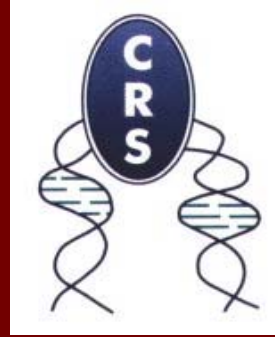
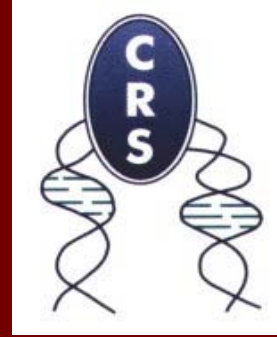


# Nitrogen Fixation by Lupins in Western Australia



Which microbes are responsible,  
from where did they originate, and  
can we intercede?

Professor John Howieson  
Centre for *Rhizobium* Studies  
Murdoch University



# A reminder..

- Lupins are unusual amongst legumes of Mediterranean origin in that they are nodulated by *Bradyrhizobium*...
- They are infected through crack entry...
- They form “lupinoid” collar nodules...
- As relics of a sub-tropical and ancient past, many Australian legumes are also nodulated by *Bradyrhizobium*...

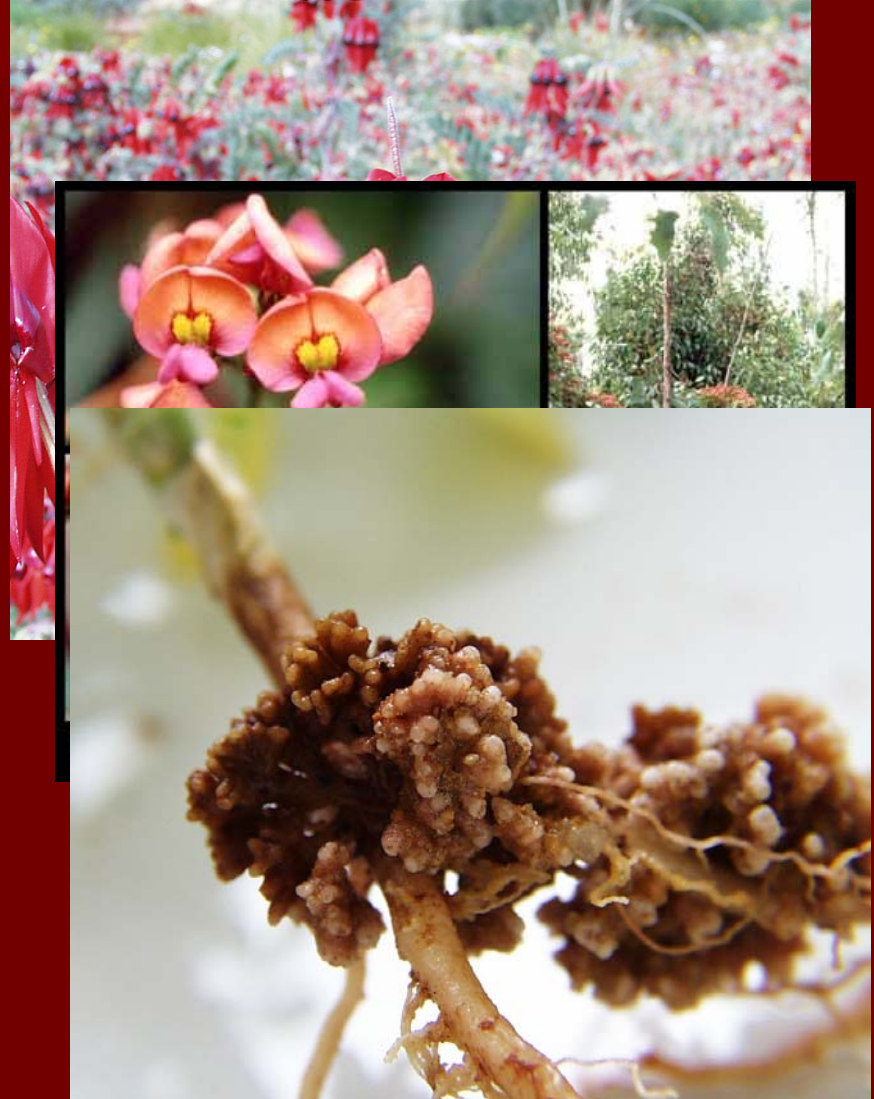
# Lupin arrived to Australia by accident

- As for all commercial Agricultural legumes in Australia, lupins were introduced to this continent, and like many, the manner of their introduction was originally accidental



# ...and the root-nodule bacteria

- Whilst we have a wealth of native legumes in WA, their root-nodule bacteria are overwhelmingly unable to interact symbiotically with the introduced agricultural legumes





Clear responses to inoculation with introduced legumes

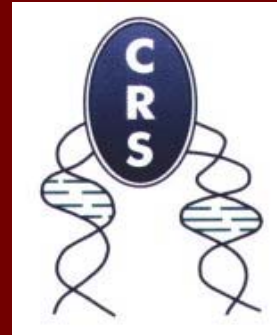
← *Lens culinaris*

*Trifolium spp*



# The situation with lupins from the early times

- 1920s –Adams and Riches noted that lupins were absolutely dependent on inoculation, except occasionally *L. cosentinii*
- 1930s - Cass-Smith and Pittman noted that *L. cosentinii* sometimes nodulated well when other species didn't
- This raised the issue of whether some lupin species could nodulate with the indigenous microflora



# Lex Parker and RT Lange

- 1960 –Lange / Parker undertook a comprehensive cross-nodulation experiment which concluded that native legume rnb could not infect lupins –they were highly **specific**



Specificity between LEGUME species nodulated by the same rnb

# SPECIFICITY...within species



There may well be specificity between and within *Lupinus* that could explain the nodulation anomalies between Parker's meticulous work and the field observations Adams et al.

- However, in the Parker and Lange experiments of the 1960s, the WA native legumes *Jacksonia*, *Gastrolobium* and *Kennedya* were nodulated by isolate D25 (and perhaps 24) originally from *L. cosentinii*.
- McInnes in 2002 provided evidence that isolates from *Daviesia* and *Mirbelia* could nodulate serradella.
- This kept the question of native legume rnb as a source of lupin nodulation open

..as the lupin industry rapidly expanded in the northern sands..

- By 1980 Dave Chatel and his colleagues could show only rare responses to inoculation of lupin.

Although rare, in 2003 at Pingrup we did observe this failure in nodulation!



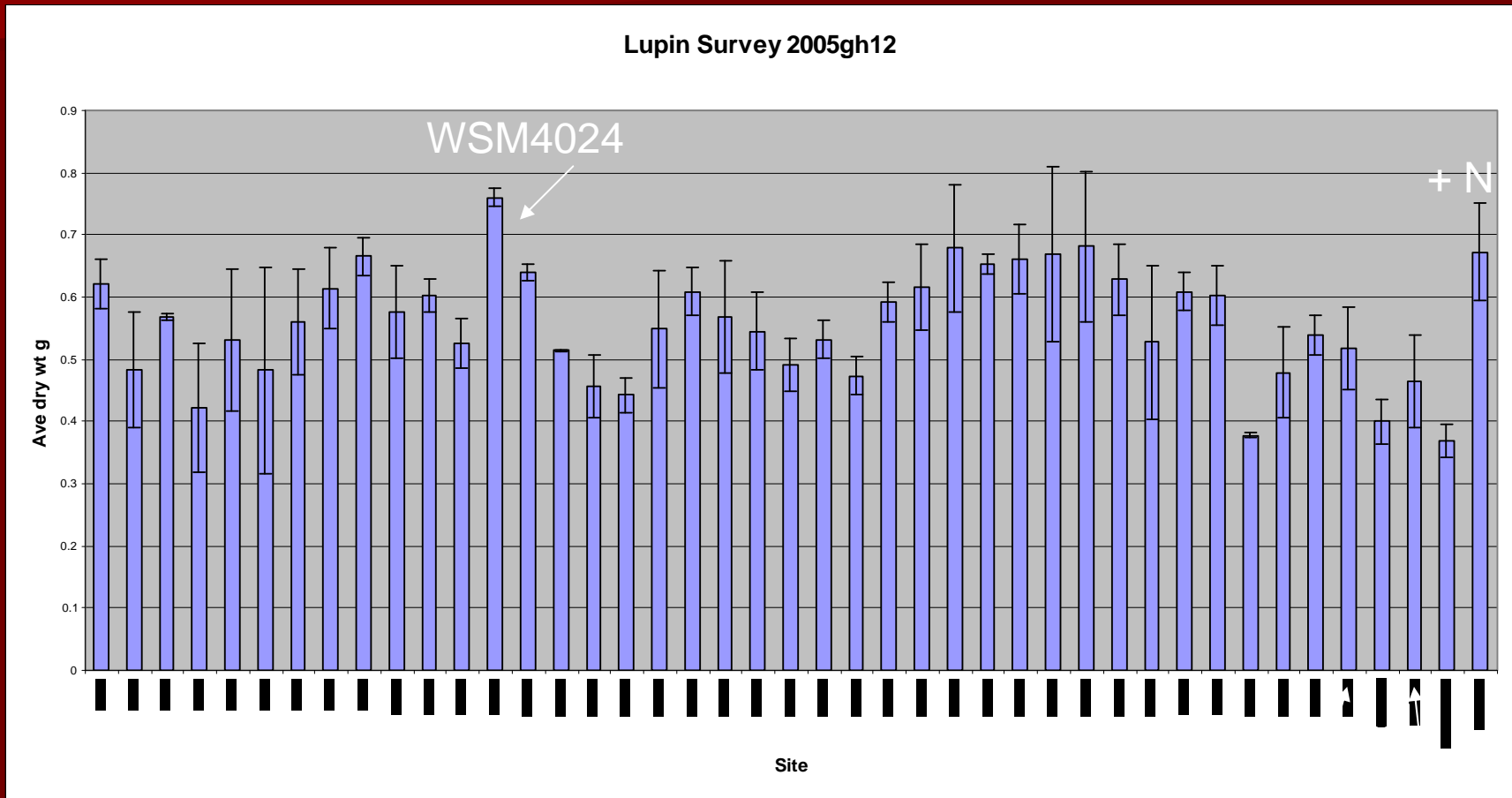
- In Chatel's work, responses to inoculation tended to disappear during the season – this in itself is most unusual – where legumes fail to nodulate in WA they usually perish
- This recovery phenomenon added to the speculation about the origins of the rnb – were lupins nodulating with a secondary source of rnb present in low number?

In 2005, CRS and DAFWA conducted a Lupin nodule occupancy survey.

## Aims

- To assess the identity and diversity of strains currently in lupin nodules
- To assess the N fixing ability of these strains

N fixation varied two-fold, although most strains were  $\geq$  commercial strains



RAPD PCR failed to identify any rnb as the commercial inoculants

## A. From where did the lupin nodule occupants arise?

1. rnb arriving to WA as contaminants?
2. escaped research isolates?
3. inoculant strains colonising the soil?
4. native rnb that could nodulate lupins (which increased in number in response to lupin cultivation)
5. native legume rnb that *acquired* the capacity to nodulate lupins?

## Some species of rnb do diversify, rapidly



- After 6 yrs in the field, 8 % of biserrula nodule occupants were chromosomally different to the inoculant strain
- The new genotypes were recipients of symbiotic DNA from the inoculant strain
- ineffective for N fixation
- Diversification occurs in rhizo, meso, sino, **but not bradyrhizobia**

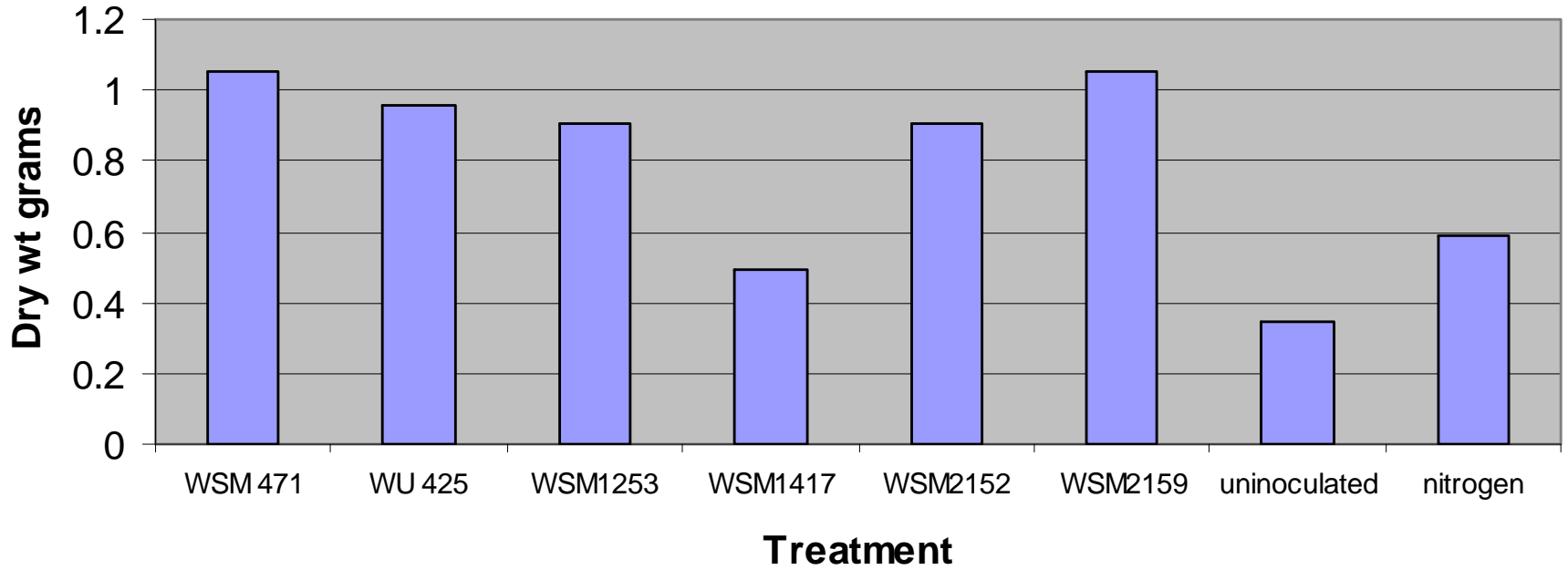
# Tomasz Stepkowski AEM 2005

- assembled many lupin strains from RSA, NA, Europe, Australia (including several early *L. cosentinii* isolates from the lab of CA Parker W72, WU140, WU8), and isolates from native legumes
- sequenced the nodulation genes *nodA*, *nodZ*, *nolL* (known to be transferable in many rnb)
- Sequenced the conserved housekeeping genes *dnaK*, *recA*, *glnII*, *atpD* and the 16S-23S rRNA ribosomal intergenic spacer,
- Conducted a range of phylogenetic analyses
- demonstrated with substantial clarity that the lupin nodulating strains in WA were **strongly aligned with a European clade and distinct from Bradyrhizobia isolated from Australian native legumes.**
- Included in the European clade were Australian inoculant strains WU425 and WSM471...millions of ha of WA soils colonised by accidentally introduced European strains of rnb.

Having solved the mystery of the origin of the strains...

- B. Is there solid evidence that Lupin rnb differ substantially in N fixation?
- Were there species x strain interactions?
- Data from the nodule occupancy survey indicated there was variation in respect of *L. angustifolius*

*Lupinus angustifolius*



Greece

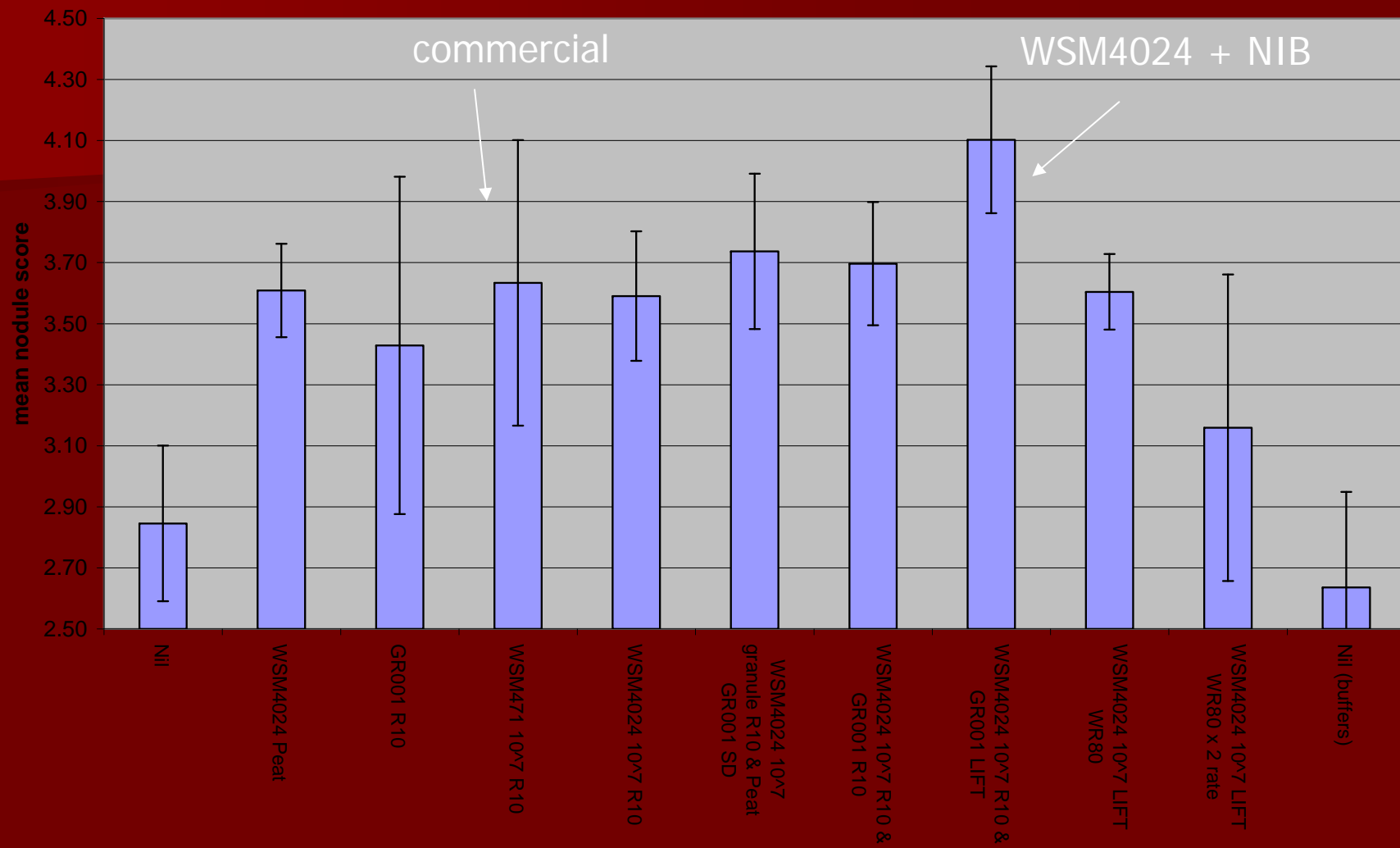
Chile

Carrabin

Yelbenie

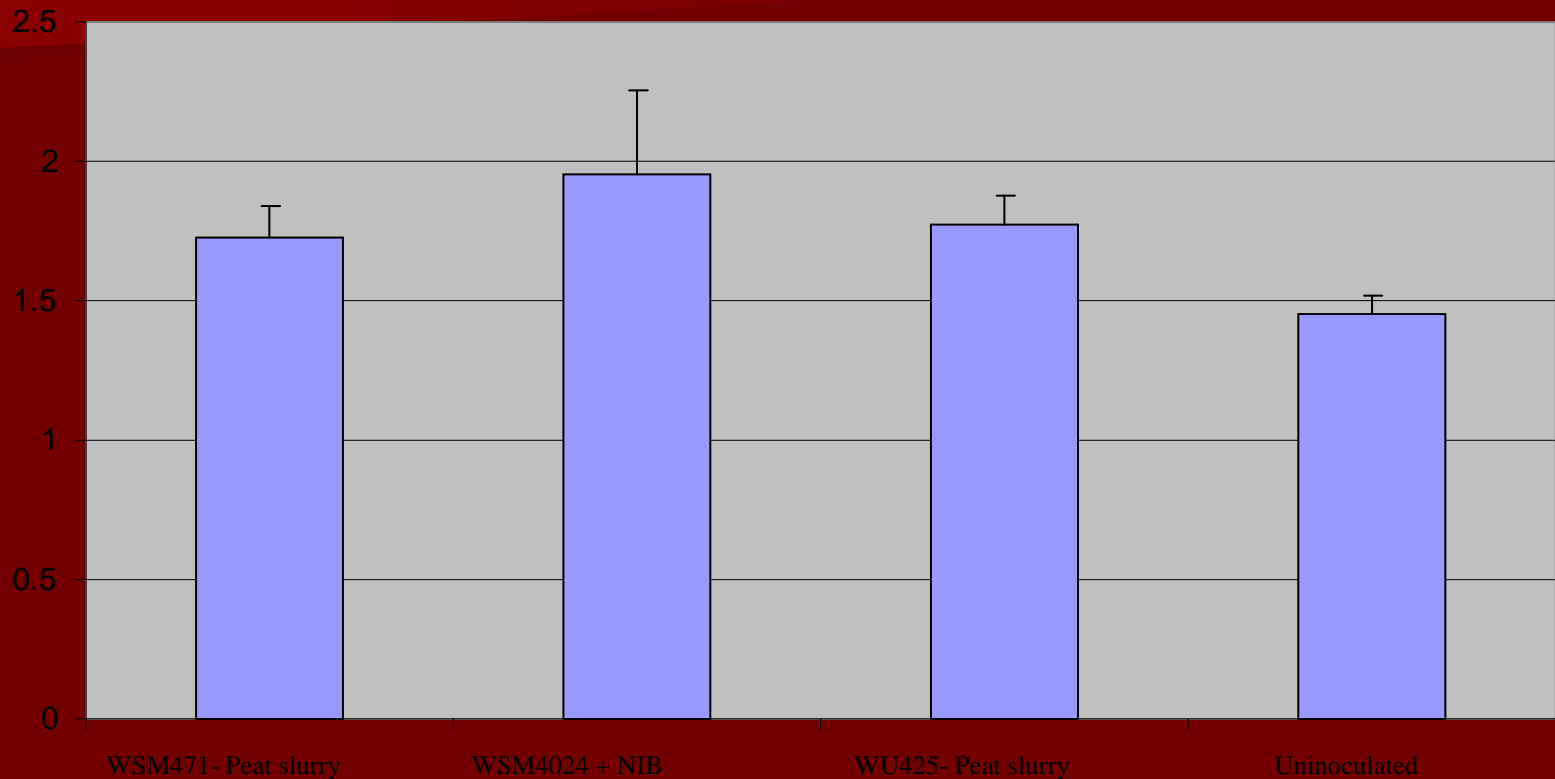
# Is there any evidence that we can influence Lupin nodulation in the field scenario?

- Lupin rnb survive in acid sandy soils at  $>10^5$  cells / g (Chatel et al 1970, McInnes et al 2002)
- It is difficult to achieve a response to inoculation when background numbers are  $>10^3$  / g soil (Thies et al 2005).



Early nodulation scores, Northern sandplains

# Seed yield kg/plot



25% increase in seed yield through inoculation with superior strain + NIB, Seed protein slightly increased

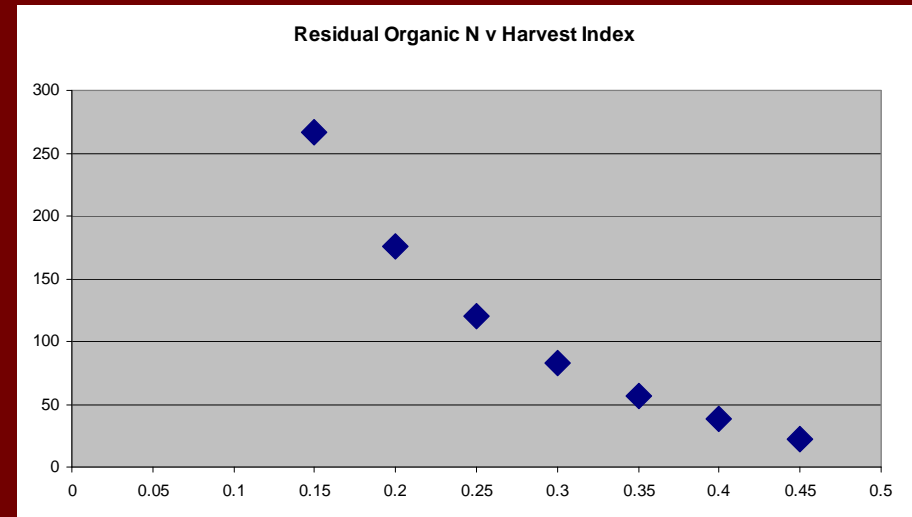
If we can influence nodulation, can we increase Lupin N fixation and will this lead to reduced N fertilisation of cereals?

N fertilisation of cereals is the single greatest contributor to greenhouse gases in cropping in Australia.

Farmers growing cereals after lupins are routinely applying 100 kg / ha N

# Residual benefits of fixed N (RON)

- Residual benefits of N fixed by lupin are very sensitive to Harvest Index
- Increasing the HI has been an aim of lupin breeding

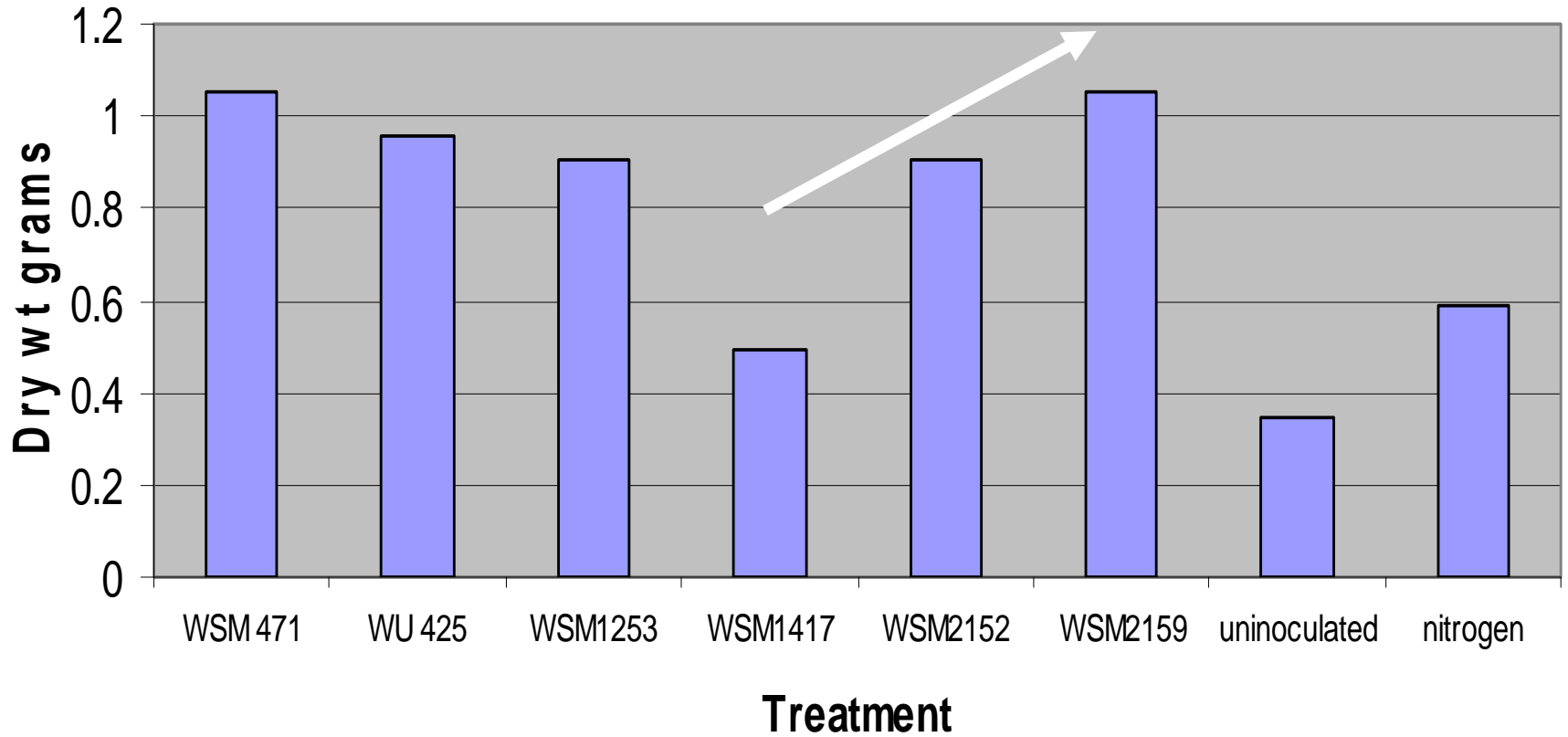


from a 2 tonne / ha crop,  
Bowden and Burgess, 1993

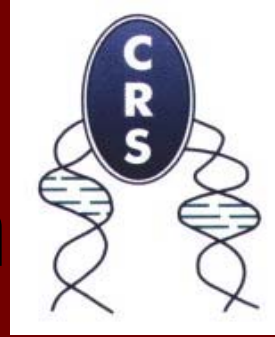
# Can we increase RON?

- Values of RON are based on 2.75% N in tops, but if this is raised to 3.25% by increased N fixation, residuals are greater.
- e.g. 4t/ha of lupin tops at 2.75% N contains 111 kgN/ha. 1t/ha grain at 5% N removes 50kg, leaving 61kg N/ha in the residues. At 3.25% N, 130 kg N/ha is fixed, leaving 80 kgN/ha after harvest, an increase of 31%.

# Lupinus angustifolius

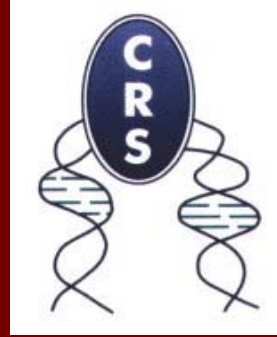


# Concluding remarks..do we have answers to the nodulation anomalies?



- *L. cosentirii* probably nodulated more readily because it was frequently found in coastal regions where the accidentally introduced rnb originally colonised,
- and perhaps because it is a more obligate symbiont?
- The cross-over from *L. cosentirii* isolates D25 and D24 to native legumes probably arose because some of the European lupin rnb have a broad enough set of nod genes to do so
- The nodulation of *O. compressus* by isolates from *Daviesii* probably represents the reverse of the above situation i.e. European rnb able to nodulate the native legumes, then being transferred back to *Ornithopus*.

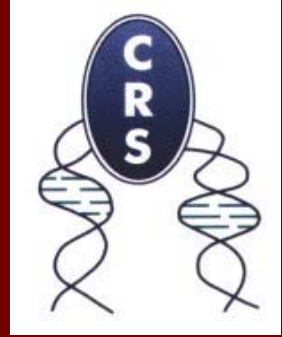
# Conclusions ctd.



- The nodule occupants of lupin crops are strains of *Bradyrhizobium* that arrived accidentally to WA, and colonised our soils
- These strains differ in their capacity to fix N with lupins by as much as 50%
- Inoculation with elite strains can affect nodulation occupancy, rate of nodulation, N fixation and subsequent N accrual into the farming system

- By combining the newer inoculation technologies with better strains of lupin bacteria, perhaps in association with NIB, we can increase N fixation in lupin crops

# acknowledgements



- Mark Seymour and Bob French (DAFWA) for surveys of lupin fields
- Greg Shea (DAFWA) for historical research
- CRS colleagues
- GRDC and MU for funding to the program
- Conference organisers