhizobial number per seed has not improved.

E. J. Hartley[1], L. G. Gemell[1] and R. Deaker[2]

NSW GOVERNMENT | Industry & Investment

Survey year	2005	2006	2007	2008
No. samples	113	50	54	55
No. passed	6	16	3	2
%	5.3	32.0	5.6	3.6

[1]Climate & Water Research, Australian Inoculants Research Group
[2]University of Sydney, SUNFix Centre for Nitrogen Fixation – Nov 09

Importance of how rhizobia is delivered

2009 ALSOCA Group C in-furrow

2010 infection/nodulation rate 90%

2010 Pasture yield – 2.8 t/ha

2009 Group C pre-coated seed

2010 Group C infection/nodulation rate 0% (independently tested)

2010 Pasture yield – 700 kg/ha

Bladder Clover 2009 establishment. Inoculation comparison Seed Coating vs. ALOSCA granules. Poor inoculant survival within seed coating fails to establish the new Group C (sub-clover) strain in 2009 for re-establishment in 2010 resulting in 2.1t/ha dry weight yield penalty.

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Survival of Rhizobia on lupin seed during planting

NSW Dept of Ag. - monitoring of farmer applied legume inoculant - lupins.

Event	Time Elapsed (**hours**)	**Number & %** Surviving per seed
At Inoculation (inoculant applied to seed in a 0.75% methylcellulose slurry)	0	1,445,000 * (100%)
Lupins **mixed** in vertical feed mixer and augered into truck	1	141,200 # (9.8%)
Transported to field and augered into air seeder box (**sampled from seeder box)**	4.75	11,200 # (0.8%)
Planted by air seeder	4.8	6,761 # (0.5%)
Recovered from soil	22.5	1,175 # (0.1%)

* Theoretical number derived from number in inoculant slurry.

Numbers determined by MPN method.

Reference: Roughley et.al 1993 Soil Biol. Biochem.25:1453-1458.

Nodule field assessment

Be prepared – Bucket, spade water

1. Mid-winter to early-spring
2. Representative sample
3. Dig, don't pull
4. Wash
5. Assess, should be abundant **PINK** nodules >90%

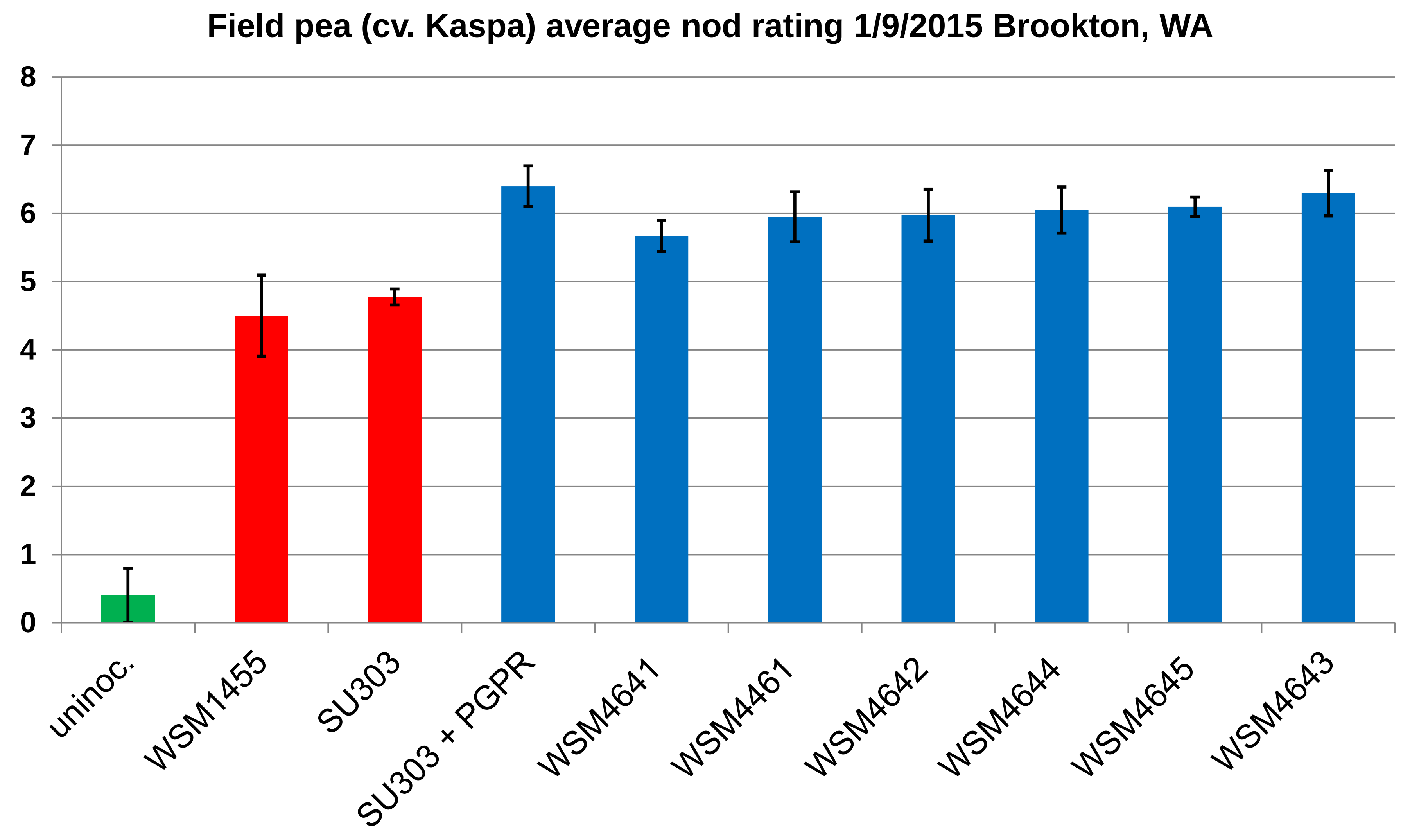
Sample timing is important, soil moisture makes it easier.

Nodule field assessment, timing important

breaking down effective ineffective

New Alosca Developments

- New Pea/Vetches strain- Group E/F pH4.7 – Ron Yates
- Head start inoculation, inoculate the year prior with Cropping Fertiliser .
- New data from the Top dressing project
  - Sub clover & medic
- Herbicide plant back issues.
- ASHEEP Mix Group AM /AL limited Qty.
- Trialling Group GS/C watch this space.



Site limed over summer (0-10 cm pH 5.1, 10-20cm pH 4.7)

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CRS

New strains lift productivity of new and old varieties alike under broader soil conditions

Group C (sub clover) development paying dividends…

WU95
1968-94

WSM409
1994-2004

WSM1325
2005

New lower soil pH tolerant strains can provide a production edge over background/paddock strains

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New Methods - Top dressing, key usage points

Pasture application only, sub-clover, medic and Lucerne.
Not an option for cropping.

Prerequisites

1. No inoculation since 1994.
2. Has adverse pH been addressed?
3. No Phosphorus Potassium or Trace element deficiencies/issues

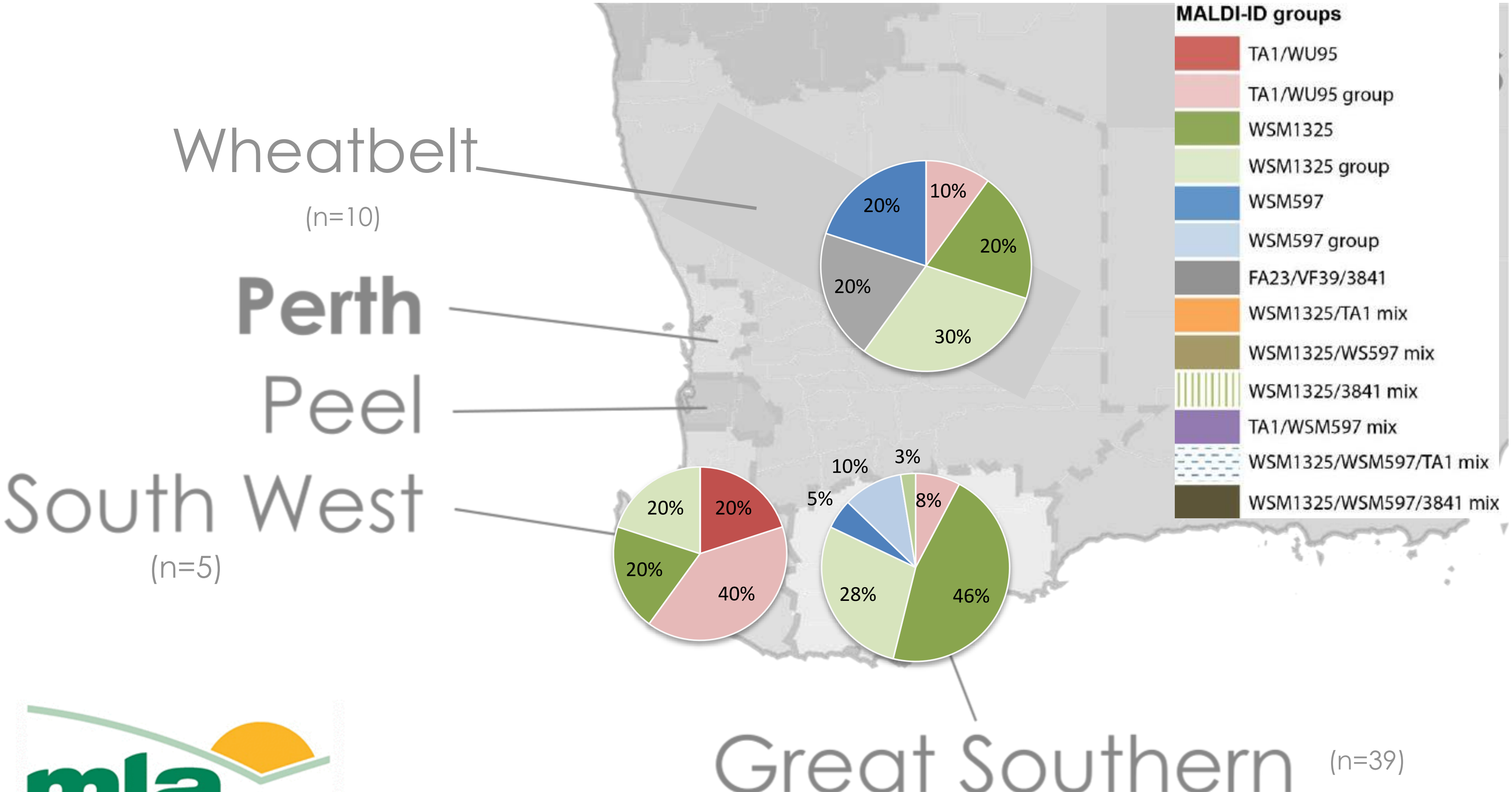
Usage tips

Currently most favoured method for even coverage- top dress from seeder carriage up or scratch into surface.

If using spinner type spreader.

- Mix with fertiliser or other product possibly lime or gypsum, be sure mix partners are not too moist.
- Reduce spreader spinner rpm and/or speed to reduce swath & to improve distribution.

MLA funded project surveying sub-clover paddock strains using the MALDI-ID technique – 2015 WA



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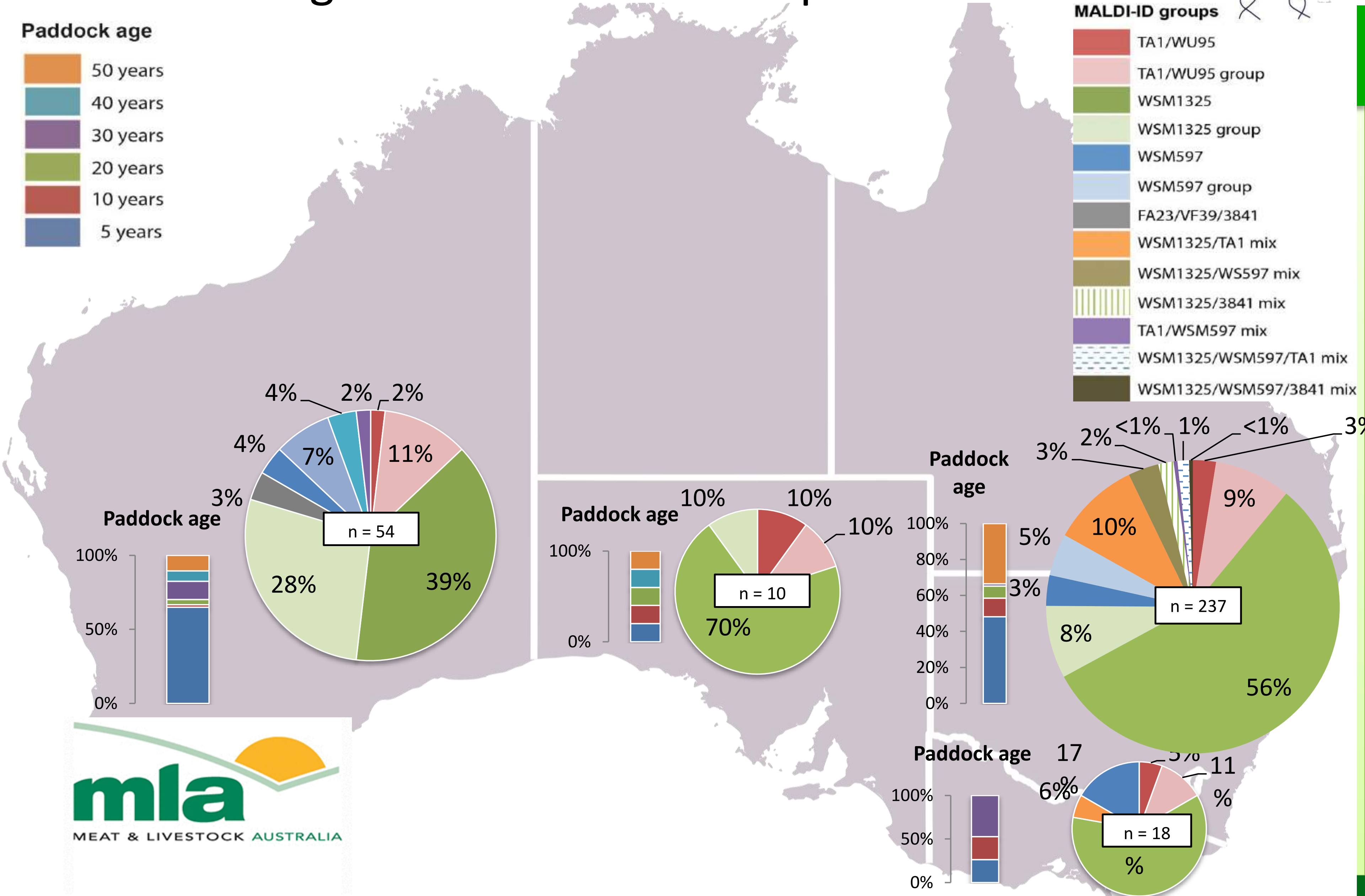
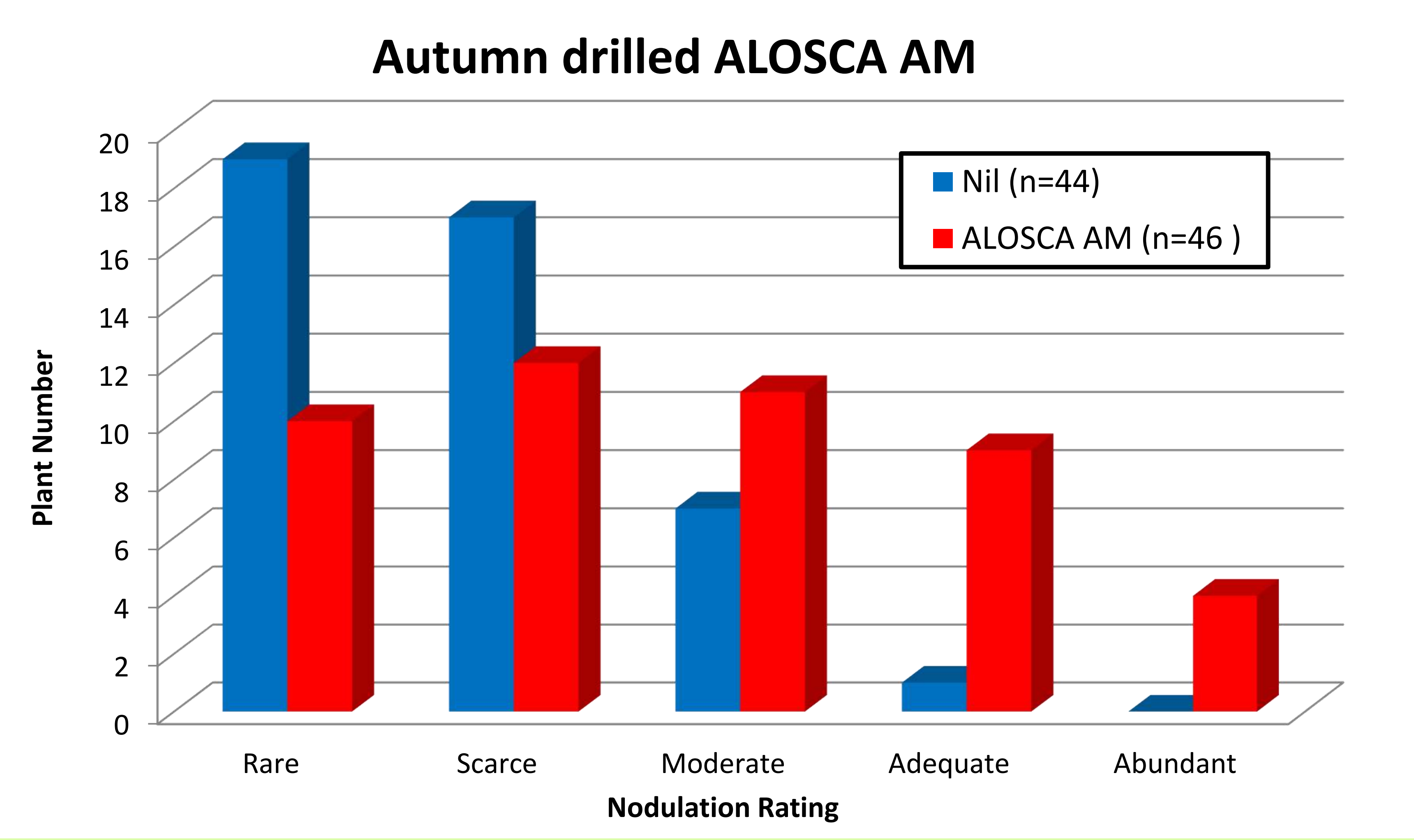
MLA funded project surveying sub-clover paddock strains using the MALDI-ID technique – 2015 National



ALOSCA Top Dressing nodulation data 2012 -14

Property	Location	Nodulation increase measured (raw data)	significant statistical difference shown
Harrington, D	Narrogin	No	No
Martin, P	Williams	No	No
Major, R	Williams	No	No
Kowald, J & B	Kojonup	No	No
Kowald, A & J	Kojonup	Yes	No
Thompson, R & M	Woodanilling	Yes	No
Wooldridge, B	Darkan	Yes	Yes
Johnston, K	Williams	Yes	Yes
South, J - 1	Darkan/Dunleath	Yes	Yes
South, J - 2	Darkan/Bokal	Yes	Yes
Angwin S	Tincurrin	Yes	Yes
Hardie, L	N Wandering	Yes	Yes
White, C (sandy)	Narrogin	Yes	Yes
White, C (clay)	Narrogin	Yes	Yes
Bradford, D	Wandering	Yes	Yes
Schroer, N	Wandering	Yes	Yes
Waters, S & M	Bridgetown	Yes	Yes
17 sites	**ANOVA t-test**	**76%**	**65%**

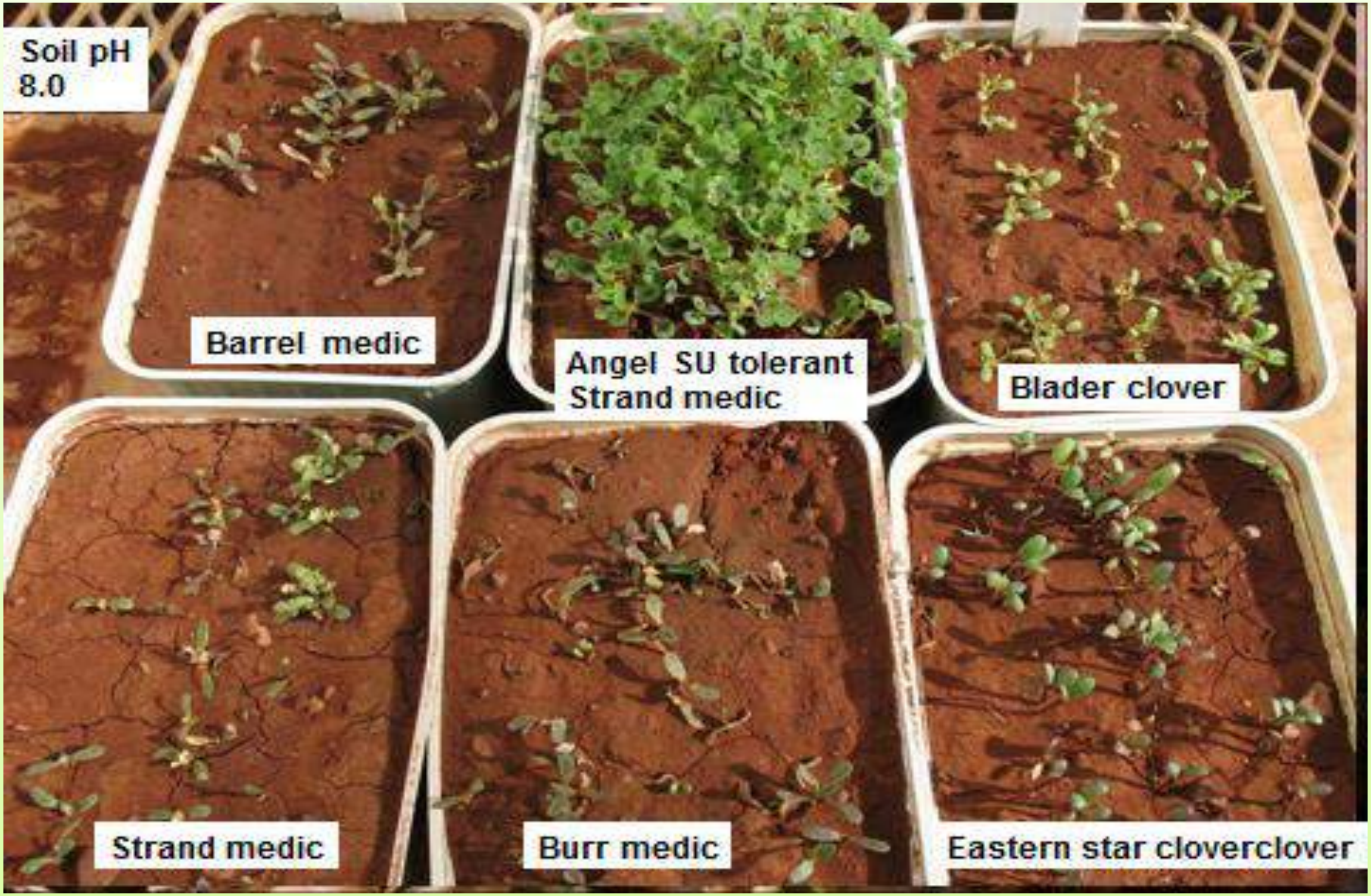
New methods – reintroduction to medic



New methods - Head Start inoculation

Beckom NSW sown wheat 2013 Alosca BS added to fertiliser

Herbicide issues



Triasulfuron application (18 days growth)

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DAFWA Crop Pasture Group (Yates, Nutt & Loi)

Dalkeith

Herald

Bartolo

Marg

Herald

Angel

Casbah

3g	0.3g	0.03g	0.003g	0.0003g	control
1/10	**1/100**	**1/1000**	**1/10000**	**1/100000 of recommended rate**	


3g
0.3g
0.03g
0.003g
0.0003g
control
1/10
1/100
1/1000
1/10000
1/100000 of recommended rate
Triasulfuron application on Sub clover (18 days growth)
DAFWA Crop Pasture Group (Yates, Nutt & Loi)

Department of Agriculture and Food
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Pyroxasulfone (Group K) (grass selective in wheat)

Crops	Re-cropping recommendation	
	Minimum re-cropping interval	Minimum interim rainfall
Wheat (not durum wheat) and triticale	0 months	0 mm
Cotton, maize, mung beans, sorghum, soybeans and sunflowers	5 months	150 mm
Barley, canola*, chickpeas, faba beans, field peas, lentils, lupins, vetch and subterranean clover	**9 months**	**250 mm**
Durum wheat, oats, lucerne and medic	21 months	550 mm

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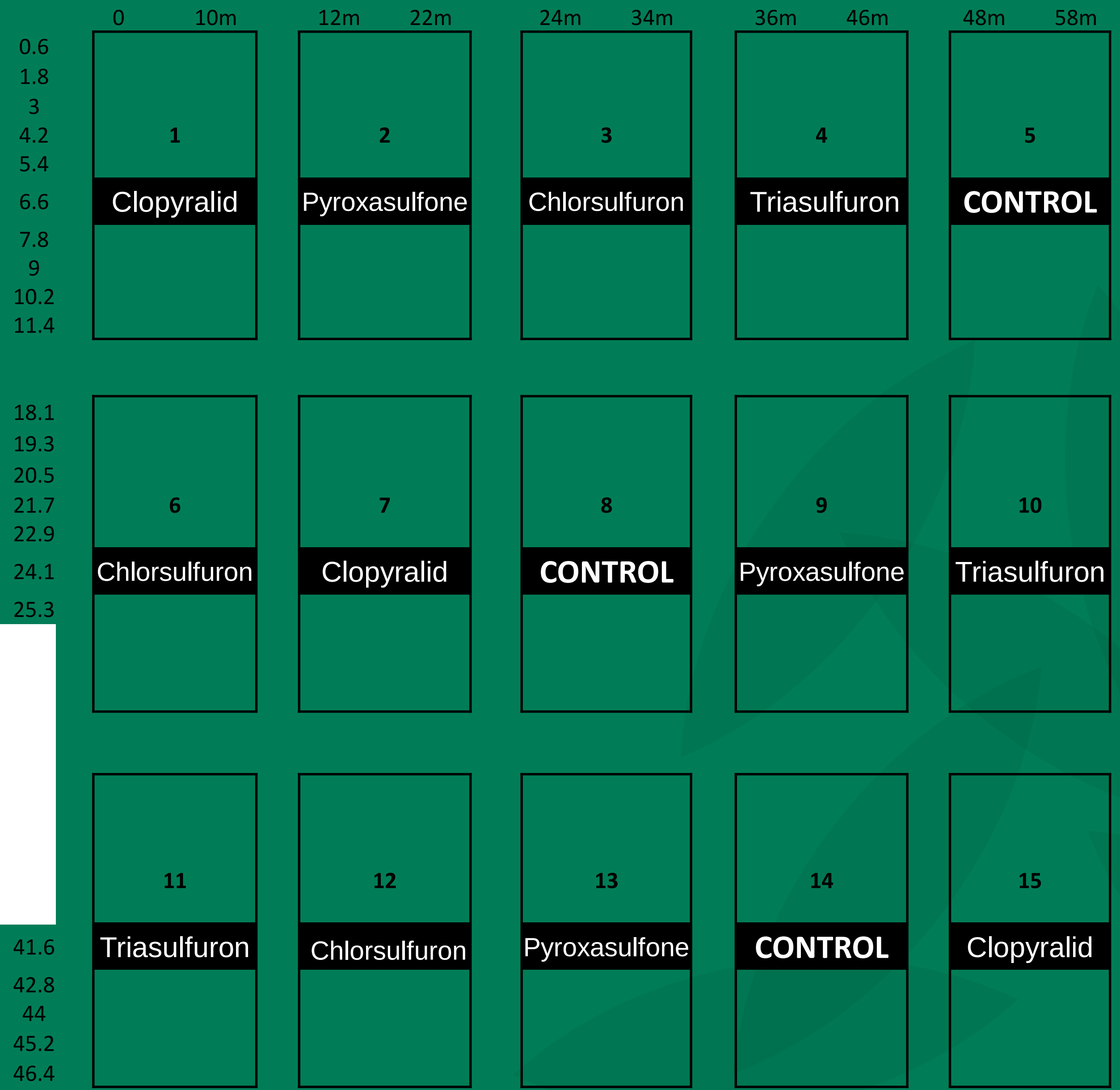

Sprayed July 2012 over 3-5 leaf wheat no incorporation

3 Core samples
- Nov 2012 (4)
- Feb 2013 (7)
- May 2013 (10)

Sown to legumes in late May 2013 (10 months post)

Peas
Lupins
Bartolo
Margarita
Prima
Casbah
Dalkeith

pH 4.6

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Plant Back!

wheat crop that reaches maturity in the season of application.

Soil pH (1:5 soil:water suspension method)	State	Replanting Interval	Minimum rainfall requirements between application and sowing the following crop	Crop
6.5 or less	Qld, NSW, Vic, Tas, SA, WA only	12 months	300 mm	Canola, Chickpeas, Faba Beans, Field Peas, Linseed, Lucerne, Lupins, Medics[Δ], Subterranean Clover[Δ]
	Qld, NSW only	15 months	700 mm	Cotton, Cowpea, Maize, Mung Bean, Sorghum, Soybean
		18 months	900 mm	Sunflowers
6.6 to 7.5	Qld, NSW only	12 months	500 mm	Canola, Chickpeas
		15 months	700 mm	Cotton, Cowpea, Maize, Mung Bean, Sorghum, Soybean
		18 months	900 mm	Sunflowers
	Vic, Tas, SA, WA only	22 months	500 mm	Canola, Chickpeas, Cotton, Faba Beans, Field Peas, Linseed, Lucerne, Lupins, Maize, Medics[Δ], Sorghum, Soybean, Subterranean Clover[Δ]
7.6 to 8.5	Vic, Tas, SA only	12 months	250 mm	Barley, Cereal Rye for grain crops, Oats
			300 mm	Barley, Cereal Rye for hay crops, Oats
	Qld, NSW only	12 months	500 mm	Canola, Chickpeas
		18 months	700 mm	Cotton, Cowpea, Maize, Mung Bean, Sorghum, Soybean
	Vic, Tas, SA, WA only	24 months	700 mm	Canola, Chickpeas, Cotton, Faba Beans, Field Peas, Linseed, Lucerne, Lupins, Maize, Medics[Δ], Sorghum, Soybean, Subterranean Clover[Δ]
8.6 and above	[illegible]	[illegible]	[illegible]	…ereal Rye for grain crops, Oats
	[illegible]	[illegible]	[illegible]	…ereal Rye for hay crops, Oats
	[illegible]	[illegible]	[illegible]	…Chickpeas, Cotton Faba Beans, Field …nseed, Lucerne, Lupins, Maize, … Sorghum, Soybean, Subterranean

Triasulfuron (ALS inhibitor)

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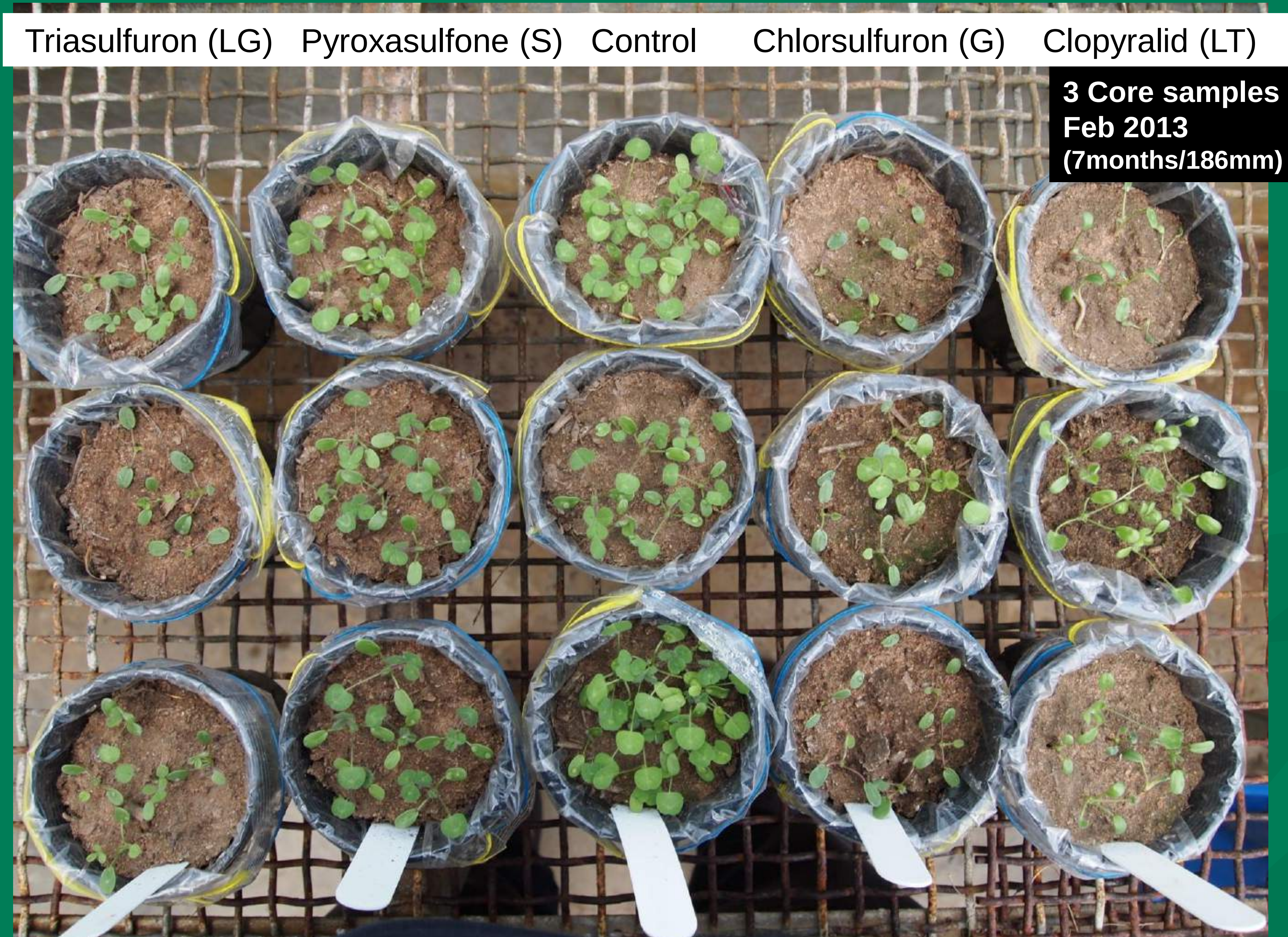
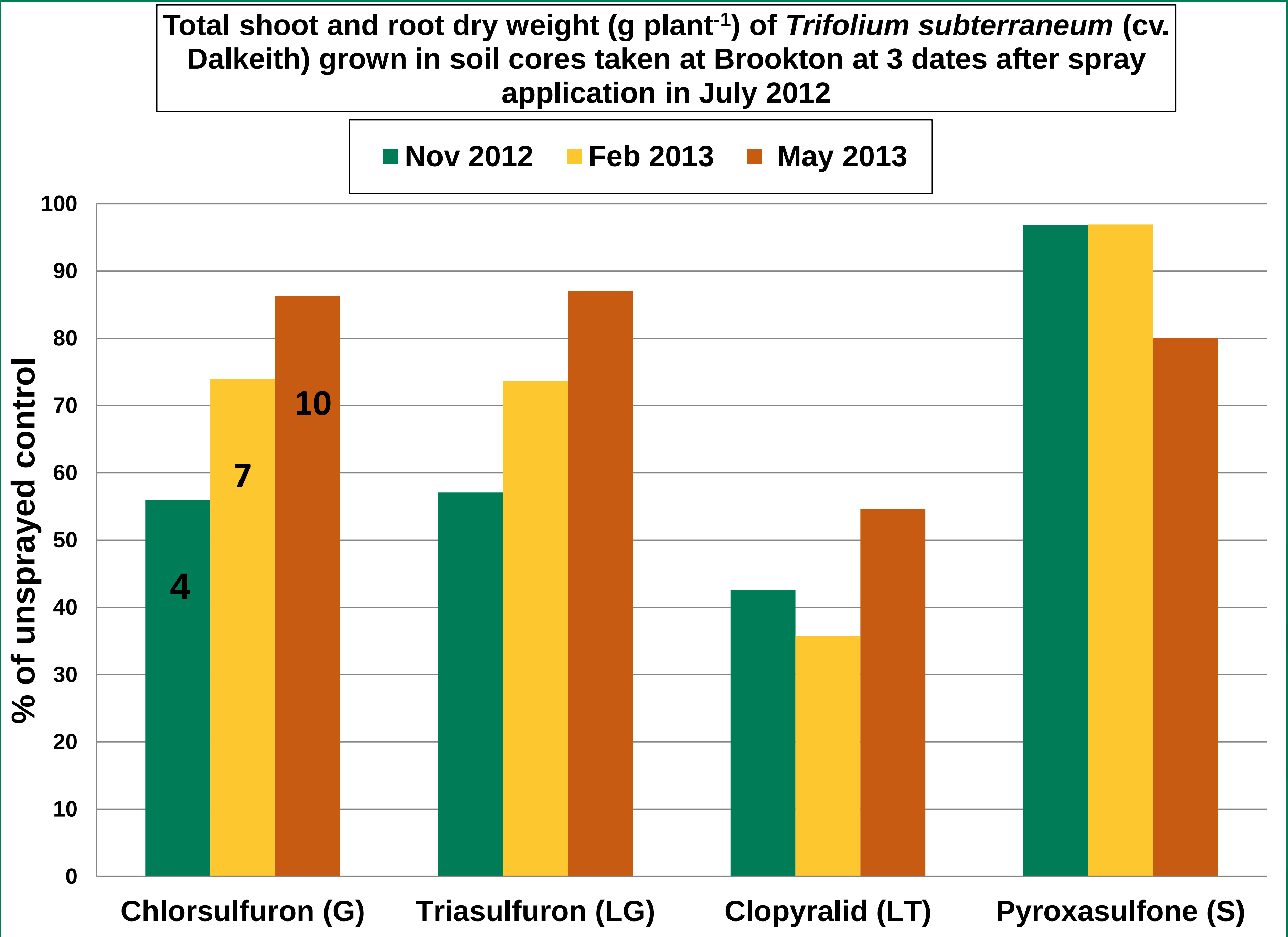
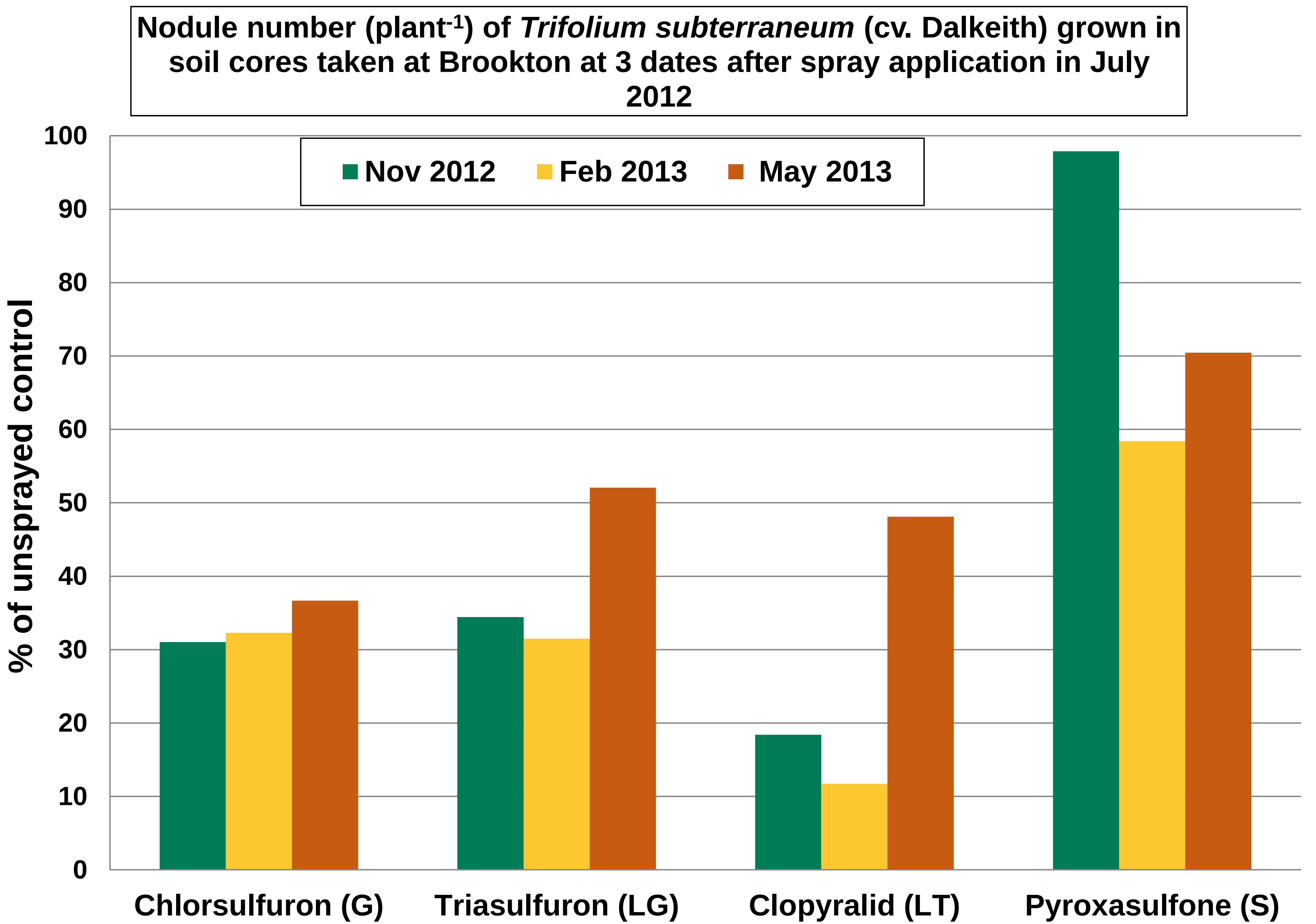
Chemical	Spray rate (g/ha)	Plant back period for pasture legumes < pH 6.5	
		time (months)	required rainfall (mm)
pyroxasulfone	125	9	250
clopyralid	115	9	150
chlorsulfuron	15	12	NS*
triasulfuron	35	12	300

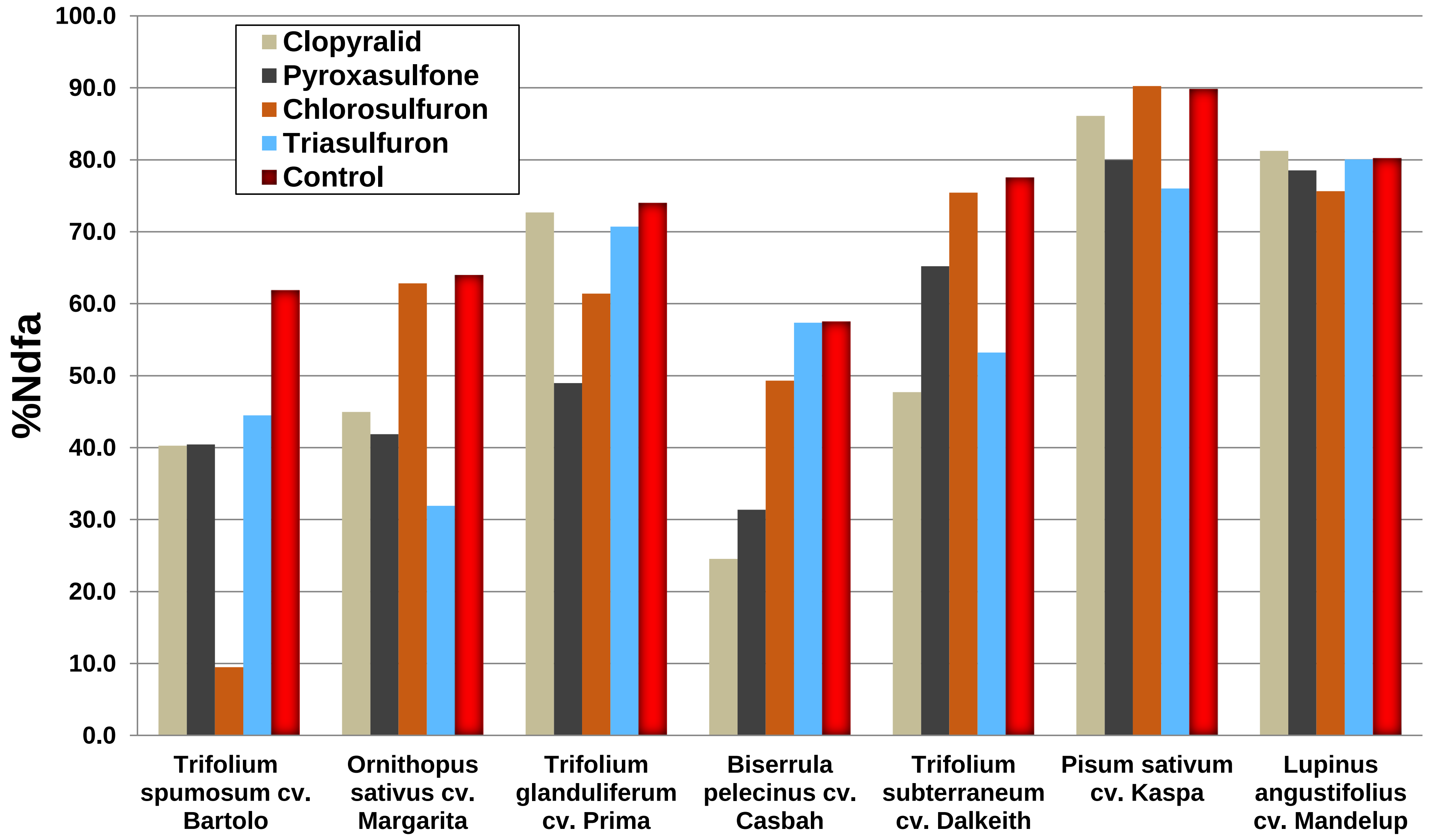
Total rainfall on the site (after the spray application) was 120mm (by November 2012), 186mm (by February 2013) and 300mm (by May 2013)


Triasulfuron (LG)
Pyroxasulfone (S)
Control
Chlorsulfuron (G)
Clopyralid (LT)
3 Core samples
Feb 2013
(7months/186mm)

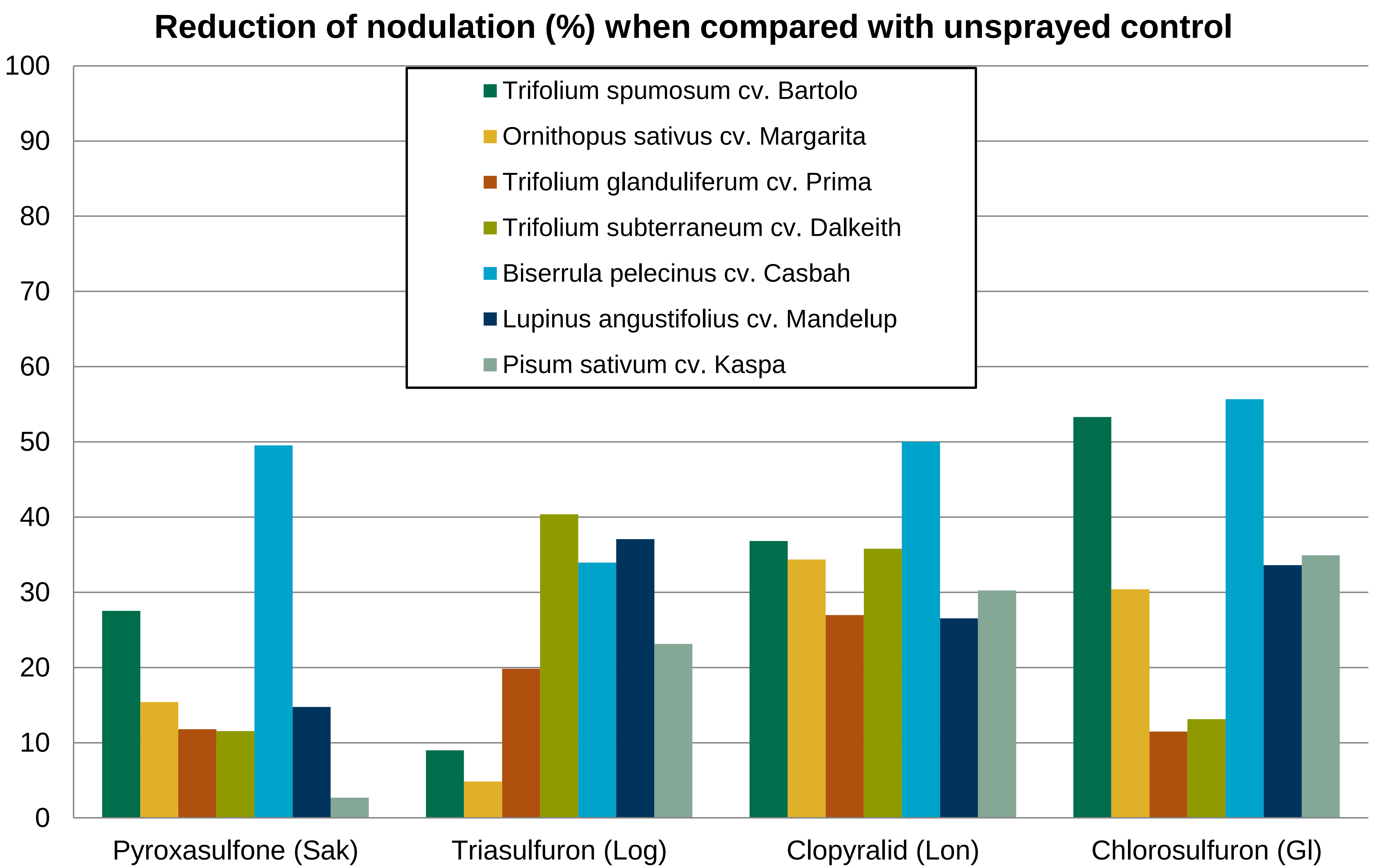

Total shoot and root dry weight (g plant-1) of Trifolium subterraneum (cv. Dalkeith) grown in soil cores taken at Brookton at 3 dates after spray application in July 2012
Nov 2012
Feb 2013
May 2013
% of unsprayed control
100
90
80
70
60
50
40
30
20
10
0
4
7
10
Chlorsulfuron (G)
Triasulfuron (LG)
Clopyralid (LT)
Pyroxasulfone (S)


Nodule number (plant-1) of Trifolium subterraneum (cv. Dalkeith) grown in soil cores taken at Brookton at 3 dates after spray application in July 2012
Nov 2012
Feb 2013
May 2013
% of unsprayed control
0
10
20
30
40
50
60
70
80
90
100
Chlorsulfuron (G)
Triasulfuron (LG)
Clopyralid (LT)
Pyroxasulfone (S)

16 months after application



13 months after application



Narrogin Trial

Effect on nodulation and biomass of post emergent sprays on Dalkeith

Dr Ron Yates DAFWA

Treatment	Group	Active Constituent	Nodulation score	% DW of unsprayed Control
Diuron	C	Ureas (Diuron)	very rare	23
Jaguar	F + C	Diflufenican + bromoxynil	rare	18
Tigrex	F + I	Diflufenican + Phenoxys	rare	36
Ecopar	G	Pyraflufen	rare	23
Terbutryn	C	Triazines (Terbutryn)	scarce	35
Broadstrike	B	Sulfonamides (flumetsulam)	moderate	54
MCPA	I	Phenoxys (MCPA)	moderate	65
2,4-D Amine	I	Phenoxys (2,4-D)	moderate	74
Raptor	B	Imazamox	adequate	51
Spinnaker	B	Imazethapyr	adequate	85
Unsprayed control			adequate	100

ALOSCA TECHNOLOGIES

Acknowledgments

- Dr Ron Yates, Murdoch CRS/DEPAIRD
- Generosity of the Centre for Rhizobium Studies (CRS) Murdoch University.
- NSW Department of Agriculture. Dr. Belinda Hackney

Check out our new website www.alosca.com.au

Root nodule bacteria (RNB), what are they?

Soil bacteria of the family Rhizobiaceae:

- *Rhizobium,* **(C, F)**
- *Bradyrhizobium* **(S)**
- *Sinorhizobium* **(AL, AM)**
- *Mesorhizobium* **(N, Bis)**

Important common characteristics

- Aerobic metabolism (don’t like water logging)
- Motile (they are mobile)
- They live as normal component of the soil microbial population (well adapted to soil)
- Mostly found on the root surface or close to the root surface (of legume species)

Survival of Rhizobia on lupin seed during planting

Lupin yield response to increasing numbers of *Rhizobium* bacteria.

Number of *Rhizobium* on seed	Nodule mass 43 days mg./plant	Dry matter production 197 days kg/Ha	Grain yield 226 days kg/Ha
1,862,087	393	9037	2088
186,209	222	8673	1802
18,621	65	7759	1899
1,862	12	7184	1648
186	5	4754	1349
19	4	4774	1126
2	4	4663	1085

NSW Dept. of Ag. Dr. R. Roughley 1986


Brookton 2013