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Pasture legumes in the mixed farming zones of WA and NSW: shifting the baseline

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Abstract

The traditional legume feedbase has diminished in quality in mixed farming zones of WA and NSW over the last 25 years as a consequence of continuous cropping and climate variability. Alternative pasture legumes with physiological characteristics that make them more resilient to perturbations have been domesticated, and are now available for implementation to improve the feedbase. This project by agronomists in WA and NSW associated with Murdoch University, Charles Sturt University and DPIRD, and has given producers the information to adopt, summer sow and manage these alternative pasture legumes. Grower Groups were able to evaluate the benefits at the paddock scale. The new pasture legumes carried stock at higher numbers through difficult winters, and provided the basis of better N nutrition to animals and crops, delivering up to $1200 per ha. in increased meat production. Three hundred tonnes of pink serradella were delivered to one seed works in WA in 2016/2017, to be prepared for summer sowing. Twenty-seven articles in rural newspapers and social media were aired on the project in 2016 and 2017, again indicating the popularity of the topic. Producers have now been given the confidence to adopt alternative pasture legumes in situations where sub clover has been unreliable.

Executive summary

This feedbase improvement project arose because of several key issues confronting producers in the mixed farming zones of WA and NSW:

- The WA wheatbelt, formerly a home to some 33 million sheep, by 2010 had seen a decline in sheep numbers to slightly more than 14 million. In the same period in NSW sheep numbers fell from 48 to 27 million.
- The ley-farming system described by Donald (1965), based upon sub-clover (or medic) growing for several seasons followed by 1 or 2 crops, had evolved (with an intensification of cropping) to 3 or 4 successive crops followed by fallow
- Sub-clover, the mainstay of this rotation on acid soils, had insufficient hard seed levels to persist strongly in these new rotations, so the fallow years between crops became, essentially, a weedy pasture with low legume content
- In NSW similar trends were seen, plus a series of drought years from 2001 also seriously mitigated against sub-clover survival.
- Rainfall became more variable, with less well-defined seasonality, adding further stresses to traditional shallow-rooted pasture production, and reproduction
- Residues of the herbicides employed in the crop sequences to keep them weed free were detrimental to clover (and other legumes) root growth and nodulation.
- A recent producer survey (n=287) found producers considered that their feedbase limited their ability to sell or maintain livestock at the weight or condition they desired 50% of the time. They cited poor persistence, lack of information on pasture species to sow and lack of pasture management information as key issues to be addressed to overcome this problem (Hackney et al. 2017b)

In the decade before these changes in land use and pasture legume performance unfolded, a pasture legume selection program initiated at CLIMA (an early CRC based in WA), followed by a national pasture program -NAPLIP (funded at times by the major RDCs) produced a series of alternative pasture legumes for acid and difficult soils (Howieson et al 2000). These legumes, such as serradella and biserrula, were shown to have morphological traits that allowed them to grow on acid sandy soils and persist through intensive crop rotations (Loi et al 2005). However, uptake of these new legumes was slow in WA, and limited in NSW (Dear et al 2002). An analysis of the barriers to adoption showed that the cost and opportunity cost of pasture renovation were the primary cause of the poor uptake, followed by uncertainty about the performance of the new legumes (Loi et al. 2008; Hackney et al. 2008). In addressing the first impediment, Drs Nutt and Loi, working at the time at DAFWA, developed a system of sowing the hardseeded legumes without the need for (expensive) scarification. The seeds/pods were harvested on-farm then sown in late February, all with conventional farmer machinery. Sowing in summer allowed the temperature extremes to break the hard seed dormancy, such that germination occurred on rains after March (Loi et al. 2012; Hackney et al. 2015). Concomitant with this was the development of rhizobia preserved in dry clay granules that could withstand being sown at the height of summer (Carr et al. 2006). Thus, the opportunity of sowing legume pastures when farmers had time available, before the winter cropping season, became reality, and two major impediments to out-of -season sowing were overcome. These were how to break seed dormancy and how to deliver live rhizobia.

Having these new “tools” available provided the incentive for MLA and AWI to invest in the current feedbase project, whose aims were to extend the use of the alternative hard seeded legumes into a wider range of regions in WA and NSW, and to evaluate which cultivars were suitable for the summer sowing (and related) approaches. The premise of the project was that mixed farmers could be convinced to focus on pasture improvement with the new technologies, and therefore arrest the decline in the sub-clover feedbase, and the slide in sheep numbers in the wheatbelt regions.

In the first years of experimentation and demonstration, Dr Hackney discovered that the hard seed breakdown patterns of the alternative legumes were very different in central and southern NSW to that pattern which had been described in WA. Dr Hackney showed that, in fact, a very wide suite of legumes were suitable for summer sowing, whereas in WA farmers are limited to summer sowing the pink serradella cv Margurita, and Bartolo bladder clover (Hackney and Quinn 2015). With this new suite of information, by 2015 researchers in the project were able to confidently extend the principles of summer sowing and twin-sowing widely throughout NSW to include areas encompassed by the Central West Slopes and Plains, North-West Slopes, South-West Slopes and Riverina and in the medium rainfall wheatbelts of WA.

To simplify the recommendations for adoption, Dr Loi developed a strong uptake message with the “Six Golden Rules” which included removal of SU herbcides from the program in the years before sowing the legumes. Youtube videos were prepared with early adopting growers providing testimony, and experts in the program addressing key approaches.

Impact: In terms of Industry benefits, the project has provided a pasture renovation program for acid soils in medium rainfall mixed farming zones of WA and NSW that is affordable and resilient. The cost of re-sowing traditional pastures in the conventional manner is too high for many producers to be considered as a frequent event. With the new legumes, if farmers grow their own seed in nursery paddocks, the renovations can be implemented for less than $60 per ha, and these costs can be amortised over 20 years. More than half of these years may be crop seasons, and the legumes and rhizobia survive in the soil until a “legume on demand” is required to break the disease cycle and restore soil fertility. Appropriate legume species for the regions (375-550 mm annual rainfall) have been identified and extension materials on how to implement them are available.

It is difficult to quantify the benefits of this impact. Carrying capacities take a few years to alter, as farmers become more confident with the new pastures. In 2017, when WA experienced a decile 1 winter drought, the summer-sown pastures “allowed us to keep sheep on the farm”, as they grew well in the autumn rains, survived the drought, then grew vigorously in the spring. This is an important and revolutionary outcome, but impossible to quantify, except in the context that farmer did not de-stock their property nor did they have to supplementary feed. However, in controlled studies, the alternative legumes delivered up to $1200 in increased meat production, per hectare. Towards the end of the project, the N benefit to cropping from the legumes became of interest, with several farmers reducing the fertiliser N applied to crops by 80% if grown on good legume residues. The case study on Mailrock Farm in WA (Butcher and Butcher 2015) provides a good analysis of the integrated benefits of summer sowing the alternative pasture legumes.

Grower group demonstration trials were utilised to assist us to quantify the benefit of the new pastures to animal production. During the difficult year 2014 at Esperance, WA, summer sown biserrula carried 7.2 DSE/ha, whereas stock were removed from the sub clover (control) paddocks. Margurita pink serradella supported 18 DSE/ha through a poor winter in 2015 at Brookton. During the millennium drought in NSW, farmer Mike O’Hare, west of Temora, planted biserrula when his sub clover pastures disappeared and reported “the introduction of hard-seeded pasture legumes, notably biserrula, was right up there with some of the biggest innovations during my time in farming, including semi-dwarf wheats, canola and direct drilling.” Mr O’Hare has recently received the 2017 NSW Farmer of the Year Award for his contribution to and innovation in agriculture which includes the adoption of hardseeded legumes and “on-demand” pastures across his entire property.

Another assessment of impact is estimating uptake. Uptake was substantial over 2015-2017 and the estimation (based on data from seed cleaners) is that 100, 000 ha of summer sown legumes are in place in WA and 20, 000 ha in NSW. In 2017, over 300 tonnes of Margurita serradella pod were delivered to one seed cleaner in WA, destined for summer sowing.

Other less tangible assessments of impact are knowledge dissemination and how the work has influenced ongoing research activities. There are several indicators from this project. Firstly, outcomes from the project are delivered annually in lectures to the >250 undergraduate students enrolled in Animal Science, and in Crop and Pasture Science, at Murdoch University and a similar number of students at CSU in Bachelor of Agriculture and B. Agricultural Economics degrees. Secondly, Dr Hayley Norman at CSIRO (Floreat) began researching the over-summer benefits of serradella residues, and provided key knowledge on the sheep health value of serradella as a conserved fodder. This was previously greatly underestimated compared with traditional pasture species, whose residues deteriorate rapidly over summer. Thirdly, based on this project, a large RRD4P application was prepared by the research team with the intention of extending the technologies to lower rainfall zones of WA, SA, NSW and Victoria. And finally, a second RRD4P program is currently researching the P efficiency of serradella across southern Australia, so agronomists can make firm recommendations on fertiliser regimes that should also lower the costs of feedbase maintenance for producers. These are major impacts of this project.

In terms of technical impacts of the project, a technique for identifying the rhizobial strain inside nodules, without the need for culturing the bacteria, was developed (MALDI-ID) in WA and its refinement as a kit for producers is proceeding. A nodulation survey in the target regions of the project found more than 90% of legume-based pastures (n=225) had poor nodulation (Hackney et al. 2017a). The pastures surveyed were principally sub clover and/or medic based and soil analysis revealed more than 50% of paddocks had a pH_{Ca}<5.0 and has raised the question of how to increase N- fixation from these pastures. This project has also mentored and directly resourced the training of PhD student Lucy Watt, at CSU (Supervisors: Professor Michael Friend, Dr Belinda Hackney, John Piltz (NSW DPI) and Gaye Krebs), PhD student Armanuel Asrat, and 3 Honours students (Tom Edwards, Samantha Lubcke and Rob Harrison) at the Centre for Rhizobium Studies / Animal Sciences Group, Murdoch University. Co-supervisors were Professor John Howieson, Dr Andrew Thompson, Dr Ron Yates (DPIRD/MU) and Dr Brad Nutt (MU).

In terms of forward planning for feedbase research, this project makes a strong case for investment in a breeding program for the alternative annual legumes. Biserrula, for example, has never been the subject of a focused breeding program, yet is considered by some to be the “sub clover of the new century”. Breeding targets for biserrula would be earlier maturing varieties with reduced phytosensitivity (the latter issue being a major disincentive to adoption for some producers). A farmer view of biserrula can be read at this link. http://www.theland.com.au/story/5048175/biserrula-rules-the-farm/?cs=4961

During this project DAFWA made a decision (in 2014) to withdraw from pasture science, and to privatise breeding of sub-clover to an International company. To ensure the skill base in pasture legume breeding for alternative species was not lost in WA, a small breeding program began at Murdoch University in 2015, with the employment of Dr Brad Nutt. The first products from this program will be commercialised in 2018 and include earlier maturing serradella varieties suited for summer sowing.

This project met all its stated eight aims (P13) and was co-funded from 2012-2016 by Australian Wool Innovation (AWI).

Conclusions:

It is clear that the contemporary pressures on pasture legumes and rhizobia, such as the intensification of cropping and variable rainfall, will remain into the foreseeable future. This is impacting the production from sub-clover and medics in some regions. Alternative legumes, with resilient rhizobia that can tolerate these stresses, must be made available for producers to have confidence in their pasture feedbase, in ley-farming systems. We believe this project has set the blueprint for feedbase research, management and sheep production in southern Australia for the current century. The various RDCs which invest in pasture research and breeding will need to decide whether the legumes that inspired Donald (1965) can, after nearly 100 years of research, be further improved to meet contemporary challenges in pasture production, or whether new pasture legumes to take the animal industries forward in southern Australia for the next 100 years, such as those reported here, have a greater potential return on research investment.

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1 Background

1.1 Ley-farming in southern Australia

1.1.1 The first 100 years

The ley-farming systems of southern Australia are considered some of the most advanced low input agro-ecosystems in the world (Keating and Carberry, 2010). They are entirely based upon annual plants and have made a fundamental contribution to society's prosperity and maintenance of soil fertility, for nearly 100 years. This is despite the majority of the regions having relatively poor soil fertility and low (250-500 mm) annual rainfall (Moore, 2001). Notwithstanding intermittent advances in farming technology and machinery during the 18th and 19th centuries, crop yields did not increase above those achieved by the first European settlers to Australia until the implementation of (annual) legume-based ley-farming in the 20th century (Donald, 1965; Puckridge and French, 1983). Perhaps as a consequence of this success, the development of well adapted legumes for similar pasture-based farming systems has become a primary focus of many research programs, throughout the developed and developing world, for over 50 years (Miles, 2001).

However, in a recent review, Crews et al (2016) voiced concerns that farming annual plants represents the most highly disturbed terrestrial activity on the planet; a practice they consider to be unstainable by many measures. Ley-farming systems that are based on self-regenerating annual legumes grown in rotation with annual crops go part of the way to addressing the disturbance and sustainability concerns about annual-plant based agriculture voiced by Crews et al (2016), because in the pasture phase the soil is not tilled, and N-rich organic residues from the pasture legumes are returned to the soil. The pasture phase traditionally extends for several years, and the disturbance to the system is greatly reduced by the absence of tillage in these years. It remains, however, a challenge to achieve growth in productivity within these particular agro-ecosystems without rescinding sustainability (Schoknecht, 2015).

In the traditional ley-farming system which rotates between crop and pasture phases as described by Donald (1965) the pasture phase is usually a mixed sward comprised of self-regenerating annual plants, including legumes. A legume dominant pasture can increase subsequent crop yields (Puckridge and French, 1983). If managed for legume dominance, this phase can increase soil fertility and impede the build-up of pests and diseases that occur in the crop phase (Angus and Peoples, 2012). Animals also graze the pasture, providing meat and wool while simultaneously helping to control weeds by eating them (Loi et al., 2005). In reviewing ley-farming in Australia, Donald (1965) wrote "*the use of fertilized, leguminous pastures stands as the greatest factor of favourable environmental change in our agriculture since first settlement*".

However, the ley-farming referred to by Donald (1965) has evolved in the 50 years since that review, in response to a series of recent biological, climatic and economic challenges (Howieson et al 2000). These are, briefly, reduced and less reliable rainfall, increased soil acidity, widespread use of herbicides and intensification of cropping (the latter because of the greater profitability of grain production enterprises relative to animal-based enterprises) (Anwar et al., 2015). As the farming systems changed because of these challenges the success of the key pasture legumes that inspired Donald's (1965) review - *Trifolium subterraneum* (sub-clover) and *Medicago* spp. (medic) has been compromised. As a result, over the past two decades the feedbase has deteriorated, and livestock numbers in the wheatbelt parts of southern Australia decreased as farmers concentrated on growing crops. In WA, sheep numbers decreased from 33 to 14 million, and in NSW from 48 to 27 million in this period (ABARE 2016). In recognition of this, a recent analysis of future breeding opportunities for the feedbase (Revell et al 2013) considered:

"The deterioration of annual legume seed banks due to the impacts of a more variable climate is likely to result in more frequent re-sowing of pastures. Indeed, the use of tactical, short- term or 'phase' pastures in cropping systems is likely to become more attractive to farmers, rather than trying to manage self-regenerating pastures over long time frames and through multiple cropping phases."

Whilst those authors were cognizant of the challenges facing conventional regenerating pastures, their suggested response (of moving to frequent sowing of phase pastures) is unattractive for many producers. Like these producers, we too disagree with the view that phase pastures, resown regularly, represent the future, simply because the opportunity cost of re-establishment of pastures is too great to be considered as a frequent event. The phase system is also relatively inflexible in terms of allowing opportunity for rapid shifts in crop to pasture and therefore crop to livestock ratios, due to the time lag in the pasture establishment phase. Such phase systems therefore do not allow producers to readily flex and change their enterprise balance to capitalise on commodity price shifts or increasingly reliable longer-term climatic forecasts. This project has set out to prove that, given the right legume species, rhizobia and management, a ley-farming system based upon regenerating legumes, is achievable in the current circumstances.

1.1.2 The next 100 years

In anticipation of the mounting challenges to the feedbase already outlined, a sustained research and development effort in alternative annual legumes began in 1993 (CLIMA 1993-

2000, NAPLIP 2000-2007). These investments set the seeds of change in the pasture legume industry. In the ensuing 25 years a number of new legumes with different physiology to sub clover and medic were domesticated. The new legumes were very hardseeded, had deeper roots, were suitable for (aerial) seed harvesting and had more resilient symbioses with acid tolerant rhizobia than clovers and medics (Howieson et al 2000, Loi et al 2005, Nichols et al 2007). Excitement grew about the potential of these alternative legumes, albeit in isolated pockets of the wheatbelt in Western Australia, as producers and scientists came to understand the behaviour of the legumes in intensive cropping systems. Biserrula, for example, was in 2005 excitedly reported to have startling regeneration after five successive crops in the Chapman valley, to the north of Perth.

This current feedbase project, then, was borne out of the need to increase the adoption rate of some of these new legumes, and explore their introduction into mainstream grazing and cropping enterprises of WA and NSW. If successful, we expected the project to help arrest the decline in animal numbers in these wheatbelt regions. It would also begin to reduce the decline in soil fertility (and the increasing financial risk to farming) posed by intensification of cropping, where yields are more frequently compromised by frost and other severe weather events.

Aims:
The specific aims of the project were to:

1. Assess hard seed breakdown patterns of alternative legumes in the regional environments of WA and NSW, and how these patterns are affected by climate
2. Assess whether these legumes can be established via summer-or twin sowing approaches, or variations on them, and monitor pasture production
3. Demonstrate, with grower groups, these novel pasture legumes, how to harvest, sow, and their resilience to climate and management
4. Demonstrate improved livestock production through enhanced growth and reproduction, and earlier access to markets, through a more resilient, nutritional feedbase and its residues
5. Assess N fixation and nodulation with the alternative establishment methodologies, and to develop a more user friendly approach to this task
6. Extend information more widely to producers under the headings of “resilient low-cost pasture legumes”, “legumes on demand” and “free N farming” (terms we invented), using modern media opportunities.
7. Develop recommendations to maintain the pasture legumes through multiple crops, to manage weeds and to minimize damage from herbicide residues
8. Increase the adoption of alternative legumes in the medium rainfall areas of WA and southern NSW.

2 Project objectives

2.1 Shifting the baseline

In this project, through changing the normal timing of the sowing operation for annual legumes, we sought to increase the uptake of hard seeded annual legume species in the pasture feedbase in mixed farming zones which receive 375-550 mm annual rainfall. The reason for the project is that the hard seeded legumes are more productive than sub-clover in many modern situations, and do not suffer the red clover syndrome. Further, they produce high quality green feed for an extended season, and the pods (particularly serradella and biserrula) are a nutritious source of protein and fats over summer. However their uptake by producers has been slow because of several barriers to adoption.

In this project we are guiding farmers to produce their own seed, and with granular inoculants, sow the pastures for forage improvement in a window between mid-February – early March, when labour and machinery on farms is available. This approach to establishment (summer sowing) should overcome the barriers to uptake of cost, opportunity and knowledge, and greatly expand the extension opportunities. The project commenced in WA in February 2013, and in NSW in summer 2013/14 (preliminary trials were sown in 2012 at Beckom to test the technology).

2.1.1 Objectives

1. Develop recommendations for reliable establishment of improved annual legume species (including serradellas, biserrula, prima and bladder clover) based on summer dry-sowing of seed (including the appropriate cultivars and rhizobia) for the drier regions of WA and NSW and the moister conditions of central and southern NSW.
2. Develop recommendations for reliable establishment of pastures based on smaller seeded legumes, such as biserrula and prima clover.
3. Quantify the animal performance from improved legume species where summer sowing has been successful.
4. Evaluate the option of grazing serradella pods as an alternative to summer grain feeding.
5. Develop grazing strategies for the improved legume species to develop a long-term seedbank, thereby ensuring persistence.
6. Investigate the population ecology of clover-nodulating rhizobia to assist in understanding of the role of rhizobia in sub-clover decline, leading to options for mitigating sub-clover pasture decline and thereby improving nitrogen fixation from existing clover stands.
7. Develop extension packages that increase the uptake of these new legumes and which demonstrate how farmers can increase the reliability of legume pastures for red meat and wool production. These will be extended to a wide range of stakeholders in the animal industries including producers, consultants and adivisors.

8. Train post-doctoral fellows and postgraduate students through the research undertaken to achieve the above objectives.

3 Methodology

A range of experimental approaches were employed. Where these are standard, a reference is given. Where these are unpublished, details of the procedures are provided. Statistical analyses, where appropriate, were with standard biometric approaches unless otherwise stated. There were broadly four types of work undertaken:

a) For comparison of sowing times we used large plots established with a tractor-drawn precision seeder, usually with 4 replicates in banks, and dimensions ranging from 1.2 m x 10 m, up to 5 m x 25 m.

b) For small trials to assess nodulation or hard seed breakdown, treatments were sown by hand into 2m x 2m plots, in randomised blocks with 4 replications. Controls were uninoculated plots, inoculum delivered as peat cultures, or local varieties for the hard seed studies. See Howieson and Dilworth (2016) Chapters 1, 8 and 10 for methods. Validation of nodulation data was by MALDI-ID complemented by isolation, purification and either a RAPD PCR reaction based on nod-directed primers or partial sequencing of the ribosomal 16S gene (Howieson and Dilworth [2016] Chapters 11-13).

c) For grower group demonstration trials we chose paddocks of 10-50 ha and implemented the available and appropriate research options in dimensions that suited the conventional, farmer-owned seeders, usually with replication. Controls were district best practice or neighbouring fields. These sites served several purposes. Animal production information was gathered where possible. Later in the project demonstration sites were more numerous and smaller (2-10 ha).

d) Field days were organised around these demonstrations, usually in June and October, and the researchers were able to assess any issues with up-scaling of outcomes in industrial-sized machinery. The sites also served as nursery paddocks for seed production for further trials in the region. Pre-season grower meetings were held in early February (2015-2017) in NSW to support new adopters in setting up seed nurseries and/or encourage improved strategies for management of existing pastures and to promote the use of hardseeded annual legumes where appropriate to soil and climatic challenges.

The following section summarises some specific methodologies for these four main approaches to delivering outcomes in the project.

3.1 Hard seed breakdown studies

Assessment of hardseed breakdown is a complex and time-consuming exercise. It involves enclosing samples of seed or pod (usually 100 units) in mesh pockets (to protect from insect predation), sowing them closed with thread, then burying them at the target depth (usually 1 cm) with 4 replicates and up to 10 times of sampling, in the target regions. The pockets are then retrieved on a monthly basis and the number of non-germinants calculated. In this project we were interested in how the regional differences in temperature and moisture interacted with hard seed breakdown of the range of legumes. For more detailed explanation of methods see Taylor (2004), Nutt (2012).

3.2 Summer v conventional sowing trials

Experiments were conducted within the regions of the main support grower groups in NSW and WA. Summer and conventional sowing time experiments were established at Alectown, Beckom, Corinella, Greenethorpe, Uranquinty and Coolamon, in NSW, and west Corrigin, Brookton, Babakin, Esperance, Cascade, Grasspatch and Condingup in WA. In later years these sites were expanded to neighbouring Shires. The legume species sown in NSW included biserrula (Casbah), French serradella (Margurita), yellow serradella (Avila, a new experimental line (NYS) and Santorini), bladder clover (Bartolo), gland clover (Prima) and subterranean clover (Dalkeith at all sites except Greenethorpe where Seaton Park was used). Arrowleaf clover (Cefalu) was added to the suite of legumes tested from Year 2 onwards. In WA, experiments were undertaken with Bartolo, Margurita, and NYS, with controls of Dalkeith sub clover where suitable. Plots were generally 5 m x 25 m, with 4 reps in randomised blocks.

Summer sowing using unscarified (clovers) or in-pod seed (serradellas and biserrula) was undertaken at all locations in the final week of February. Conventional sowing using scarified seed of all species was undertaken at all locations in the final week of May. This time was chosen for the conventional sowing time as it represents the average mid-season sowing window for pastures, after the cropping program is in place.

Data collected included seedling establishment, winter biomass, total seasonal biomass, seed yield, hard seed breakdown, and seedling re-establishment after crop. Some alternative strategies to summer sowing were also investigated as the needs arose. See Loi et al (2012) for detailed methods.

3.3 Nodulation surveys and strain ID methodologies

Nodule sampling was undertaken from a range of sites as operations allowed. See Howieson and Dilworth (2016) Chapters 2 and 8 for methods. The point of the surveys was several-fold – firstly to assess whether nodulation was achieved with the out-of season sowing methods, secondly to assess nodulation after the cropping phase, and thirdly to validate the strain in the nodules, using methodology under development such as MALDI-ID. To further support the development of MALDI-ID, a comprehensive survey of 225 paddocks was undertaken as part of this project in 2015 and 2016 and supported by Local Land Services (Central West, Central Tablelands, Riverina and South-East). Further sampling has occurred and was funded by various Local Land Service regions in 2017 with a total of more than 400 paddocks across NSW sampled.

The optimisation of bacterial identification using MALDI-TOF MS

Techniques for bacterial identification of nodule occupancy in legumes have relied upon cultural methodologies since 1895. This is time-consuming and expensive. In this project we took the opportunity to invoke the newest technologies in mass spectrometry and next gen sequencing available in the laboratories of the CRS at Murdoch University, to build a more time-efficient approach, using clover-nodulating rhizobia as a model.

Several rhizobial strains (including TA1, WU95, WSM409, WSM1325) have been used in the past as commercial inoculum for clover species in Australia. TA1 was isolated in Tasmania and served as an inoculant for perennial clover species and initially sub clover. However, this strain did not survive in the hot and dry summers of WA, where “second year clover mortality” was frequent (Chatel et al 1968), and therefore alternative strain were investigated. Naturalised clover plants were surveyed in WA in the 1960s and this produced a series of strains including WU95, which was commercialised in the 1970s. However WU95 has a very narrow host range for N fixation -its N fixation is confined to *T. subterraneum* (Howieson et al 2005), and so WSM1325 was released as an inoculant for all clovers used in southern Australia in 2005. As neither TA1 nor WU95 are effective at N fixation with the new aerial seeding clovers being evaluated in this project, but are frequent soil inhabitants and competitive for nodulation, we sought to develop rapid methods for assessing which strain was actually forming the nodules. We used modern mass spectrometry and sequencing methods that we reported in (Zeigler et al 2014). This is a brief description of the second approach that was developed in this project to validate the MALDI-tof approach:

Identifying RNB occupants of *Trifolium* spp. nodules, using universal primers and a Next Generation Sequencing approach

Strategy: Identify genes that are unique to WSM1325 (not found in other sequenced Rlt strains) and design primers for these genes that can be used in multiplex PCR to identify the presence/absence of WSM1325 in clover nodules.

PCR design and conditions (for general description of methods see Howieson and Dilworth 2016, Chs 11-13).

For the NGS a 2-step PCR was used (adapted from Gohl 2016). The first reaction was designed to amplify the selected gene and add 12bp of a known and conserved sequence to the flanking regions of the amplicon. Dual-barcoded primers in the second reaction of PCR were binding these flanking regions attaching a sample specific DNA barcode combination (Caporaso et al. 2012). PCR conditions were as follows: step 1, 98C for 1 min, 35 cycles of 98C for 15sec, 58C for 15sec and 72C for 30sec with a final extension of 72C for 7 mins. In step 2 cycle number was reduced to 25x and annealing temperature increased to 61C. Reactions were performed using Phusion Hi-Fidelity polymerase using HF buffer (Thermo

Fisher Scientific) in 20ul total volume. PCR products were pooled and sent for Illumina Miseq v3 300PE sequencing.

Data processing

Pair-end reads were joined using FLASh (Magoc 2011), and processed using custom-made Bash and Python scripts. Due to the presence of degenerated nucleotides in the primer sequence, reads were trimmed both from the sample-specific barcodes and primer binding sites. The resulting sequences were selected for the expected sequence length (443bp for thrC and 328bp for gyrB) and clustered using USEARCH with implemented UCHIME for chimera detection and removal (Edgar 2010) and Edgar et al (2011). Sequences obtained were aligned against local database of gyrB and thrC gene sequences.

Clover species used in glasshouse and field trials to validate nodule ID:

Trifolium spumosum (Bladder clover) cv Bartolo: Symbiotically specific; samples of field-grown nodules available

Trifolium subterraneum (sub clover) cv Dalkeith: More promiscuous; samples of field-grown nodules available

Trifolium vesiculosum (Arrowleaf clover) cv Cefalu: More promiscuous; samples of field-grown nodules available

International collaboration: Andrzej Tkacz (Post-Doc in Prof Phil Poole's lab, Oxford University) developed the primers and ran the MiSeq.

3.4 Demonstration trials with grower groups

These trials became our most valuable tool for engaging farmers, and also for building knowledge on animal performance when farmers had the opportunity for grazing the new pasture legumes. They usually took two forms:

- Large scale (10-50 ha) participatory on-farm sowings throughout the medium rainfall regions on major soil types with participating grower groups and individual farmers. They involved the use of most suitable species for that soil type-rainfall area and comparison of its performance against the farmers current rotation system. These were used as case studies and to facilitate wider scale adoption
- Optimal sowing and rotation strategies – by 2015 we had sufficient experience to sow a suite of smaller (2-10 ha) experimental / demonstration sites. A range of rotation strategies were selected that included different lengths of cropping (e.g. 1:1, 1:2, 1:4, 2:1, 2:2) to assess any developing weed issues and impact on legume seed reserves, and rhizobial survival.

3.4.1 Economic implications

Where made possible by data collection, we took the opportunity to assess the economic implications of changes in the pasture composition and production on animal performance and value. These assessments were restricted to the large demonstration experiments where growers were able to give estimates of changed outcomes for their animals. Examples are provided below.

3.4.2 Grazing summer sown Biserrula at Esperance to assess animal performance

Four adjoining paddocks were monitored for stock production, three of which were summer sown into biserrula (Casbah) in 2012, cropped in 2013, and regenerated into biserrula in 2014. The comparator paddock was improved sub clover cropped in 2013, regenerating in 2014. Growing conditions at Esperance during the winter of 2014 were very harsh, with an early break but followed by a significant winter drought. Paddock 46B, the control paddock, was initially sub clover dominant, but the incidence of red clover syndrome reduced the vigour of this species, to the point where capeweed eventually dominated. The three replicates of biserrula paddocks were able to continue growth through the dry winter months. From the animal grazing records for the winter, for each paddock, grazing days per ha were calculated.

Paddock sizes were as follows

45C – 149ha –biserrula cv Casbah

45D – 108ha - biserrula cv Casbah

45E – 61ha - biserrula cv Casbah

46B – 132ha – sub-clover / capeweed

Experimental protocol: Stock Manager to graze paddocks according to their winter performance whilst ensuring animal quality score remains high. Grazing days and production were recorded.

3.4.3 Replicated meat and wool production experiment at CSU

This experiment at Wagga Wagga consisted of four replicates each of the biserrula varieties Casbah and Mauro. These varieties differ slightly in terms of early vigour, time to maturity and hard seed levels. Three classes of sheep were assessed simultaneously in this experiment – lactating merino ewes, their lambs, and weaned prime lambs (white Suffolk cross and black Suffolk first cross). The rationale for use of the pigmented and non-pigmented lambs in this study was to assess the potential of pigmentation to mitigate against clinical signs of photosensitisation (see MLA funded project ‘Understanding photosensitisatrion in livestock grazing the pasture legume *Biserrula pelecinus*’ led by Dr Jane Quinn). Sheep were grazed for a period of six weeks commencing in late September through to the end of October (plots were in the early stages of flowering at the commencement of the experiment). The average stocking rate across the site for the duration of the experiment was 17 DSE/ha. In addition to sheep grazing the biserrula plots, the remainder of the merino ewe-lamb flock, grazing on naturalised pastures at CSU (5 DSE/ha) (predominately annual ryegrass, barley grass, sub clover and volunteer legumes), were also weighed to compare performance.

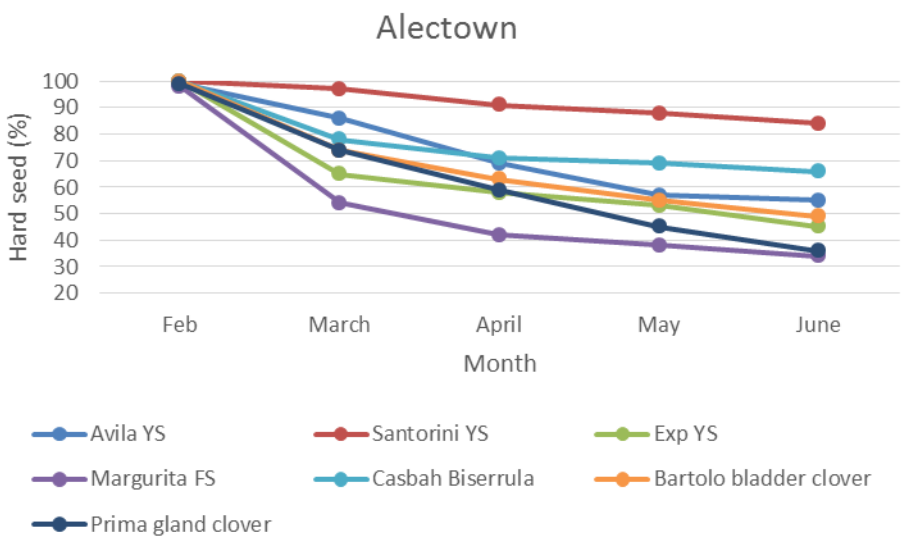
4 Results

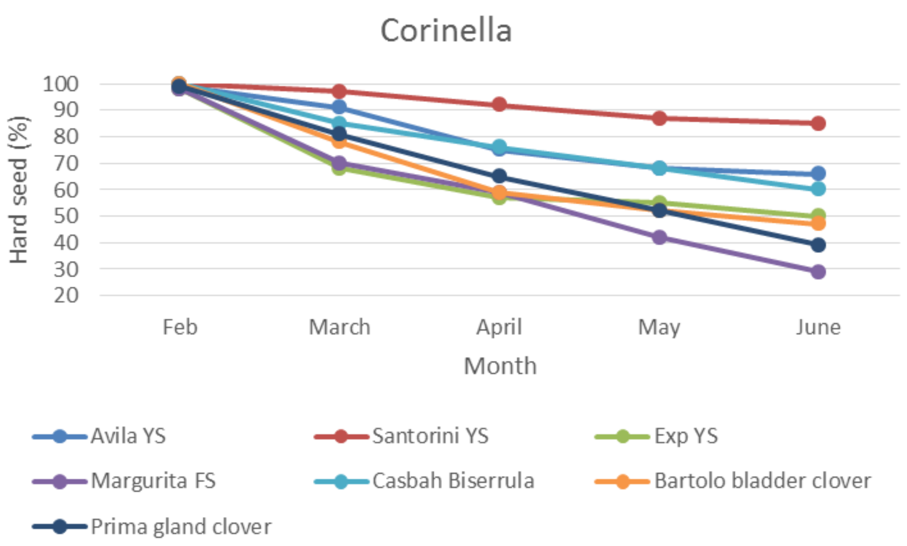
In this section a set of highlights of the research are provided, under each of the main headings, to give a flavour of the outcomes. Reference will be made to appendices or publications for the full data.

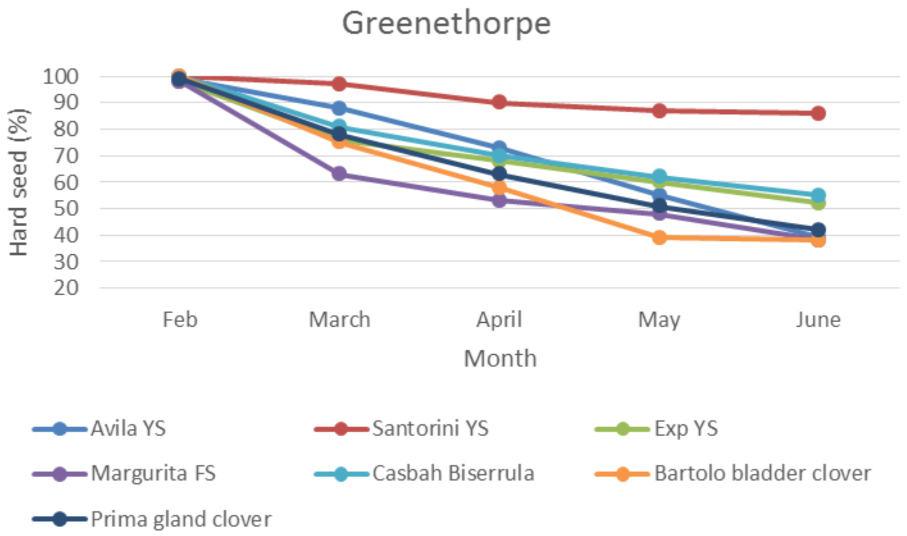
4.1 Hard seed breakdown studies in NSW

These studies were undertaken early in the project to ascertain which of the alternative hardseeded annual legumes might be suitable for summer sowing in NSW. At the time, Bartolo bladder clover and Margurita were established options in the WA regions, with the new yellow serradella (NYS) as a near commercial option. However little was known about how the hard seed would form, then break down, under the milder spring and summer conditions of inland NSW.

Results varied considerably between sites (Fig. 1). The consistent finding was that Santorini yellow serradella maintained very high hard seed levels, even at the Alectown site, which received more than 250 mm rainfall in the period February to April alone. Many of the other species/varieties varied considerably between locations. Casbah biserrula for example, maintained more than 70% hard seed at Uranquinty (the driest site over summer) but had reduced to 50% hard seed at Greenethorpe at the end of sampling period. Similarly, varieties such as Margurita French serradella and Prima gland clover also showed considerable variability maintaining 30-50% hard seed depending on location. Additionally, the pattern of softening varied within variety, between locations. For example, at Alectown, varieties such as Margurita French serradella had much more rapid softening in the first month compared to the same variety at Uranquinty. Softening patterns in NSW, based on this one year of data, differed considerably from previous research in WA (Loi et al 2012). For biserrula, it explains why summer sowing with this species appears to be an option in NSW but not in WA, and also why second year regeneration is consistently observed in NSW but not in WA. It is critical that hard seed breakdown patterns be well understood as this is fundamental to maintaining annual legumes in the pasture or pasture-crop rotation. Hard seed breakdown patterns are represented below as an example.


Alectown
Hard seed (%)
100
90
80
70
60
50
40
30
20
Feb
March
April
May
June
Month
Avila YS
Santorini YS
Exp YS
Margurita FS
Casbah Biserrula
Bartolo bladder clover
Prima gland clover


Corinella
Hard seed (%)
100
90
80
70
60
50
40
30
20
Feb
March
April
May
June
Month
Avila YS
Santorini YS
Exp YS
Margurita FS
Casbah Biserrula
Bartolo bladder clover
Prima gland clover



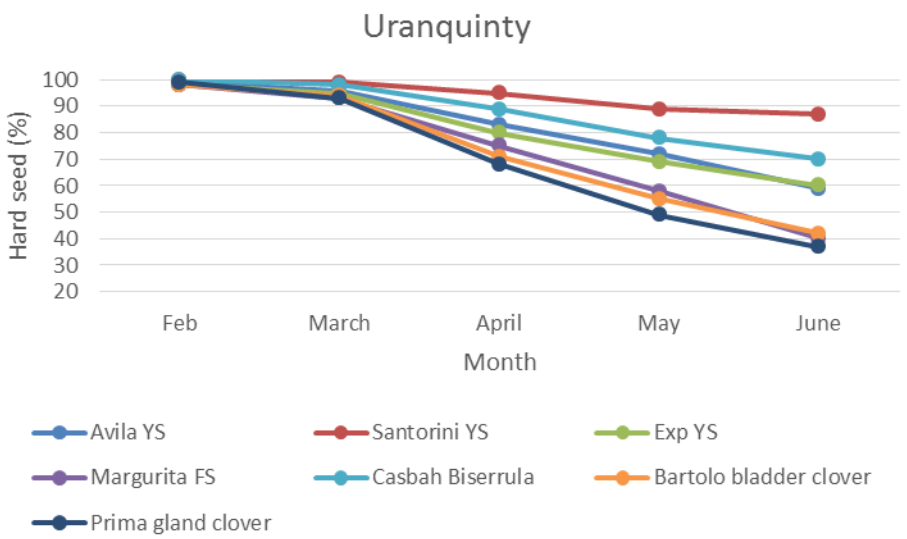


Fig. 1 Hard seed levels and breakdown patterns of a range of annual legume species/varieties measured on five occasions at four locations in NSW.

While a sound understanding of hard seed break down patterns was required for choosing specific species x region associations for use of establishment technologies such as summer sowing, it was equally important to understand how different species perform in grazed situations and how this may influence their potential for on-going recruitment in subsequent seasons. It is well known that residue levels in pastures affect the rate of hard seed break down and opportunity for annual legume recruitment. Further, there has been much speculation around the capacity of aerial seeding legumes to produce sufficient seed under grazing regimes of various intensity with the fear being that aerial legumes may not be able to produce sufficient seed for on-going recruitment. These hypotheses were tested by measuring the end-of-spring biomass along fixed transects in paddocks of new legume species and returning following the autumn break to assess the recruitment of annual legume species.

Strong relationships were found between residual spring herbage and plant density in the following autumn (Fig. 2).

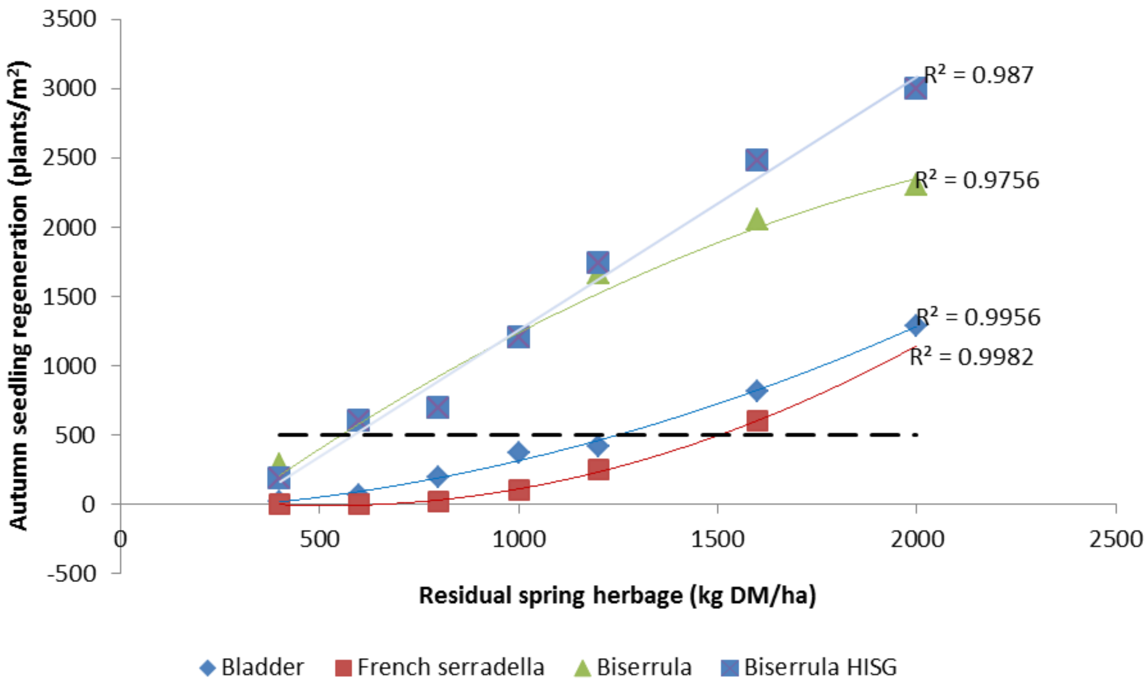


Fig. 2 The relationship between residual spring herbage (kg DM/ha) and the following autumn seedling regeneration (plants/m^2) for bladder clover, French serradella and biserrula at Beckom NSW with summer grazing at 6 DSE/ha and for biserrula at Charles Sturt University, Wagga Wagga where residue was grazed at high intensity over the summer period (HISG).

Higher quantities of residual spring herbage were required for bladder clover and French serradella in order to reach an acceptable plant density (500 plants/m^2) for subsequent herbage production. Both bladder clover and French serradella have larger seed than biserrula and hence a higher proportion of ingested seed of these species is digested by grazing livestock in comparison to biserrula. Even at the high summer grazing pressure used at CSU, biserrula was still able to achieve high plant regeneration counts across the range of spring residual herbage levels recorded.

4.2 Summer v conventional sowings

Using knowledge from the hardseed breakdown studies a range of trials comparing summer sowing, twin sowing and conventional winter sowing were undertaken. In WA, where there was a limit to the number of cultivars that could be summer sown (Bartolo and Margurita) because hard seed does not soften sufficiently, we also attempted some “strategic” dry sowing options. The driver for this was farmers wanting to accrue the benefits of summer sowing, but to include biserrula because of its popularity. The approach here was to sow inoculated and scarified seed in advance of the first autumn cold front.

Some examples of outcomes are presented below. For published studies refer to Nutt et al (2017) and Hackney and Quinn (2015).

4.2.1 Summer Sowing in NSW to establish the relative advantages and disadvantages of the approach to pasture establishment

The initial field trials comparing summer and conventional sowing were undertaken in NSW in 2014. Results from two of these sites are shown below (Figs. 3 and 4). With the exception of Santorini yellow serradella at Uranquinty, summer sowing of legumes resulted in a significant increase in peak spring herbage production compared to conventional sowing at both Uranquinty and Greenethorpe with a 1.7-15 fold increase in production measured where sowing occurred in February compared to May, depending on species and location. These results show that there is significant potential to increase first year pasture production through the use of summer sowing and that there are a wide range of species/varieties suitable for this purpose in NSW. Field trials in subsequent years also identified arrowleaf clover as highly suitable for this purpose and some of these results are shown later in this document.

The Greenethorpe site also showed the potential of summer sowing to compete strongly against major cropping weeds such as annual ryegrass (Fig. 4). Where legume species were sown in summer, they not only produced significantly more herbage, but they were also highly suppressive against annual ryegrass with negligible quantities found in the summer sowing treatments. In contrast, significantly higher quantities of annual ryegrass were found in conventionally sown treatments (knock down used at sowing) though both Casbah biserrula and Prima gland clover had significantly less annual ryegrass than other species sown in May. It should be noted that a knockdown herbicide (glyphosate 2 L/ha) was applied prior to both sowing events to control any weeds present. The farm in this study, like many through the mixed farming zone of WA and NSW, has a high level of resistance to Group A herbicides clethodim and haloxyfop and therefore the ability to use summer sowing to reduce annual ryegrass pressure is highly advantageous.

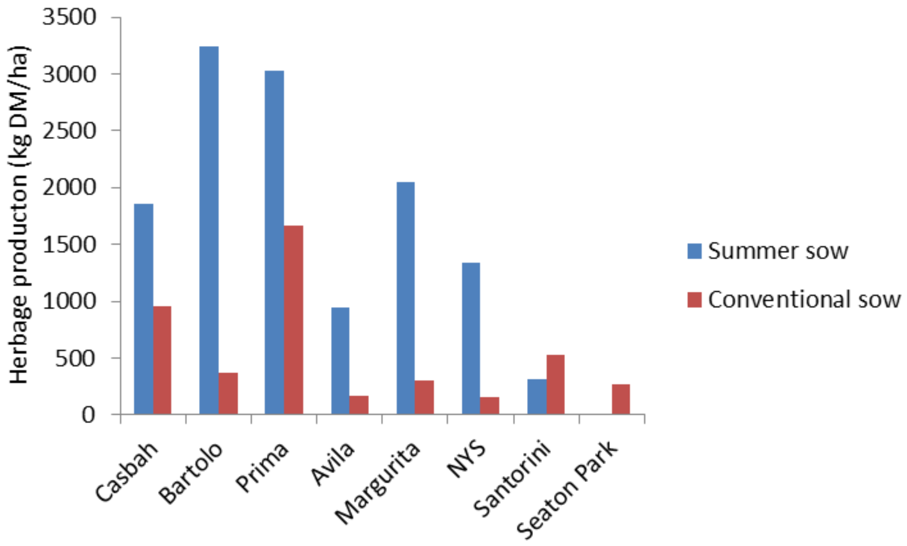


Fig. 3 Cumulative dry matter production of a range of annual legume species sown as a hard seed or in-pod in the last week of February 2014 (summer sow) compared to a conventional scarified seed sowing in the last week of May 2014 at Uranquinty, NSW. Final herbage measurements taken 14 October 2014.

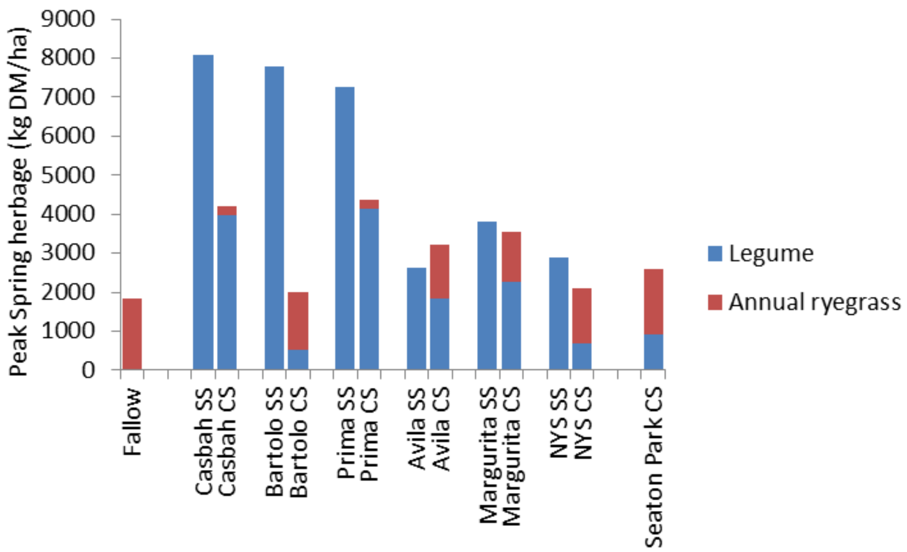


Fig. 4 Herbage production (kg DM/ha) of a range of annual legumes sown as hard seed or in-pod in the final week of February 2014 (Summer sown, SS); the same legumes sown as scarified seed in late May (Conventional, CS), and the dry matter (kg/ha) of annual ryegrass present at sampling on 2 October 2014 at Greenethorpe, NSW.

4.2.2 Strategic dry sowing

4.2.2.1 Babakin

Strategic dry sowing was examined to determine the relative advantages and disadvantages of sowing scarified seed (of species which cannot be summer sown in WA) in advance of a cold front in autumn, compared to sowing into wet soil after weed control. There are major issues of how to deliver the inoculant in a variably wet soil, and also of weed control. Strategic dry sowing has been problematical for small seeded species in the past because of the competition from weeds. So, weed numbers and nodulation are important parameters to assess in this approach.

Strategic dry sowing should achieve earlier establishment at higher soil temperatures, therefore enabling greater herbage production prior to winter. It is potentially better suited to recently developed annual legumes because of their more rapidly developing and adventitious root systems enabling better early season survival. It is also undertaken before the cropping program, when labour is available. Of course, as with summer sowing, it is essential to keep the rhizobia alive during the dry period. This experiment also looked at whether any biserrula inoculant strains are better suited to dry sowing than WSM1497 (the commercial inoculant).

Methodology: A site was selected at Babakin (north Corrigin), WA. Average annual rainfall 290mm, soil type was a loamy sand with gravel at the surface, with soil pH_{Ca} 5.1. Two sowing dates were selected – an early dry sowing (8 April) and a second post-rainfall sowing (14 May). Plots used for the first sowing were sprayed with 500 g/ha Fusion plus wetter and second sowing times sprayed with 1.5 L/ha glyphosate plus wetter prior to sowing. Species sown were, arrowleaf clover (cv. Cefalu), bladder clover (cv. Bartolo), biserrula (cv. Casbah), gland clover (cv. Prima), French serradella (cv. Margurita), yellow serradella (cv. Santorini) and subterranean clover (cv. Dalkeith). All treatments were sown with 15 kg/ha scarified seed with 10 kg/ha ALOSCA granules of appropriate group. Uninoculated strips of Casbah biserrula, Bartolo bladder clover and Margurita French serradella were sown at each end of the trial at the first time of sowing, as were inoculated lines of Biserrula using four different rhizobial strains. Plots were grazed continuously from June 3. Measurement of establishment, nodulation, winter and total season herbage production and composition were made along with seed yield and any impact of any losses due to pests and/or diseases.

Weeds and productivity in strategic dry sowing

Winter herbage production varied considerably between strategic dry sowing and the conventional sowing time, as did pasture composition (Fig. 5). The key finding of winter herbage assessment was the higher weed burden in the strategic dry sowing treatment compared to the conventional sowing treatment, and the lower percentage contribution of sown legumes to total herbage production. However, winter production was always higher I SDS.

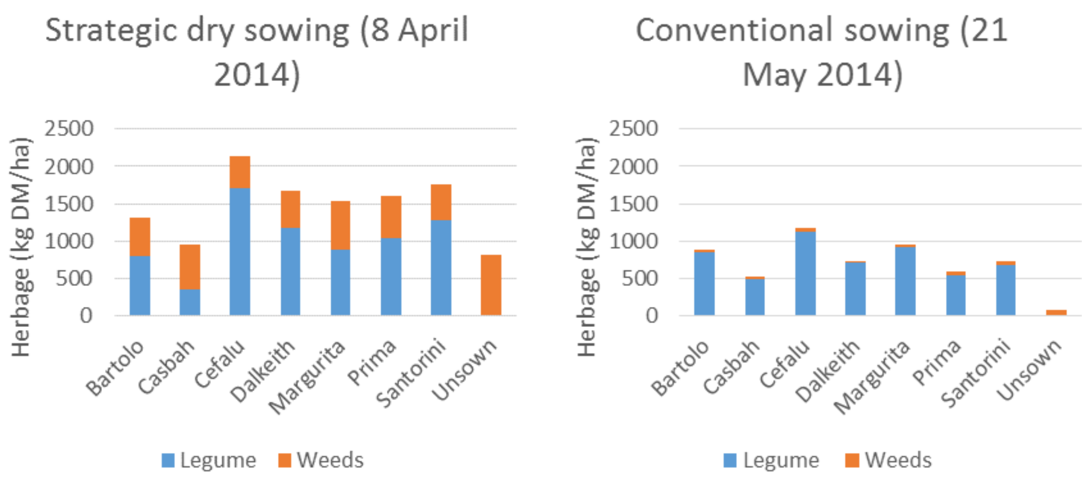


Fig. 5 Sown legume and weed species contribution to winter herbage production (9/7/2014) under strategic dry sowing (8 April 2014) and conventional sowing (21 May 2014) times at Babakin, WA for a range of annual pasture legumes and an unsown control treatment.

In general, weed contribution to total herbage present at senescence was lower in the conventional sowing time treatments compared to the strategic dry sowing treatment (Fig. 6). However, weed contribution to production was low at both sowing times for Margurita, Prima and Santorini with weeds accounting for less than 5% of total herbage present. Weed contribution to total herbage declined proportionally between the winter assessment and the senescence assessment for the strategic dry sowing treatment which may indicate capacity of early germinating plants to compete well against weeds.

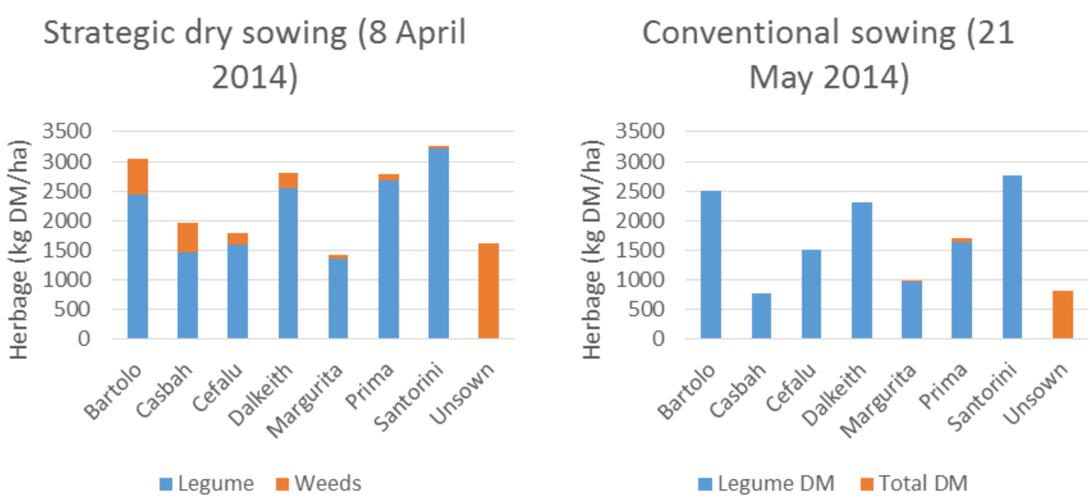


Fig. 6 Sown legume and weed species contribution to herbage present at senescence under strategic dry sowing (8 April 2014) and conventional sowing (21 May 2014) times at Babakin, WA for a range of annual pasture legumes and an unsown control treatment.

In terms of weeds present, the actual total number recorded per unit area differed little between the strategic dry sowing and conventional sowing treatments (240 and 209 plants/m^2 respectively) (Fig. 7). However, the species found at each sowing time varied considerably. Capeweed population was ten time greater in the early compared to late sowing time while the number of grass weeds increased at the later sowing time. Such changes in weed populations with germination time are associated with temperature requirements for germination with species such as capeweed requiring a higher critical temperature for peak germination compared with annual grass species (Turner et al. 2001).

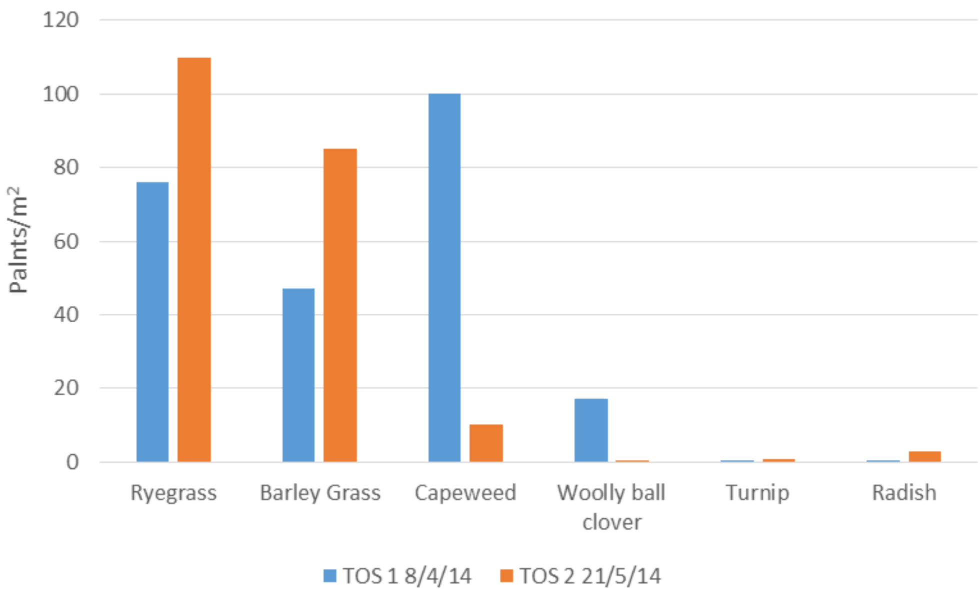


Fig. 7 Weed species and density (plants/m2) recorded in strategic dry sowing (TOS 1) and conventional sowing (TOS 2) treatments at Babakin, WA in 2014.

There was generally little difference in seed yield between strategic dry and conventional sowing time treatments (Fig. 8) with the exception of Santorini where conventional sowing produced 400 kg/ha less seed than the strategic dry sowing treatment. Native budworm caused 25% loss in seed production in Margurita and 5% in Santorini for both sowing time. Seed losses in Casbah due to native budworm were more than double (15%) in strategic dry sowing treatment compared to the conventional sowing treatment (7%).

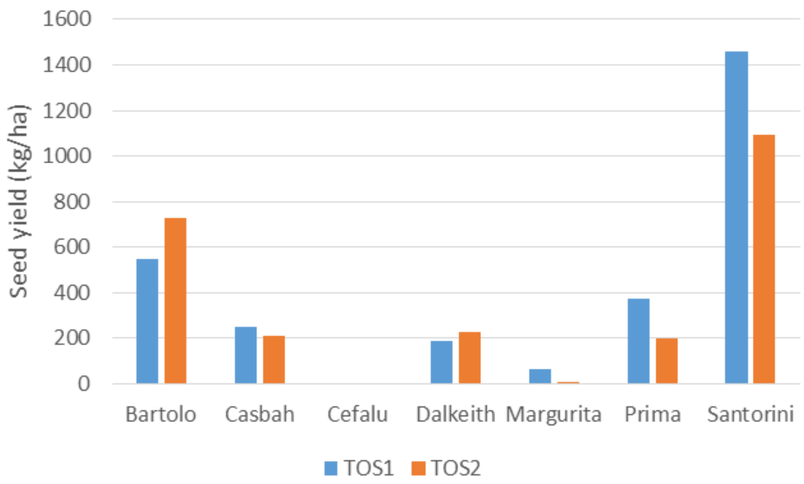


Fig. 8 Seed yield (kg/ha) of a range of annual legumes sown as a strategic dry (TOS 1) or conventional (TOS 2) time of sowing at Babakin, WA in 2014.

4.2.2.2 Greenethorpe NSW

A field site at Greenethorpe was used to evaluate the efficacy of strategic dry sowing in NSW (7th April 2016 and 2017) compared to conventional sowing (30th May 2016, 31st May 2017). A knockdown herbicide (glyphosate 2 L/ha) was used prior to each sowing time. The site used in 2016 had previously been sown to wheat (2015), canola (2014) and oats (2013). The paddock used in 2017 had previously been sown to wheat (2016), canola (2015) and oats (2014). There had been good weed control practised in all crop years, though there is known resistance of annual ryegrass to both clethodim and haloxyfop across the farm.

In 2016, a considerably wetter than average year (946 mm cf. 600 mm), there was little difference in legume productivity between sowing times with the exception of Bartolo bladder clover and Casbah biserrula where the later sowing time (May) produced significantly more herbage (Fig. 9). Arrowleaf clover produced significantly more herbage than all other species at both times of sowing (exceeding 8 t DM/ha) and had the lowest proportion of annual ryegrass indicating it was highly competitive against weeds. Dry sowing of Bartolo, Casbah, Avila and Seaton Park resulted in a higher proportion of annual ryegrass compared to the later sowing time. These results reflected the WA experience.

In 2017, a drier year (480 mm at time of writing in December 2017), there was a greater effect of sowing time on herbage production with significantly more herbage produced by all species sown dry in April compared to late May with the exception of subterranean clover where there was no difference (Fig. 9). Contamination of the plots by annual ryegrass was lower in 2017 compared to 2016.

There was negligible occurrence of broadleaf weeds in either 2016 or 2017 at these sites.

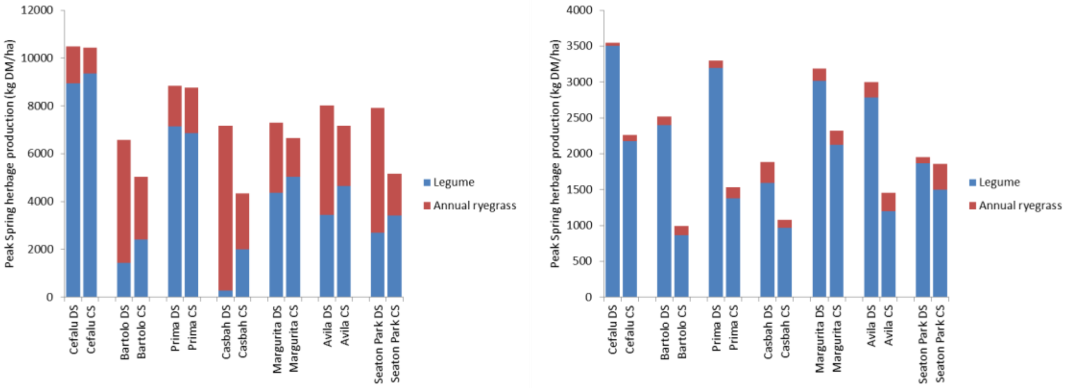


Fig. 9 Peak herbage production (kg DM/ha) of a range of annual legume pasture species sown either in early April prior to rain (DS) or late May (CS) and volunteer annual ryegrass at Greenethorpe NSW in 2016 (left) and 2017 (right)

Interpretation of dry sowing data

- Small seeded species (e.g. Cefalu, Casbah and Prima) can be successfully established by dry sowing but they can have lower establishment efficiency when sown dry compared to larger seeded species, particularly where weed pressure is high.
- Dry sowing can result in higher weed competition and lower relative legume content but this appears to be affected by the timing of opening autumn rainfall and the weed seed bank (total weed competition and type of weeds).
- Dry sowing can significantly increase establishment success particularly in years where climatic conditions (poor growing season rainfall and severe frosts e.g. 2017 in NSW, retard the growth of late sown treatments).
- It is critical to know paddock history, especially weed control and weeds likely to be present in choosing a paddock for possible dry sowing
- Sowing time had some effect on budworm damage but this relationship may be seasonal.

4.2.2.3 Nodulation outcomes when legumes were established with alternative timing and strategies

a) At Babakin the strategic dry-sowing experiment with inoculated Biserrula was sown in March, preceding a cold front. Several different strains of biserrula inoculant were investigated, including previous commercial options WSM1558 and WSM1271.

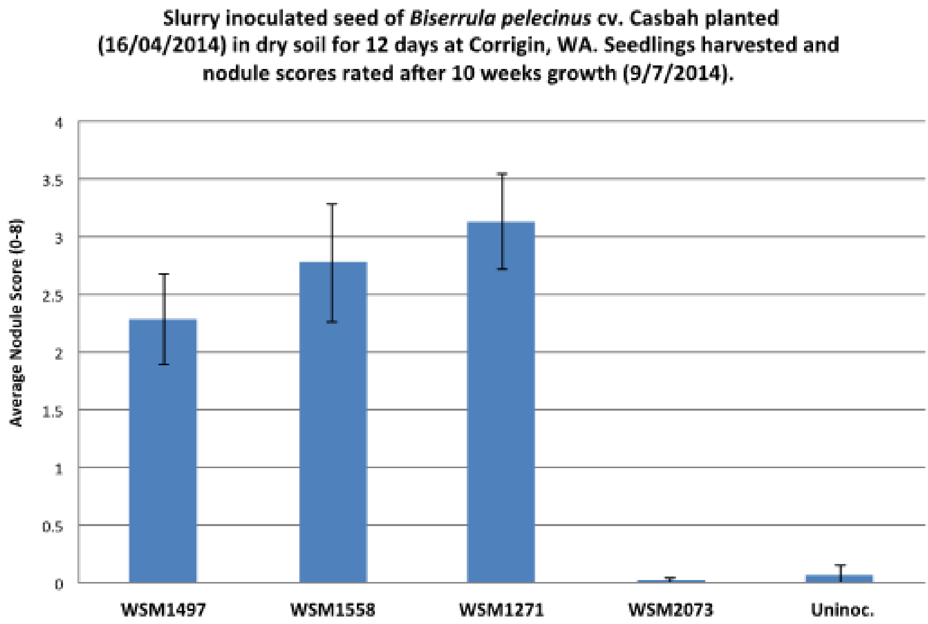


Fig. 10 The average nodule score of biserrula 10 weeks after germination, following dry sowing as a seed slurry. The strains spent 12 days in a dry soil before rains.

The inoculant quality strains were able to survive the 12 days in dry or autumn -damp soil preceding the cold front, giving an indication that inoculation failure would not be an impediment to a possible strategy for establishment of biserrula in this manner. WSM2073 is a species of Mesorhizobium described by CRS (Nandasena et al 2007) that naturally inhabits WA soils. It appears there may be strain differences in the ability to survive on seed in dry soils, and this could be further investigated.

b) At Beckom - inoculant applied with the preceding cereal crop

Because there has been some uncertainty in the minds of farmers about the survival of summer sown inoculant, we decided to evaluate whether it would be possible to inoculate the field during winter, whilst summer sowing the seed in the normal manner in the following year. This would allow the inoculant to be delivered into moist and cool soil.

Biserrula was chosen as its inoculant strain WSM1497, is a very good soil saprophyte and would be expected to colonise the rhizosphere of the cereal crops. In the example below, the farmer had left a strip un-inoculated for demonstration purposes, with the rest of the cereal crop sown with granules of biserrula inoculant. The uninoculated biserrula had no nodules present, while the inoculated area had 100% nodulation on plants sampled.

Fig 11. Non-nodulated biserrula plants (left) where inoculant was deliberately omitted in the sowing of the winter cereal in 2013, and nodulated plants (right) where ALOSCA granules for biserrula were applied in the final crop year, with the crop. Biserrula seed was summer sown across both areas in the following year (February 2014) at Beckom, NSW. This photo was taken in September 2014.

4.2.3 Smaller scale demonstration sowings in 2015 and 2016

Additional sites were established in conjunction with local farming groups and agribusiness in 2015/16. Footage of sowings and interviews with farmers were recorded by a professional videographer. The footage from WA and NSW was edited to produce visual and audio learning aids for adoption to complement written adoption packages. Field days were held at sites throughout 2016; for the first time we held an autumn field day -on April 18 at Brookton, WA, to demonstrate the outcomes from summer sowing in a wet autumn. In NSW, several of the sites were selected in conjunction with local agribusiness including

CRT and AgNVet. This approach is enhancing the transfer of technology to private consultants and allowing access to their client base.

4.2.3.1 Winter production data from pastures sown in March 2016 using newly developed technologies

Three sites were sown in NSW to evaluate the effect of time of sowing on herbage production. These were located at Greenethorpe, Collingullie and Tullibigeal. Sowing time was spaced on six weekly intervals with the methods used being:

1. Summer hard seed/in-pod sowing (SS)– 3rd week of February 2016
2. Strategic dry (pre-autumn break) scarified seed sowing (ES)– 1st week of April 2016
3. Late scarified seed sowing (LS) – 3rd week of May 2016

Following rainfall in January 2016 of 40-51 mm across all sites, no rainfall was received in February at any site except Collingullie where 15 mm fell on February 1. March rainfall was 33, 39 and 20 mm at Greenethorpe, Collingullie and Wagga Wagga respectively. Very little rain was received at any location over the April period with less than 15 mm recorded at all locations with this occurring following the strategic dry sowing at all locations. The autumn break occurred on May 8-12 at all locations with rainfall totals of 47, 69 and 45 mm received at Greenethorpe, Collingullie and Wagga Wagga over this period respectively. An additional 20-25 mm was received at all locations in the final week of May. From this point forward, rainfall was significantly higher at all sites than the long-term average with annual totals being 920, 762 and 720 mm for Greenethorpe, Collingullie and Tullibigeal respectively. Lon-term average rainfall for these locations are 620, 520 and 440 mm respectively.

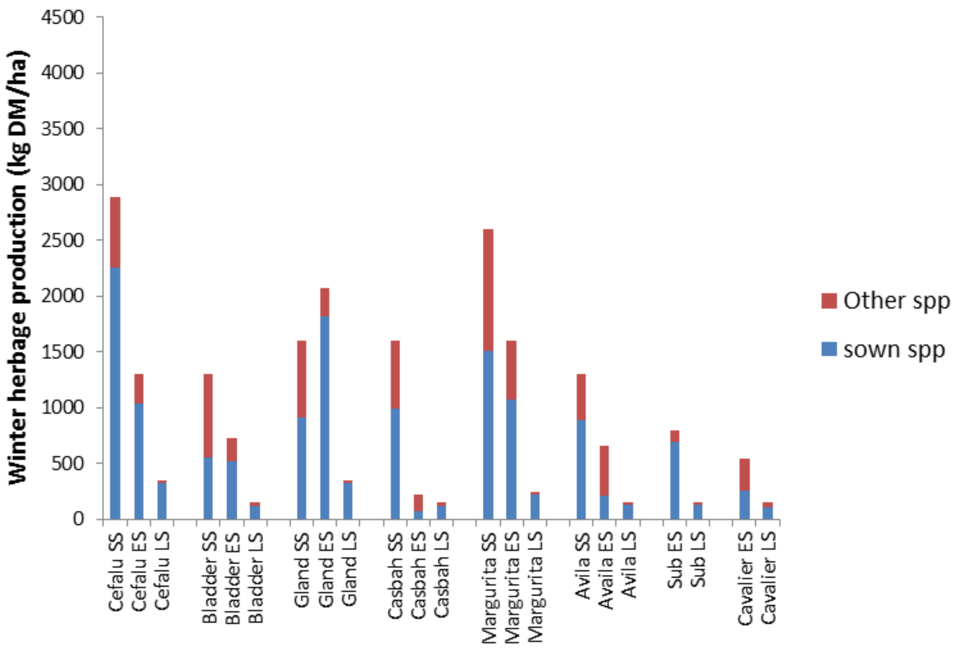


Fig. 12 Winter herbage production (measured on 2nd September 2016) at Greenethorpe, NSW for a range of annual legume species sown as hard seed/in pod in summer (SS), as a strategic dry sowing with scarified seed (ES) or as a late sown scarified seed sowing (LS). SS treatments sown in third week of February, ES in first week of April and LS in third week of May. LSD for comparison of sown species 274 kg DM/ha; for other spp. LSD is 385 kg DM/ha.

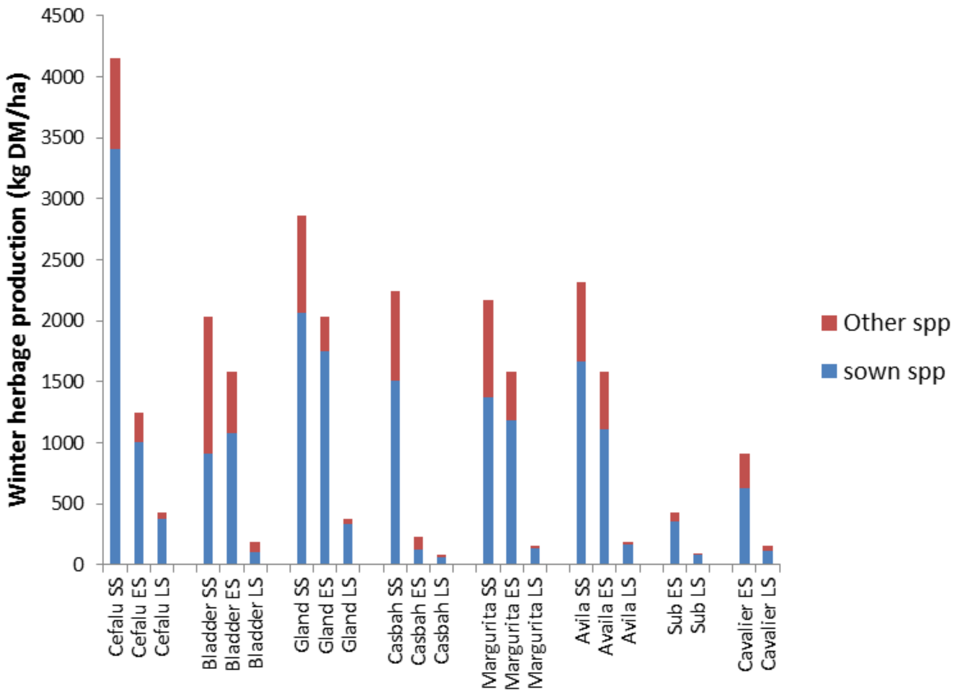


Fig. 13 Winter herbage production (measured on 2nd September 2016) at Collingullie, NSW for a range of annual legume species sown as hard seed/in pod in summer (SS), as a strategic dry sowing with scarified seed (ES) or as a late sown scarified seed sowing (LS). SS treatments sown in third week of February, ES in first week of April and LS in third week of May. LSD for comparison of sown species 267 kg DM/ha.

The rainfall received in March was sufficient to commence germination of the summer sown treatments, but germination was more staggered than in preceding years where earlier substantial rainfall was received. This meant there was ongoing germination in these plots at least up until mid May. Unfortunately, substantial and prolonged flooding through the Central West meant that the Tullibigeal site could not be accessed for nearly eight weeks. While the site was salvaged and spring measurements were taken, it was impossible to access the site to obtain end of winter herbage production.

Even though substantial rainfall was not received until later in autumn than in previous years of this project, the early germination in the summer sowing (SS) treatment and subsequent survival of these early emerging seedlings resulted in a significant increase in winter herbage production compared the traditional late May –post autumn break sowing time (LS) at all locations (Figs. 12 and 13). Additionally, SS yielded higher winter herbage production compared to strategic early sowing (ES) times at Greenethorpe and Collingullie (Figs. 12 and 13) for Cefalu arrowleaf clover, Casbah biserrula Margurita French serradella and Avila yellow serradella respectively. Summer sowing also resulted in significantly higher herbage production of gland clover at Collingullie, but the ES treatment outperformed SS treatment for gland clover at Greenethorpe (Figs. 12 and 13). Rainfall in April at all sites occurred after the ES treatment was in place so presumably the significantly higher herbage production in the species mentioned above was due to the response of the already established early germinating seedlings in the SS treatments.

There was a less clear-cut outcome in terms of competitiveness of sown species against weeds than in other years of the project. In previous years, many of the top performing summer sown species (biserrula, arrowleaf clover, gland clover) had very low densities of weeds in SS treatments. However, results this year were much more variable and may be attributable to the more staggered germination of the sown species. This was likely as a consequence of reduced early autumn rainfall, resulting in delayed formation of a dense canopy. Also, the effect of a wetter than average winter had an adverse effect on some species such as biserrula, which is relatively intolerant of the waterlogging which was experienced at these sites over winter.

Spring herbage production

Summer sowing resulted in significantly higher spring herbage production compared to LS for Cefalu arrowleaf clover, Casbah biserrula and Avila yellow serradella at the Greenethorpe site (Fig. 14).

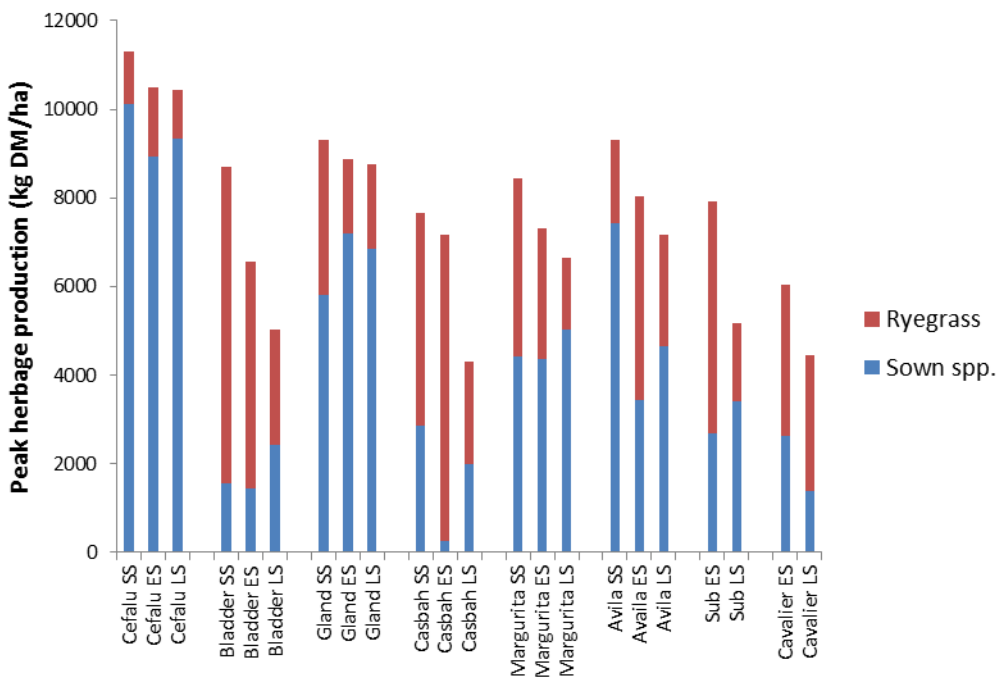


Fig. 14 Spring peak herbage production at Greenethorpe, NSW for a range of annual legume species sown as hard seed/in pod in summer (SS), as a strategic dry sowing with scarified seed (ES) or as a late sown scarified seed sowing (LS). SS treatments sown in third week of February, ES in first week of April and LS in third week of May. LSD for comparison of sown species 396 kg DM/ha.

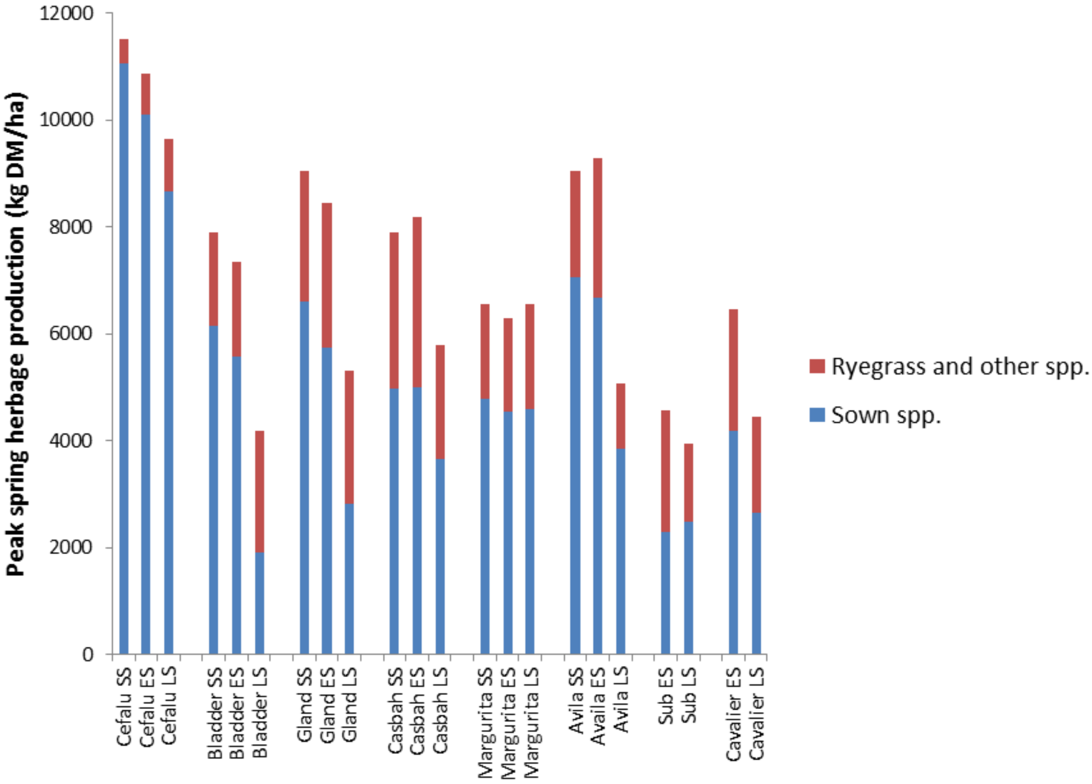


Fig. 15 Spring peak herbage production at Collingullie, NSW for a range of annual legume species sown as hard seed/in pod in summer (SS), as a strategic dry sowing with scarified seed (ES) or as a late sown scarified seed sowing (LS). SS treatments sown in third week of February, ES in first week of April and LS in third week of May. LSD for comparison of sown species 326 kg DM/ha.

Herbage production of the arrowleaf clover was particularly impressive at this site and regardless of sowing time, its herbage production was significantly higher than for any other species. Strategic dry (ES) and LS treatments resulted in significantly higher herbage production of gland clover compared to SS while LS of Margurita French serradella resulted in significantly higher herbage production than either the SS or ES. Higher weed burdens in earlier sown treatments likely resulted in greater competition against the sown species for these treatments. For the traditional legume species, highest production was achieved with LS treatment for subterranean clover compared to the ES treatment for burr medic. This site was quite badly affected by waterlogging due to the significantly higher than average rainfall year and this impacted considerably on the overall performance of the highly waterlogging sensitive species, biserrula and bladder clover.

At Collingullie, significantly higher herbage production was recorded in SS compared to LS treatments for all species except French serradella where sowing time had no impact on herbage production (Fig. 15). Strategic dry sowing (ES) also resulted in significantly higher herbage production compared to LS for all species except subterranean clover. This was less impact of waterlogging at this site compared to Greenethorpe, however, there was some evidence of this in late winter and early spring where bleaching of some biserrula plants and stunting of bladder clover was observed. Affected plants grew out of this as soil dried out. Arrowleaf clover was again a stand out at this site and also very competitive against weeds.

Peak production was generally considerably lower for all species at Tullibigeal compared to other sites (Fig. 16). Severe flooding prevented access to this site for approximately two months and while the site itself was not inundated, soil moisture levels were likely in excess for much of the growing season. It would be premature to draw conclusions on the effect of sowing time from this site due to inability to access it throughout the growing season to observe the effect of very wet season on plant performance. However, earlier sowing times (SS or ES) favoured production of arrowleaf, bladder clover, gland clover, subterranean clover and burr medic compared to LS treatments.

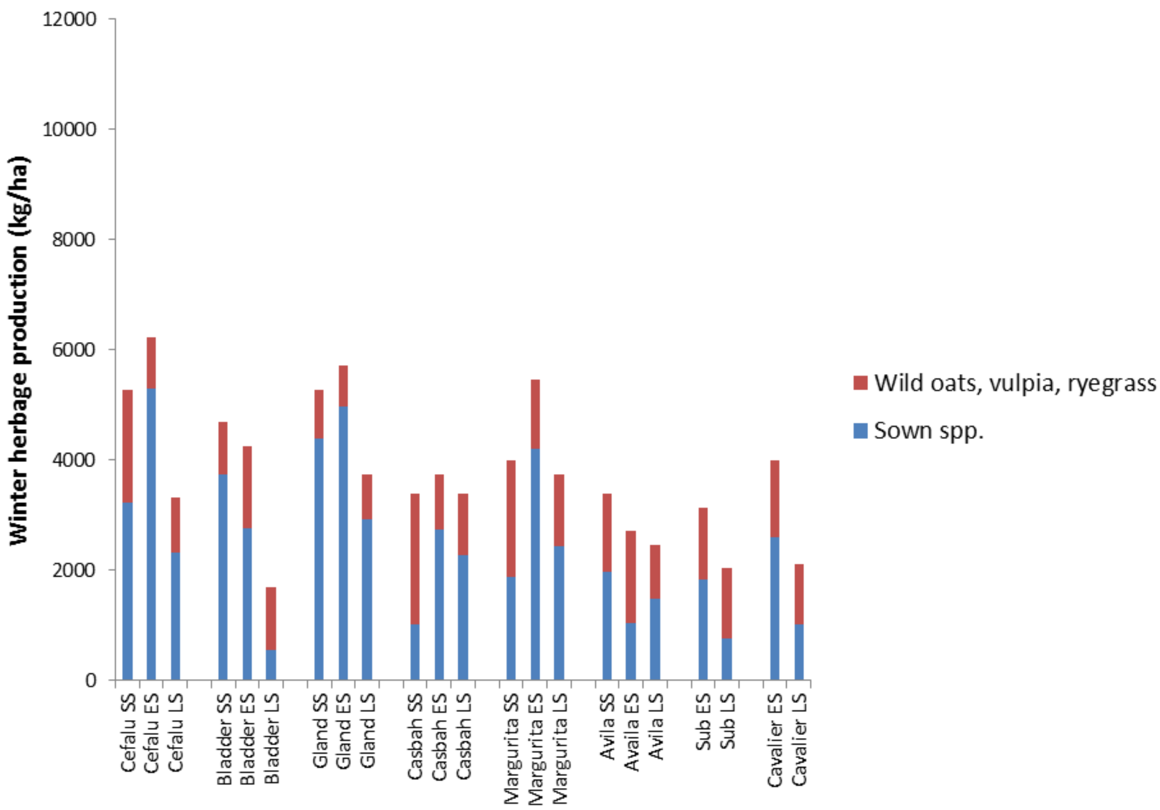


Fig. 16 Spring peak herbage production at Tullibigeal, NSW for a range of annual legume species sown as hard seed/in pod in summer (SS), as a strategic dry sowing with scarified seed (ES) or as a late sown scarified seed sowing (LS). SS treatments sown in third week of February, ES in first week of April and LS in third week of May. LSD for comparison of sown species 401 kg DM/ha.

Seed production

At Greenethorpe, sowing time had a significant effect on seed production for arrowleaf clover (SS>ES,LS), bladder clover (LS>SS,ES), Casbah biserrula (SS> LS>ES), gland clover (LS>SS), Avila yellow serradella (SS>LS>ES) and Cavalier burr medic (ES>LS) (Fig. 17).

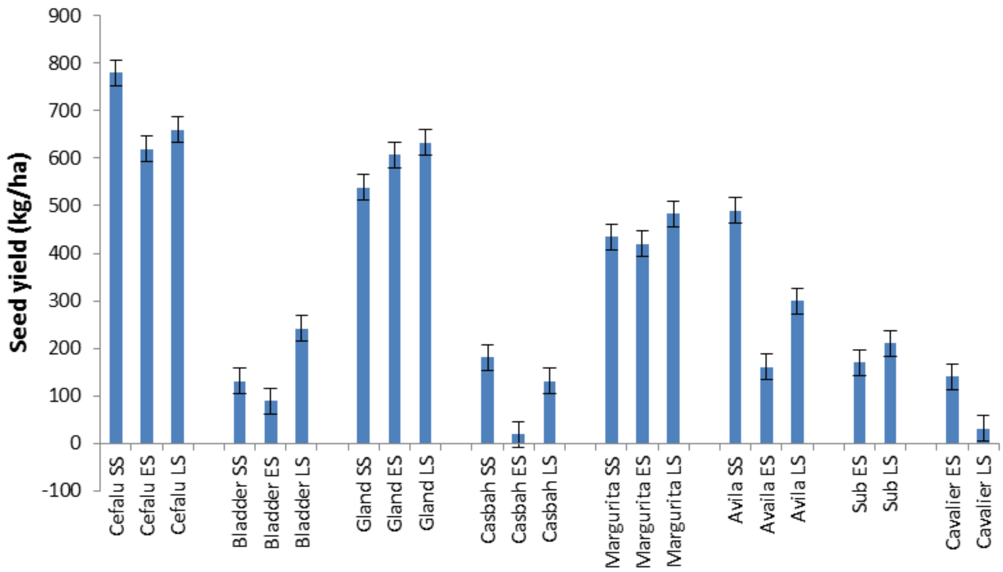


Fig. 17 Seed production of a range of annual pasture legumes sown as either a hard seed/in-pod summer sow (SS), in third week of February, a strategic dry scarified seed sow (ES) in first week of April or late autumn scarified seed sow (LS) in third week of May 2016 at Greenethorpe, NSW.

Seed production of arrowleaf, gland clover, Margurita French serradella and SS Avila yellow serradella coupled with the ability to harvest seed with a header again illustrated the potential of these species for on-farm seed nursery production compared to conventional species such as subterranean clover and burr medic.

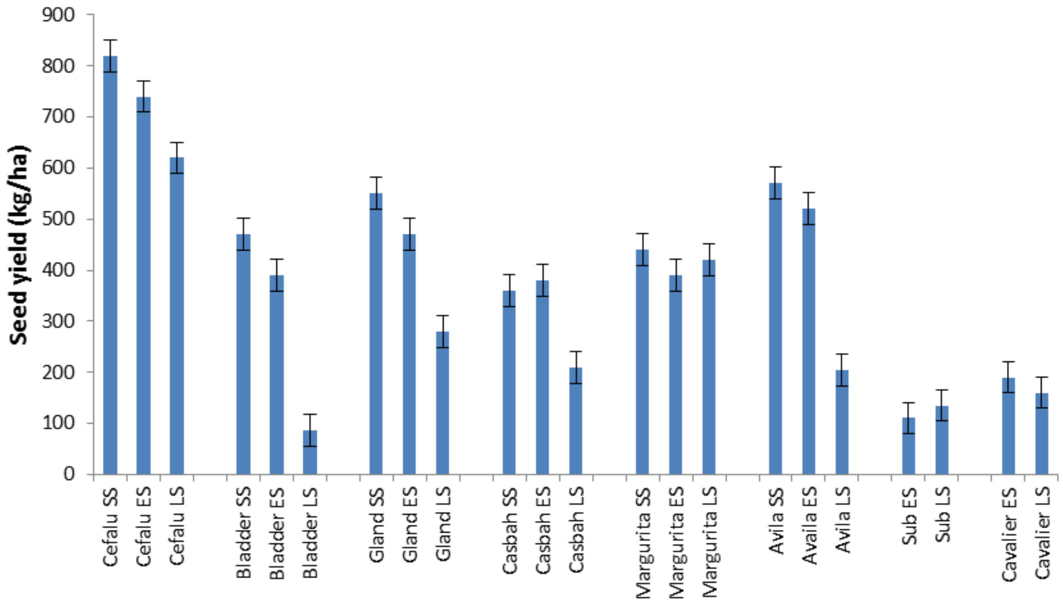


Fig. 18 Seed production of a range of annual pasture legumes sown as either a hard seed/in-pod summer sow (SS), in third week of February, a strategic dry scarified seed sow (ES) in first week of April or late autumn scarified seed sow (LS) in third week of May 2016 at Collingullie, NSW.

At Collingullie, either SS or ES resulted in significantly higher seed production compared to LS except for subterranean clover and burr medic where sowing time had no impact (Fig. 18). Summer sowing (SS) produced significantly more seed of Cefalu arrowleaf cover, bladder clover and gland clover compared to ES treatments. Earlier sowing times (SS or ES) always resulted in significantly higher seed production of all species in comparison to ES or LS treatments for subterranean clover and burr medic. The ability to sow newer legumes as SS treatments or achieve greater seed production as an ES treatment compared to these conventional species is a considerable advantage for producers looking to either produce their own seed or for the purpose of building up large resilient seedbanks in longer term pastures, or pastures used for crop-pasture rotations where the seedbank can act on an on-demand pasture at any point in the cropping rotation.

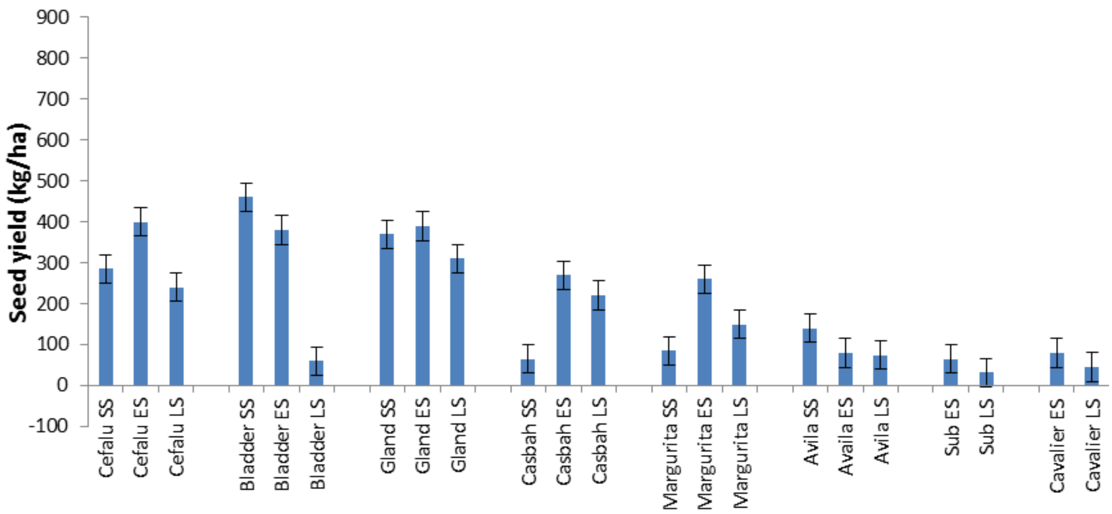


Fig. 19 Seed production of a range of annual pasture legumes sown as either a hard seed/in-pod summer sow (SS), in third week of February, a strategic dry scarified seed sow (ES) in first week of April or late autumn scarified seed sow (LS) in third week of May 2016 at Collingullie, NSW.

Maximum seed production at Tullibigeal (Fig. 19) was considerably lower than for other sites. There was no clear effect of sowing time on seed yield across all species at this site and as for herbage production, caution should be exercised in drawing conclusions from this site as it is unclear the effect the exceptionally wet growing season may have had on plants during the period the site could not be accessed due to flooding.

Seed production of arrowleaf, gland clover, Margurita French serradella and SS Avila yellow serradella coupled with the ability to harvest seed with a header again show the potential of these species for on-farm seed nursery production compared to conventional species such as subterranean clover and burr medic.

These examples present a sub-set of the data produced in the project. Full data sets are available in the milestone reports, and on the CRS website.

4.3 Nodulation studies and surveys utilising MALDI-tof, RAPD PCR and Next Generation sequencing to identify nodule occupancy

In WA TA1, WU95, WSM409 and WSM1325 have all been commercial inoculant strains over the last 60 years. Up until 2005 WU95 provided the best persistence and growth for annual sub-clover following its development in the 1970s, however it has a narrow host range. It does not fix Nitrogen well with the newer aerial seeding clovers. In contrast, WSM1325, which was developed in the 2000s, does fix N well with many aerial seeding clovers (Howieson et al 2005). After 7 years of testing across Australia WSM1325 became commercialised. Currently, Australian the inoculant group C contains WSM1325 and this is the inoculant for most annual clovers, including *T. subterraneum* and *T. spumosum*. We have investigated the ability of MALDI-TOF MS to distinguish between WSM1325 and these different clover nodulating strains, because producers often enquire whether they have the most efficient strain in their bladder clover nodules. Further to this, sub-clover pastures remain a significant component of mixed farming systems in southern Australia. However, we have had little understanding of the population of clover nodulating rhizobia in sub-clover nodules. With subclover decline apparent in the different States, we have begun to investigate the dynamics of nodule occupancy between the current inoculant and previous inoculant strains, as well as native strains (strains evolved from inoculants or inadvertent strain introductions).

4.3.1 Across States

With the help from the MLA pillar meetings, NSW Local Land Services, farmer groups and agribusiness we were able to engage with several grower groups around the country and collect nodule samples from 340 field sites (243 from NSW, 10 from SA, 20 from VIC and 66 from WA, Fig. 20).

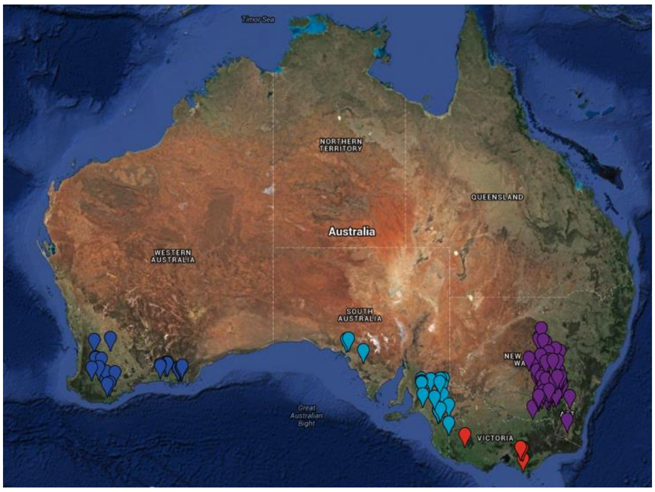


Fig. 20 Map of Australia showing the survey sample points.

The MALDI-ID results show that there is a dominance of WSM1325-like strains in each of the States (56% in NSW, 70% in SA, 61% in VIC and 39% in WA, (Fig. 21). However, State differences are noticeable in the overall composition of clover nodule rhizobia. NSW and WA seem to have the largest diversity in nodule occupants with 10 and 7 different types respectively, however sample size may have influenced these results (Fig. 21). To put these results into perspective of paddock history, bar graphs of each State were generated indicating the paddock establishment age (see insert Fig. 21). These results indicate that the majority of WA samples were taken from paddocks up to 5 years old, for NSW it was largely either young (up to 5 years) or very old (50 years and older) paddocks, SA was evenly sampled for all paddock ages and in VIC the majority came from 30 year old paddocks (Fig. 21). The data suggests that paddock age does not seem to be a determinant factor for rhizobia occupancy, however we have not yet received the inoculation history from all farmers and hence it is difficult to draw firm conclusions.

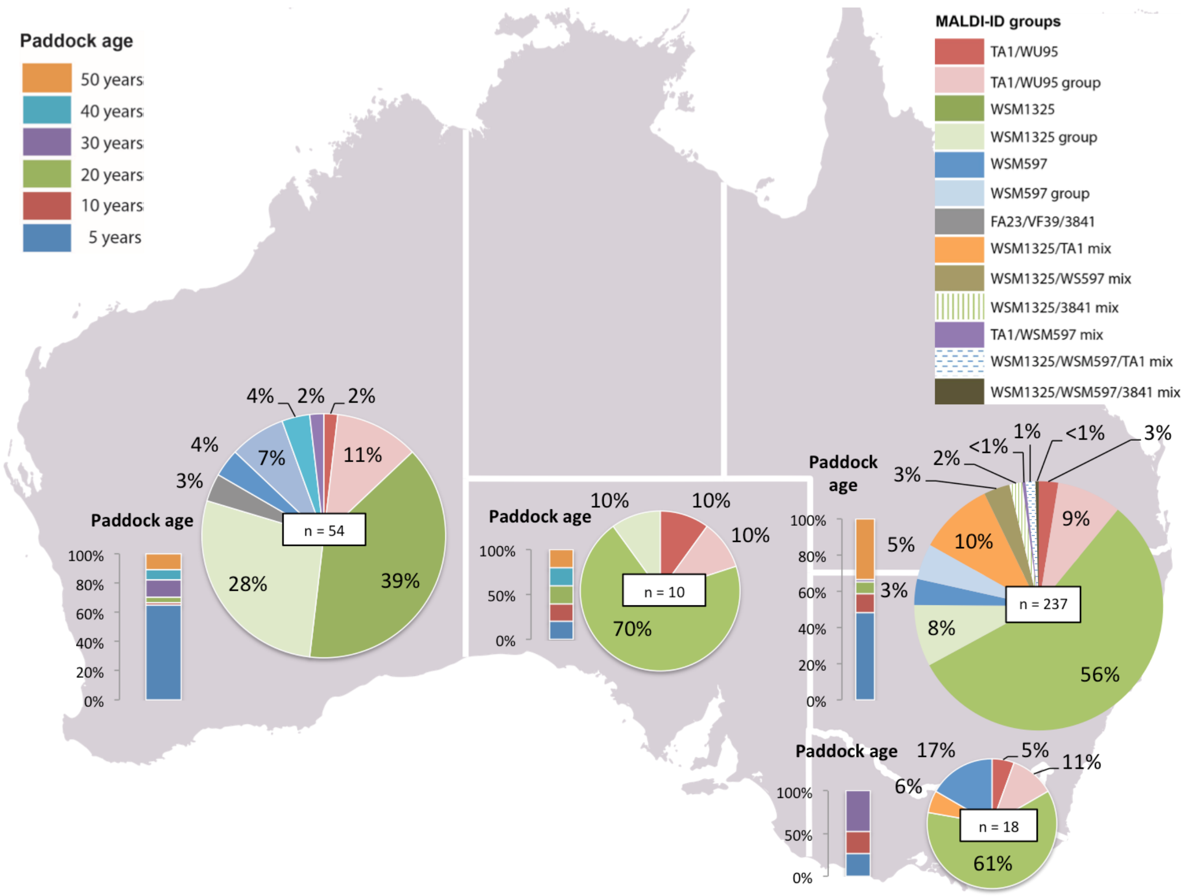


Fig. 21 MALDI-TOF MS identification results shown as percentages for each of the identified groups (see MALDI-ID groups legend). Each pie chart is overlaid on its respective state and the bar graphs indicate the percentage of paddock age (see legend colours for paddock age) within the sample set. n refers to the number of samples.

Our analysis provided evidence that we are currently able to differentiate TA1 and WU95/WSM1325 using L36 ribosomal mass as a marker. Additionally, ribosomal mass L24 can be used as a marker to distinguish between WU95 and WSM1325. This is critical as these two strains represent the most widely sown of the commercial inocula in the last 60 years.

All other ribosomal proteins were identical between the different strains. Separation of WSM1325 from WSM1317 and WSM409 was not possible and hence we are investigating a Next gen Miseq approach to separating these strains, and to validate MALDI ID.

Next Gen sequencing approach

Results from this approach are displayed in Table 1 below. Next gen sequencing allowed considerable accuracy in nodule strain ID, although it could not always separate WSM1325 from SARDI943. Where the cells are highlighted in red, we believe the identification to be spurious. For example, samples 2 and 8 were identified as WSM1325, but we had insufficient reads (<100) to be confident of this identification. The data highlights that further research is needed to fine-tune the primer selection as well as the processing to increase the quality of the reads, but it also shows that this technology is very promising for the future as an adjunct to MALDI-ID.

Table 1. Strains analysed with the NGS approach, the number of reads (red coding shows insufficient reads) and likely identification

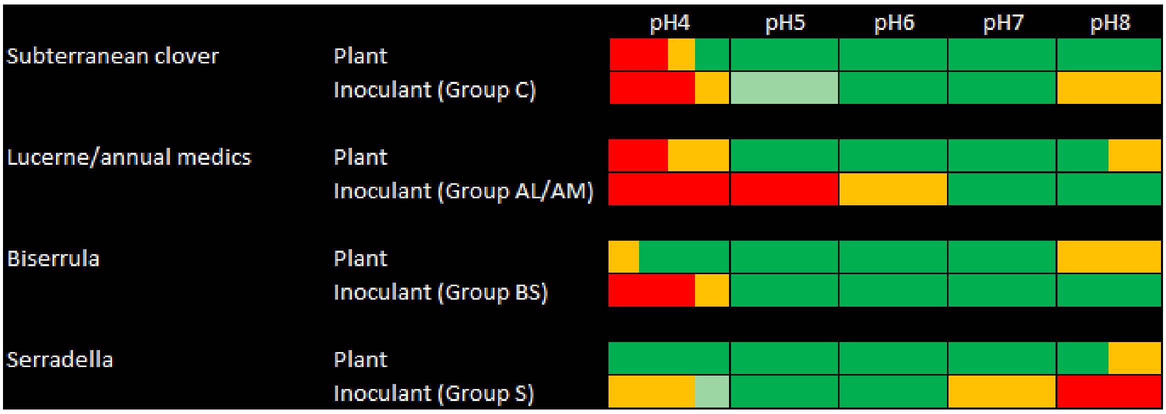
Sample	NGS-GyrB	# reads	NGS-ThrC	# reads	Summary
1	WSM1325 (100%)	21	SRDI943 (98.87%)	149	WSM1325/SRDI943
2	WSM1325(100%)	80	WSM1325	30	WSM1325
3	WSM1325 (100%)	125	WSM1325 (99.77%)	396	WSM1325
4	WSM1325 (100%)	71	WSM1325 (100%) + SRDI943 (98.87%)	102/47	WSM1325/SRDI943
5	WSM1325 (100%)	90	SRDI943 (98.2%) + WSM2304 (97.97%)	157/11	WSM1325/SRDI943
6	WSM1325 (100%)	123	WSM1325 (97.07%)	91	WSM1325
7	WSM1325 (100%)	5698	WSM1325 (100%) + SRDI943 (97.97%)	348/75	WSM1325/SRDI943
8	SRDI943 (100%)	12	SRDI943 (99.77%)	5	ND
9	SRDI943 (100%)	1589	WSM1325/SRDI943 (99.77%)	23	WSM1325/SRDI943
10	WSM1325 (100%)	3469	WSM1325 (99.77%)	204	WSM1325
11	WSM1325 (100%) + SRDI565 (98.78%)	1906/278	WSM1325 (99.77%) + WSM597 (100%)	29/6	WSM1325/SRDI565
12	WSM1325 (100%)	4707	WSM1325 (97.75%)	377	WSM1325
13	WSM1325 (100%) + SRDI943 (99.39%)	146/9296	SRDI943 (98.87%)	344	WSM1325/SRDI943
14	WSM1325 (100%) + SRDI943 (99.39%)	211/7808	WSM1325 (100%)	242	WSM1325/SRDI943
15	WSM1325/SRDI943 (100%)	99/2498	SRDI943 (97.97%)	641	WSM1325/SRDI943
16	SRDI943 (99.39%)	75	WSM1325 (99.32%) + SRDI943 (98.87%)	42/44	WSM1325/SRDI943
17	SRDI943 (100%)	2738	SRDI943 (98.87%)	105	SRDI943
18	SRDI943 (100%)	3123	WSM1325 (99.77%) +SRDI943 (97.97%)	118/104	WSM1325/SRDI943
19	SRDI943 (99.37%)	3058	SRDI943 (99.1%)	213	SRDI943
20	WSM1325 (100%)	3257	WSM1325 (99.77%)	161	WSM1325
21	WSM1325 (100%) + SRDI943 (99.39%)	807/293	WSM1325/SRDI943 (98.87%)	43/56	WSM1325/SRDI943
22	WSM1325 (100%)	109	WSM1325 (99.77%)	268	WSM1325
23	WSM1325 (100%) + SRDI943 (99.70%)	72/832	CC275e (96.84%)	228	WSM1325/SRDI943/CC275e
24	SRDI943 (95.12%)	1586	SRDI943 (98.20%)	450	SRDI943
25	WSM1325 (100%)	566	WSM1325 (100%)	55	WSM1325
26	SRDI943 (100%)	2520	SRDI943 (100%)	168	SRDI943
27	SRDI943 (100%)	5593	x	1	SRDI943
28	WSM1325 (100%) + WSM2304 (97.87%	54/4416	WSM2304 (97.74%)	70	WSM1325/WSM2304
29	WSM1325 (99.7%) +WSM2304 (95.72%	512/986	WSM1325 (100%)	13	WSM1325/WSM2304
30	SRDI943 (95.12%) + CC275e (93.88%) +	360/78/930	WSM2304 (97.29%)	302	WSM2304/SRDI943/CC275e
31	WSM1325 (100%) + SRDI943 (99.39%)	670/314	SRDI943 (99.1%)	141	WSM1325/SRDI943
32	SRDI943 (99.39%)	1138	SRDI943 (98.87%)	354	SRDI943
33	WSM2304 (97.87%)	873	WSM2304 (97.97%)	117	WSM2304
34	WSM1325/SRDI943 (100%)	1337/731	WSM1325 (99.77%)	154	WSM1325/SRDI943
35	WSM1325 (100%)	2905	WSM1325 (100%)	92	WSM1325
36	WSM1325/SRDI943 (100%)	511/336	SRDI943 (100%)	118	WSM1325/SRDI943
37	WSM1325 (100%)	3836	WSM1325 (97.3%)	74	WSM1325
38	WSM1325/WSM2304 (100%)	108/5386	WSM2304 (97.97%)	393	WSM1325/WSM2304
39	WSM1325 (100%) + SRDI943 (99.39%)	63/4582	SRDI943 (99.77%)	187	WSM1325/SRDI943
40	SRDI943 (95.12%)	1146	WSM2304 (97.97%)	46	SRDI943/WSM2304
41	WSM2304 (97.87%)	106	WSM1325 (97.97%) + SRDI943 (98.87%) +	4	WSM2304
42	WSM1325 (100%) + WSM2304 (95.72%	298/3105	WSM1325 (99.77%)	25	WSM1325/WSM2304
43	WSM1325 (100%)	2383	WSM1325 (99.77%)	123	WSM1325
44	WSM1325/SRDI943 (100%)	591/200	WSM1325 (low)	2	WSM1325/SRDI943
45	WSM1325 (99.7%) +WSM2304 (95.72%	716/2469	WSM1325 (100%) + SRDI943 (98.87%)	149/74	WSM1325/WSM2304
46	WSM1325 (100%)	7089	WSM1325 (98.42%)	426	WSM1325
47	WSM1325 (100%)	4370	WSM1325 (100%)	390	WSM1325
48	WSM1325 (100%)	229	SRDI943 (98.20%)	111	WSM1325/SRDI943
49	WSM1325 (100%)	364	WSM1325 (99.32%)	32	WSM1325
50	WSM1325 (100%) + TA1 (99.7%)	147/87	WSM1325 (99.77%)	7	WSM1325
51	WSM1325 (100%) + WSM2304 (97.87%	138/8004	WSM2304 (97.97%)	110	WSM1325/WSM2304
52	WSM1325 (100%) + WSM597 (98.78%)	970/142	WSM1325 (100%)	69	WSM1325/WSM597
53	WSM1325 (100%)	8487	WSM1325 (99.77%)	390	WSM1325
54	SRDI943 (100%)	2360	SRDI943 (98.87%)	199	SRDI943
55	WSM1325/SRDI943 (100%)	115/2195	WSM1325 (99.77%)	423	WSM1325/SRDI943
56	SRDI943 (99.39%)	913	SRDI943 (98.87%)	502	SRDI943
57	WSM1325 (100%)	1273	WSM1325 (98.65%) + SRDI943 (98.2%)	17/34	WSM1325/SRDI943
58	WSM1325 (100%)	7656	SRDI943 (100%)	218	WSM1325/SRDI943
59	WSM1325/SRDI943 (100%)	215/6092	SRDI943 (99.55%)	189	WSM1325/SRDI943
60	SRDI943 (99.39%) + WSM1325 (100%) +	781/127/700	WSM1325 (100%) + WSM597 (98.19%)	3	WSM1325/SRDI943/WSM2304
61	WSM1325 (100%) + SRDI565 (99.39%)	213/6005	SRDI565 (98.42%)	674	WSM1325/SRDI565
62	WSM1325 (100%)	8098	WSM1325 (99.77%)	438	WSM1325
63	WSM1325 (100%) + WSM2304 (97.87%	133/5077	WSM597 (98.19%)	1074	WSM1325/WSM2304/WSM597
64	SRDI565 (96.65%)	1303	SRDI565 (100%)	245	SRDI565
65	SRDI943 (100%)	1369	WSM1325 (100%)	44	WSM1325/SRDI943
66	WSM1325 (100%)	158	SRDI943 (98.87%)	207	WSM1325/SRDI943
67	WSM1325/SRDI943 (100%)	2225/632	SRDI943 (99.77%)	126	WSM1325/SRDI943
68	WSM1325 (100%)	1187	SRDI943 (99.77%) + WSM597 (98.42%) +	4/11/2	WSM1325
69	WSM1325 (100%) + SRDI943 (99.39%)	1162/527	WSM1325 (100%) + SRDI943 (98.87%)	54/65	WSM1325/SRDI943
70	WSM1325 (100%)	7125	WSM2304 (98.87%)	439	WSM1325/WSM2304
71	WSM1325 (100%) + SRDI943 (99.09%)	741/180	SRDI943 (98.87%)	89	WSM1325/SRDI943
72		1	SRDI943 (98.87%) + WSM597 (98.42%) +	7/2/2	ND
73	WSM1325/SRDI943 (100%)	29/38	WSM597 (100%)	18	WSM597
74	WSM1325 (100%) + SRDI565 (99.09%)	128/350	SRDI565 (99.1%)	729	WSM1325/SRDI565
75	WSM1325 (100%)	5668	WSM1325/SRDI943 (100%)	61/11	WSM1325/SRDI943
76	WSM1325 (99.7%)	2182	WSM1325 (100%)	198	WSM1325
77	SRDI943 (99.39%)	2635	WSM1325 (100%) + SRDI943 (98.87%)	120/114	WSM1325/SRDI943
78	WSM1325 (100%)	2143	WSM1325 (99.77%) - Low	3	WSM1325
79	SRDI943 (99.39%)	3520	WSM1325 (100%)	3	SRDI943
80	SRDI943 (100%)	2815	SRDI943 (99.77%)	660	SRDI943
81	SRDI943 (100%)	121	SRDI943 (98.87%) + SRDI565 (98.65%)	14	SRDI943/SRDI565

4.3.2 Nodulation surveys 2015 and 2016

In NSW extensive nodulation surveys were undertaken with the financial and staff support of Local Land Services (LLS) across the Central West (n=60 paddocks) and Riverina (n=81) LLS regions in 2015 and the Central Tablelands (n=30) and South-East (n=54) LLS regions in 2016. In total, 225 paddocks representing pastures used in crop-pasture rotation systems and permanent pastures were sampled. Paddocks sampled predominately contained traditional legumes (subterranean clover and/or annual medics), however, a number of paddocks, in the Central West and Riverina contained either arrowleaf, bladder, gland or biserrula while white clover, red clover, arrowleaf and Caucasian clover were also encountered in the South East LLS. Mature lucerne paddocks were excluded from the survey due to the diffiultly in extracting the root systems of established plants. However, where lucerne had been sown in the year of sampling, these paddocks were included. A minimum of 15 legume plants were collected from a representative area of the paddock (20 m x 20 m in area) and nodulation on each individual plant assessed using the 0-8 scoring system described in Howieson and Dilworth (2016, Ch 8) where a score of 4 (more than 20 nodules) is considered adequate. Soil samples (0-10 cm) were collected for analysis from all paddocks while assessment of botanical composition via the rod-point method was also undertaken along with collection of as much information on paddock management as possible. More details on methodology can be found in Hackney et al. (2017a).

Overall, paddocks in the Central Tablelands and South-East LLS regions had a lower legume content (26%), compared to the Central West and Riverina (51%), though there was significant variation within regions (4-95%). Ninety-three percent of paddocks sampled had less than adequate nodulation. In terms of soil factors that may influence nodulation, 40% of paddocks had a Colwell P below the critical level based on Phosphorus Buffering Index while 73% of sampled paddocks had inadequate sulphur (< 8 mg/kg). In terms of soil pH_{Ca}, 78% of sampled paddocks had a pH of less than 5.5 and 50% were below pH 5. Where soil pH is less than 5, it would be expected that there would be some compromise in the performance of the subterranean clover (and other annual Trifolium spp.) symbionts, while there would be expected to be poor performance of the lucerne symbiont at the soil pH recorded in the majority of paddocks in this survey (Table 2).

Table 2. The effect of soil pH_{Ca} on a number of annual legume species and their associated rhizobia. Green indicates optimal plant/rhizobia function, yellow indicates sub-optimal function and red poor function.



Source: Drew et al. (2012), R. Yates (pers. comm.), various government departmental publications

Data was analysed using a regression tree approach (Genstat 18) to understand the impact of soil and host/rhizobia associations on legume nodulation. Soil available P is shown as the ratio of available P to critical P based on PBI, and soil S is shown as ratio of available S relative to the critical value of 8 mg/kg in this model.

The regression tree (Fig. 22) accounted for 73% of variation in the data. Italicised numerical values shown at the terminal nodes indicate the nodulation score. The initial split in the data occurred on the basis of host plant with lucerne and annual medics split from clovers and biserrula. For lucerne and annual medics the range in nodulation score across paddocks where they were found ranged from 0-2.5; a very limited and sub-optimal score range and therefore subsequent splits are relatively meaningless in this model.

For the clovers and biserrula, the next split in the regression tree occurred on the basis of soil available P with paddocks having an available:critical P ratio of less than 0.7 having a low nodulation score (1.9). The next split occurs on the basis of host plant with biserrula split from clovers. Biserrula had a more-than-adequate nodulation score of 5.6. This does not necessarily imply that biserrula is a super-nodulator, but rather subsequent interrogation of the data found that the biserrula paddocks sampled generally had soil pH and available nutrients conducive to the growth of the host plant and performance of its associated rhizobia (Table 2) and this is most likely a reflection of good attention to fundamental agronomic principles by the early adopters (i.e. leading farmers) of this plant species.

Within the clovers, the primary data split occurred on the basis of soil pH with paddocks having a pH>5.6 having virtually adequate (score 3.9) nodulation. This split equates well to the parameters described in Table 2 which suggests optimal function of Group C symbionts at pH>5.5. Adequate nodulation could also be achieved where soil pH was less than 5.6, however this required other parameters (CEC, P) to be more favourable and pH to be greater than 5.3. This accords with our understanding of how elevated P and Ca levels can ameliorate the impact of low pH on rhizobial growth (Howieson et al 1993). The results of this survey suggest that soil acidity is a primary factor affecting the nodulation of clovers. It also shows that alternative species such as biserrula have the capacity for excellent nodulation where they are grown in appropriate conditions. Pleasingly, all biserrula paddocks sampled in this survey are used in 'on-demand' roles in crop-pasture rotations and were sampled in this survey in years following a cropping phase and this data suggest that the biserrula symbiont is surviving well through the cropping phase in the absence of the host plant. More detail of results of this survey can be found in Hackney et al (2017a).

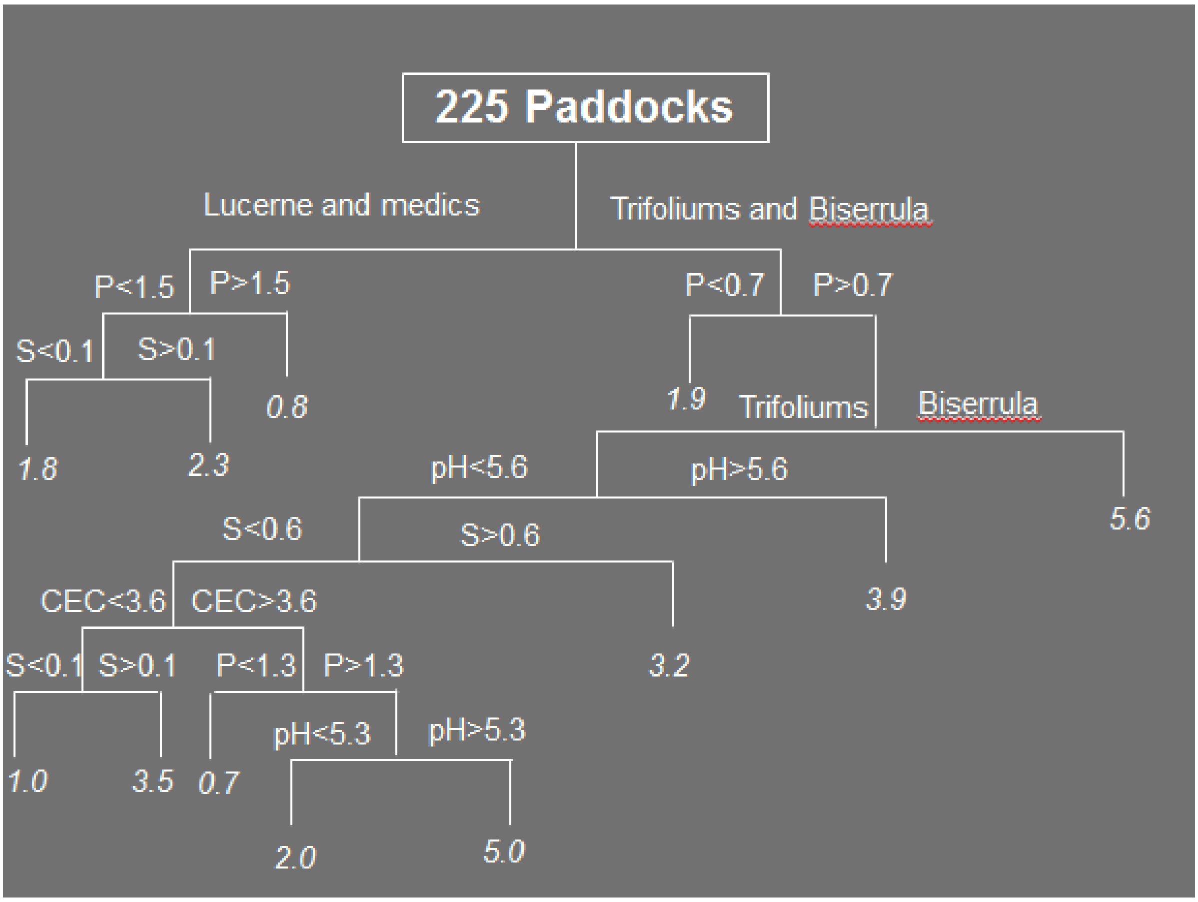


Fig. 22 The influence of host legume-rhizobia associations and soil chemical parameters on legume nodulation for 225 paddocks sampled across the Central West, Central Tablelands, Riverina and South-East Local Land Service regions in 2015 and 2016. R^2=0.73.

4.4 Animal production on improved pastures

A number of on-farm and replicated grazing studies were undertaken as part of this project. Initial grazing studies involved predominately on-farm measurements to determine differences in carrying capacity, liveweight gain and reproductive performance on alternative pasture legumes or the current (or historically used) pastures on-farm, which were originally subterranean clover-based. But in many cases these had degraded over time due to the susceptibility of subterranean clover to false breaks, dry spring conditions and/or red clover syndrome. The compelling case for adoption is based on changes in livestock production achievable from current compared to new legume pastures. While we were often confronted with feedback indicating comparison on farm should be made between alternative legume pastures and 'good' subterranean pastures, the reality was that such subterranean pastures did not exist for comparison and producers were not willing to sow them due to their lack of persistence, and the cost of sowing (subterranean clover $8-10/kg compared to $0.70/kg for alternative legumes produced on-farm). For WA producers, their experience with subterranean clover being wiped out intermittently as a result of red clover syndrome also weighed against the appetite for their re-sowing. In subsequent PhD studies of Ms Lucy Watt, comparison was made between alternative legumes and lucerne which is increasingly replacing subterranean clover in pastures in the <600 mm mixed farming zone of NSW (see 4.4.2).

4.4.1 On-farm studies

4.4.1.1 Comparison of carrying capacity during winter – Esperance 2014

In the case study below, grazing days through the winter, average winter DSE/ha and stocking rates carried by the paddocks were calculated (using DSE ratings provided in Table 3).

Table 3. DSE ratings used to calculate carrying capacity of biserrula and subterranean clover-based paddocks at Esperance in 2014

Animal	Liveweight (kg)	DSE rating
Wether	50	1
Dry cow	500	8
Pregnant cow last 3 months	500	11
Lactating cow-calf 0-3months	500	18
Lactating cow - calf 3-6 months	500	22
Twin bearing ewe - last 2 months	70	3.2
Single bearing ewe-last 2 months	70	2.4
Lactating ewe with twins at foot	70	3.6
Lactating ewe with single lamb	70	2.4
Yearling cattle 350 kg	350	8

The data shows the greatly improved carrying capacity of the paddocks improved with biserrula in this seasonal scenario and reflects the unique ability of biserrula to grow through moisture limited conditions.

Balancing by the farmer of FOO with livestock production resulted in an increase in carrying capacity over the winter period of 54-86% for biserrula paddocks compared to the subterranean clover paddock (Fig. 23). This increase is very significant for red meat production systems, particularly at a time of year when FOO generally limits pasture productivity. In addition to increased carrying capacity over winter in the biserrula paddocks, the farmer reported no incidence of adverse animal health issues on biserrula compared to subterranean clover paddocks. While red clover disease did affect the subterranean clover potential production, there is currently no way to predict an outbreak of this disease or to control it. What is clear however, is the ability of biserrula to significantly increase productivity in a real industry setting in the winter period. Biserrula pastures carried more stock than the degraded subterranean clover-based pasture, for longer, and achieved, on average, 7.3 DSE/ha over the winter across the three replicated paddocks whereas the subterranean clover-based pasture carried 4.2 DSE/ha over winter.

The reason for the increased carrying capacity on the biserrula was because it was more able to tolerate the semi-drought conditions over winter when growth was water limited, than the subterranean clover-based paddock. Added to this the higher nutrition provided by the legume dominant biserrula pasture allowed the farmer to retain stock on the paddocks whilst they maintained condition.

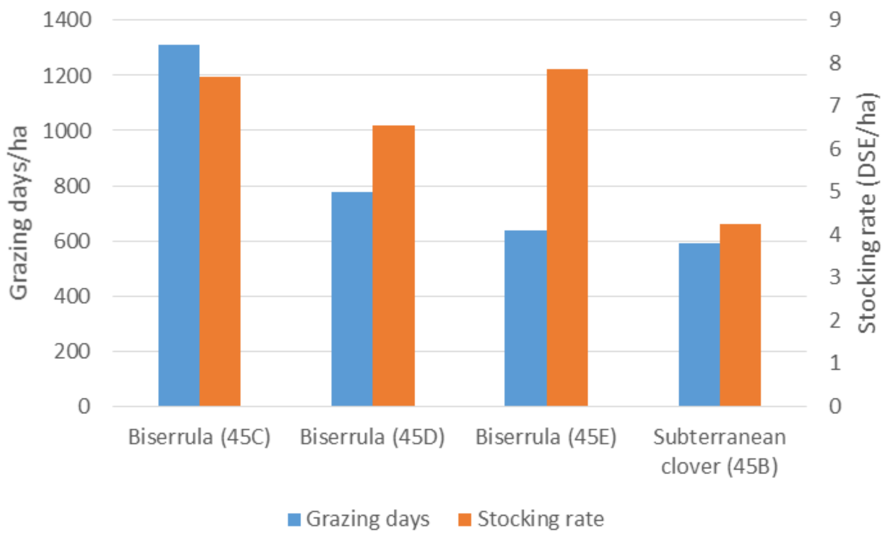


Fig. 23 Number of grazing days per hectare and stocking rate (DSE/ha) over the period 30 April to 1 September 2014 for three regenerating biserrula and one regenerating subterranean clover pasture at Esperance, WA.

4.4.1.2 Spring live-weight gain of prime lambs on biserrula – Beckom NSW 2014

At Beckom, in early September of the same year, four week old Wiltipoll x Australian White lambs and their mothers were placed on a biserrula pasture which had regenerated after a three-year cropping phase. Average entry weight of the lambs was 10 kg. Biserrula accounted for 90% of the feed on offer at commencement of grazing with the reminder of the pasture was made up of annual ryegrass and barley grass. Average stocking rate over the duration of the grazing period (56 days) was 7.4 DSE/ha (district average grazing pressure 4 DSE/ha). Sheep were mustered off the camp (therefore, no intake since the previous evening) on the day of weighing and had been locked in yards for four hours prior to weighing. Liveweight gain differed between sexes and averaged 350 g/head/day overall (Fig. 24).

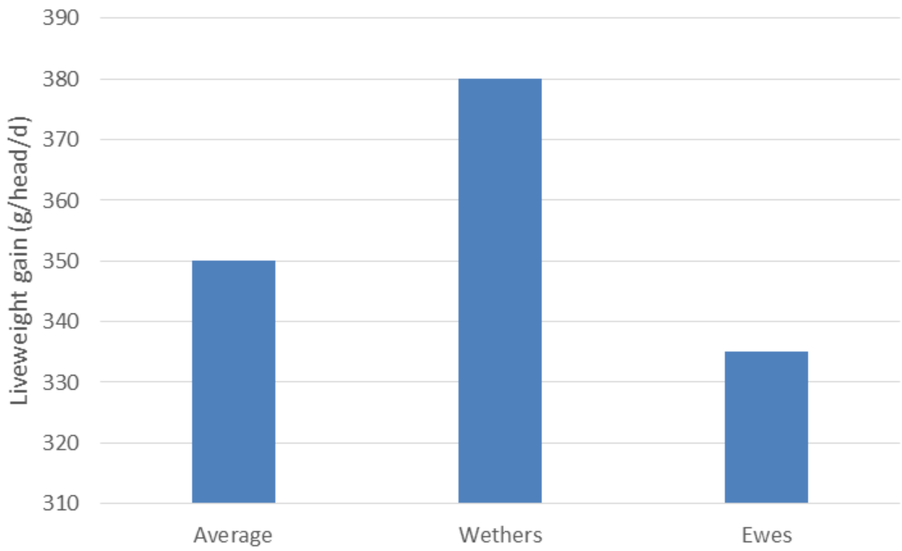


Fig. 24. Daily weight gain of crossbred lambs shown as average, for wether lambs and for ewe lambs at Beckom NSW in spring 2014.

Feed on offer at the end of winter in this paddock was 4000 kg DM/ha (Fig. 25). Seasonal conditions deteriorated considerably from late August onwards. Ewes and lambs were introduced to pasture on 3 September 2014 and feed availability at that time was 3880 kg DM/ha. At the conclusion of measurements and when lambs weaned, approximately 1700 kg DM/ha remained. Feed samples were collected for quality analysis throughout the experiment and are now in the laboratory for testing.

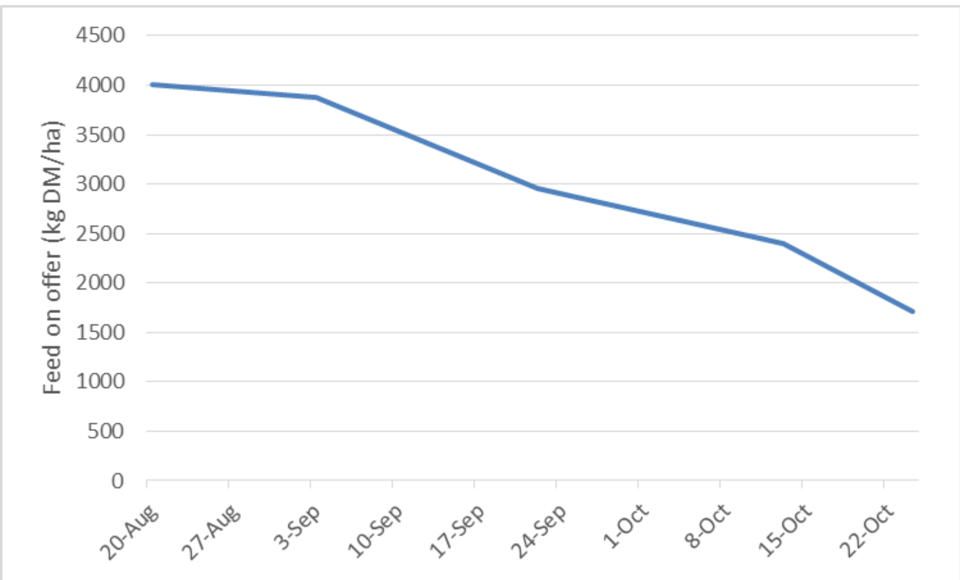


Fig. 25. Feed on offer in regenerating biserrula paddock grazed by crossbred ewes and lambs at Beckom, NSW. Sheep were introduced to pasture on 3 September 2014 and removed on 24 October 2014.

4.4.1.3 Reproductive efficiency – Brookton WA 2015

Parts of Western Australia, including Brookton, experienced a declie 1 rainfall year in 2015 which resulted in many producers having to sell stock or provide supplementary feed to valuable breeding stock. Anna and Colin Butcher who operate a mixed faming business covering 2700 ha (1600 ha of which is sown to winter crops) with 2500 Dorper ewes began growing French serradella in 2012 as an 'on-demand' pasture in the cropping rotation. Prior to growing serradella, the Butcher's frequently supplementary-fed their ewes through summer, due to lack of paddock residues and also through winter as a result of poor reliability of subterranean clover-based pastures and its susceptibility to red clover syndrome. Their historic ewe pregnancy scanning under this system shows they averaged 9% empty ewes. In 2015, despite the very poor seasonal conditions, no supplementary feeding of ewes was required. Rams were de-pastured with ewes (at 2.5%) on the same day the previous drop of lambs were weaned. Scanning six weeks after the rams were removed showed only 1.7% of ewes empty. If we estimate a weaning rate of 100% and value each lamb produced at $120/head, then the return to the livestock enterprise as a consequence of reduced number of dry ewes in that year compared to the historic records is in excess of $21 000 or almost $20/ha of land utilised for grazing (1100 ha). Some of these outcomes were reported at the Nhill Field Days (Butcher and Butcher 2015).

4.4.1.4 Additional data for on-farm carrying capacity of alternative legume and legume mixes

Uranquinty – arrowleaf clover 2016

A 100 ha paddock of arrowleaf clover was established via summer sowing in 2015. Seed was harvested in the establishment year (400 kg/ha) and the paddock grazed over summer 2015/16 and left to regenerate. The paddock was grazed with 1000 lambs (0.8 DSE/hd) for 34 days (8 DSE/ha) in late June/early July and subsequently 600 in-lamb crossbred ewes (1.6 DSE/hd) for 42 days (9.6 DSE/ha) in August early September. The paddock was then locked up in mid-September and cut for hay in early November yielding 6.2 t/ha.

Greenethorpe – Bladder clover, lucerne, oats mixed pastures 2016

A paddock of 40 ha had been sown to lucerne in 2013. As the lucerne stand had thinned considerably, a mixture of oats and bladder clover (unscarified seed harvested on farm) was sown into the lucerne in February 2016. The paddock was grazed by 400 Crossbred ewes with twin lambs (5.1 DSE/unit) for 21 days (51 DSE/ha) in early June followed by 1000 crossbred ewes and lambs (3.8 DSE/unit) for 28 days (95 DSE/ha) in mid July/early August and subsequently 1000/750 cross bred ewes and lambs (4 DSE/unit) for 28 days (100 DSE/ha) in September.

Greenethorpe – French serradella, arrowleaf clover mixture 2016

A 40 ha paddock of serradella was established in 2011 via twin-sowing with Canola. The paddock had four consecutive years of crop following initial seed set. Unscarified arrowleaf clover was summer sown into the paddock in February 2016 to complement the serradella. Both legumes established strongly (>600 plants/m^2) in autumn 2016 with serradella accounting for more than 80% of seedlings indicating good persistence of the seedbank despite no seed set since 2011. The paddock was grazed by 600 in-lamb crossbred ewes (2.3 DSE/unit) for 14 days (34.5 DSE/ha) in early June followed by 1000 crossbred ewes and lambs (3.8 DSE/unit) for 28 days (95 DSE/ha) in early July, and subsequently 1000 crossbred ewes and lambs (3.8 DSE/unit) for additional 14 days (95 DSE/ha) in late August.

These carrying capacities far outweigh the district averages. In the Greenethorpe example above, serradella provided 4473 grazing days when winter grazed, compared with the district average of less than 1500. This does not include the extra benefit from the higher value residues of serradella over summer compared with conventional pastures.

4.4.2 Replicated grazing and feeding studies

4.4.2.1 The role of biserrula for livestock production and as an integrated weed management tool
A replicated (n=4) grazing trial was established at Charles Sturt University Wagga Wagga in 2014 to assess the potential of both Casbah and Mauro biserrula to support livestock production. A comparator, typical subterranean clover-annual grass paddock was used as a control. Sheep (Lactating merino ewes and lambs) were introduced to the biserrula and volunteer pastures on 22 September 2014. Suffolk x Dorper lambs were also run on the biserrula pastures. Crossbred lambs were not included in the volunteer pasture treatment due to the presence of annual grasses such as barley grass which may have resulted in carcass damage. Stocking rate on the biserrula was 18 DSE/ha and 5 DSE/ha on the volunteer pasture

There was no difference between Mauro and Casbah in terms of liveweight performance of any sheep group (Fig. 26) with all animals gaining in excess of 200 g/head/day. However, there was considerable difference between the merino's grazing biserrula-based pasture and those grazing volunteer pastures. Lambs on the volunteer pasture grew at less than half the rate of those on biserrula. The merino ewes grazing biserrula gained over 200 g/head/day while lactating, while those on the naturalised pasture lost 75 g/head/day.

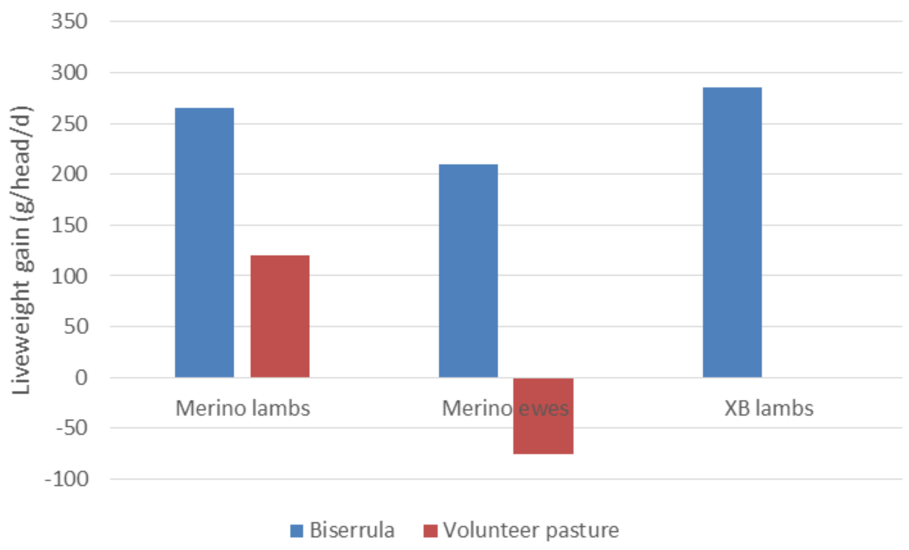
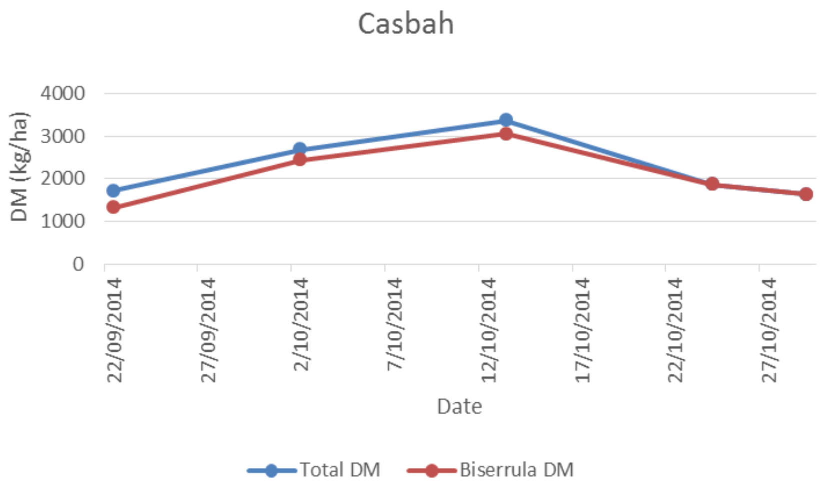


Fig. 26 Liveweight gain of merino ewes and lambs grazing biserrula-based pasture and those grazing subterranean clover- volunteer pasture (containing legumes) and crossbred lambs grazing biserrula at Wagga Wagga, NSW.

Increased weight gain was seen in plots where there was presence of other pasture species; this was predominately annual ryegrass. In plots where annual ryegrass was present as a volunteer species at more than 10% of feed on offer, weight gains were up to 30 g/head/day greater than the average weight gain suggesting presence of other species in the pasture, even at relatively low levels, give improved growth performance over and above that observed on biserrula alone. Thus, there may be merit in deliberately using biserrula in a mixed pasture rather than in a monoculture for improved livestock performance.

While there was no difference in weight gain between biserrula varieties, there were considerable differences in pasture composition over the duration of the grazing trial (Fig. 27). In Casbah plots, sheep grazed out volunteer species over time, resulting in convergence of total herbage and proportion of herbage contributed by biserrula over time. In Mauro plots, where volunteer species were present, this convergence did not occur. This may indicate a fundamental difference in palatability of the two cultivars, meaning that Casbah may be the preferred option where grazing will be used as a technique to remove unwanted weed species, whereas Mauro made be preferred where a more mixed pasture is desired.



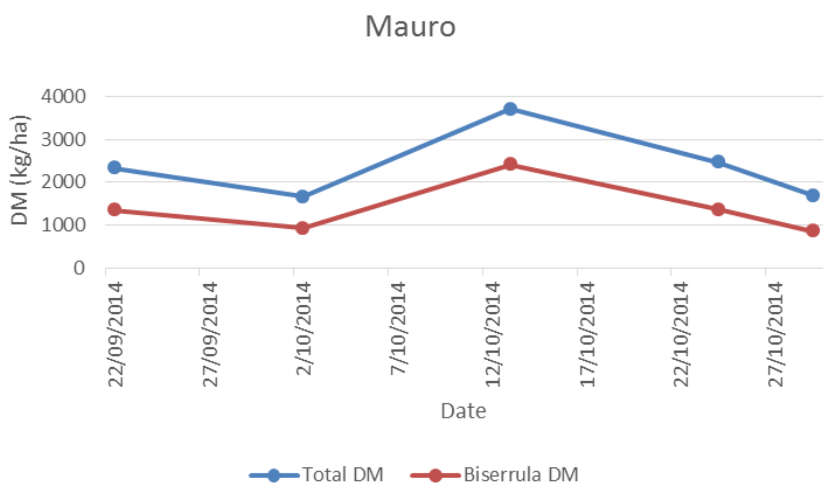


Fig. 27 Total herbage biomass and biserrula biomass (kg DM/ha), sampled five times during spring 2014 in Casbah and Mauro biserrula-based pastures at Wagga Wagga, NSW.

4.4.2.2 The value of biserrula as a grazing residue over summer

Dry merino ewes were grazed on biserrula and biserrula-ryegrass mixed residues in summer 2015/16 (where ryegrass had been sown the previous autumn at 30 or 120 kg/ha) at a stocking rate of 12-14 DSE/ha depending on feed on offer at the commencement of the experiment. All treatments were replicated three times. The ewes used in the experiment were cast-for-age and in very light body condition (score 1-1.5). The ewes grained more than 225 g/head/day on all residues regardless of whether ryegrass residue was present in the mix (Fig. 28). Ewes grazing Casbah and Casbah-120 Ryegrass treatments gained significantly more weight than those on the other treatments (>300 g/head/day). Weight gain of this magnitude on dry residue is very high and is probably a reflection of the low body condition score of the ewes initially. With such animals in such condition, a higher proportion of the ingested diet can be partitioned to growth rather than maintenance in the initial grazing period. Our analyses for indicated the metabolisable energy (ME) of the total feed on offer was 7.5 MJ ME/kg DM with protein of 12%. The seed however, had a ME of 12 MJ ME/kg and protein of 32% while the pod was 9 MJ ME/kg DM and 15% protein. Thus it is probable that animals are selecting a diet of higher quality than the average of the pasture.

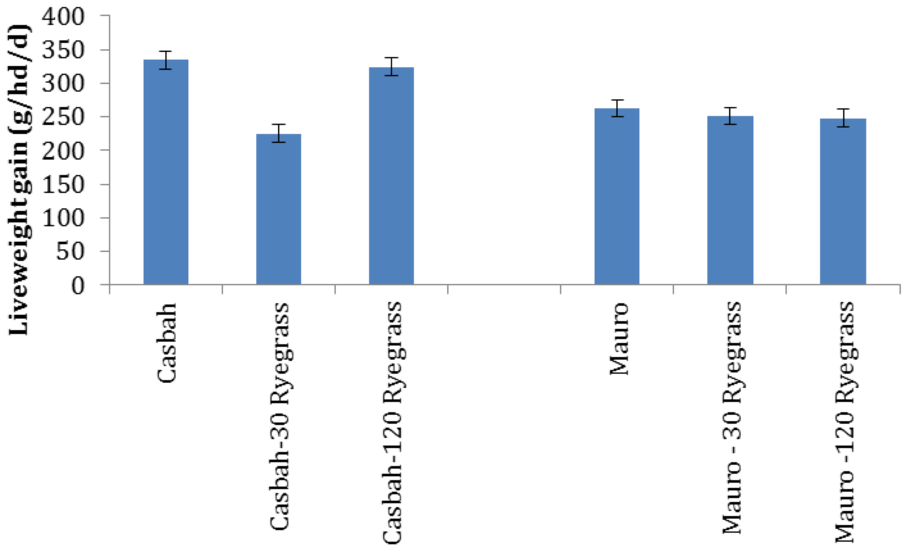


Fig. 28 Growth rate of merino ewes in summer grazing Casbah or Mauro biserrula-based pasture oversown with 30 or 120 kg/ha of annual ryegrass (ARG).

4.4.2.3 Comparison of livestock production on alternative legumes compared to traditional pasture species

a) Grazing trials – winter

This experiment undertaken at Charles Sturt University investigated the impact of arrowleaf clover, biserrula and bladder clover compared to lucerne, lucerne/phalaris and a 'commercial' (volunteer pasture) on livestock productivity using weaner merino and crossbred lambs to determine if there was any impact of breed on productivity in winter 2015. The first grazing experiment commenced in late winter (18 August) and ran for a period of five weeks. Sheep (18 DSE/ha equivalent) were weighed at intervals of 14, 28 and 35 days after commencement of grazing.

Liveweight gain varied significantly between legume species and weighing period (Fig. 29). In the first two weeks of the grazing experiment (14 days), liveweight gain was significantly lower on arrowleaf clover and biserrula than for all other treatments. There was however, no difference in liveweight gain between bladder clover, the commercial volunteer plots and lucerne while the lucerne/phalaris mix was significantly lower than the bladder clover.

Sheep were removed from the arrowleaf clover and biserrula plots following the first weighing period. Arrowleaf clover growth appeared to be more severely restricted by the cold winter conditions than other treatments. The decision was made to remove sheep from the biserrula plots at this time due to the incidence of photosensitisation. Twenty-five percent of sheep at this stage were exhibiting moderate photosensitisaton (ear swelling and scabbing, facial swelling, red eyes) while the remaining seventy-five percent were exhibiting minor symptoms of photosensitisation.

During the second monitoring period (28 days), liveweight gain on bladder clover was significantly higher than for all other remaining pasture species. During this period sheep on the commercial plots began to lose weight. It should be noted that the commercial plots were sprayed with a knockdown (glyphosate) to prevent barley grass in these plots producing seed and causing eye and carcase injury and as a result of heavy August rain, feed quality declined very quickly resulting in weight loss, despite more than adequate feed availability. Consequently, sheep were removed from the commercial plots at 28 days. Sheep were also removed from the bladder clover plots at 28 days due to declining feed availability . Sheep continued to graze the lucerne and lucerne/phalaris plots for a further week (35 days). During this period, weight gain on the lucerne was significantly higher than on the lucerne/phalaris, which is most likely a consequence of low legume content limiting weight gain in the lucerne/phalaris plots.

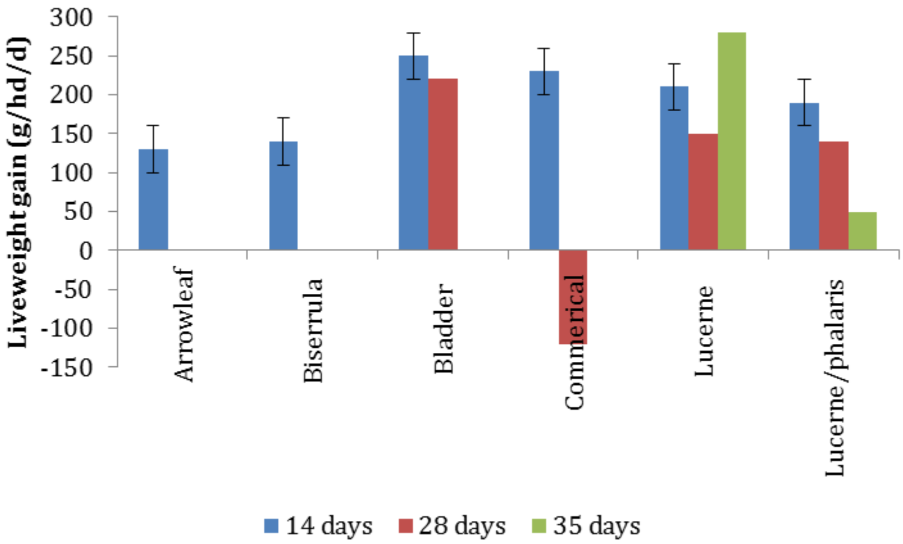


Fig. 29 Liveweight gain of lambs grazing a range of pastures during winter-early spring 2015 at Wagga Wagga, NSW. Lambs were weighed at 14, 28 and 35 days after commencement of grazing. Animals were removed from arrowleaf plots due to lack of feed availability and from biserrula due to photosensitisation after 14 days and from bladder clover and commercial plots after 28 days due to declining feed availability.

Changes in feed quality over the grazing period (Fig. 30) provide some explanation for differences in liveweight gain. For example, bladder clover had relatively high digestibility early in the grazing period resulting in initial high weight gains while the lucerne-phalaris mixture declined significantly in quality over the grazing period resulting in lower weight gains towards the end of the experiment. Some species such as arrowleaf clover had high quality at the commencement of the experiment but insufficient feed on offer meant that liveweight gains were restricted.

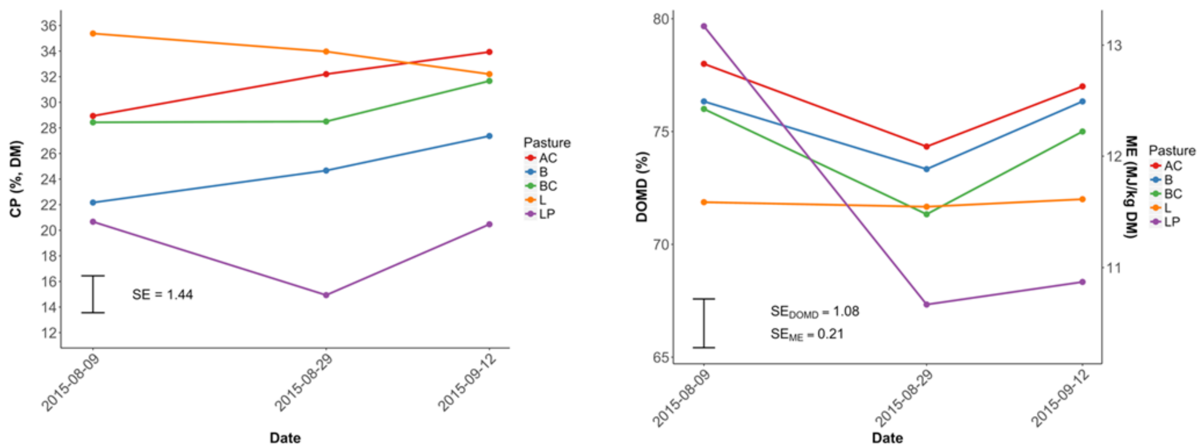


Fig. 30 The crude protein (CP%) and digestibility (DOMD%) of arrowleaf clover (AC), biserrula (B), bladder clover (BC), lucerne (L) and lucerne-phalaris pastures at Wagga Wagga during a six week grazing experiment in winter 2015.

In winter 2016 a further grazing experiment was undertaken. Plots had been split in summer with half of each plot having oats sown at 30 kg/ha. This was done to determine if there was merit in adding oats to a paddock with a strong legume seedbank to increase winter feed on offer and also to mitigate against animal health disorders such as photosensitisation (biserrula) and red gut (lucerne). The commercial pastures were replaced by French serradella in 2016 by sowing a heavy rate of serradella seed (70 kg/ha) to mimic a regenerating pasture in April 2016. The autumn break in 2016 was quite late resulting in poor growth of the legume-only plots and there was insufficient feed on offer to graze these over winter. However the oat-legume plots were grazed for a six week period at stocking rates of 20-30 DSE/ha (using merino lambs) depending on feed on offer),

There was no significant difference in weight gain between the legume-cereal mixtures (Fig. 31) with lambs gaining over 120 g/head/day over the grazing period. This weight gain is lower than previously reported on such mixtures but winter of 2016 was significantly wetter than average which is likely to have impacted considerably on grazing behaviour Importantly, inclusion of the oats resulted in no issue being recorded due to photosensitisation in biserrula.

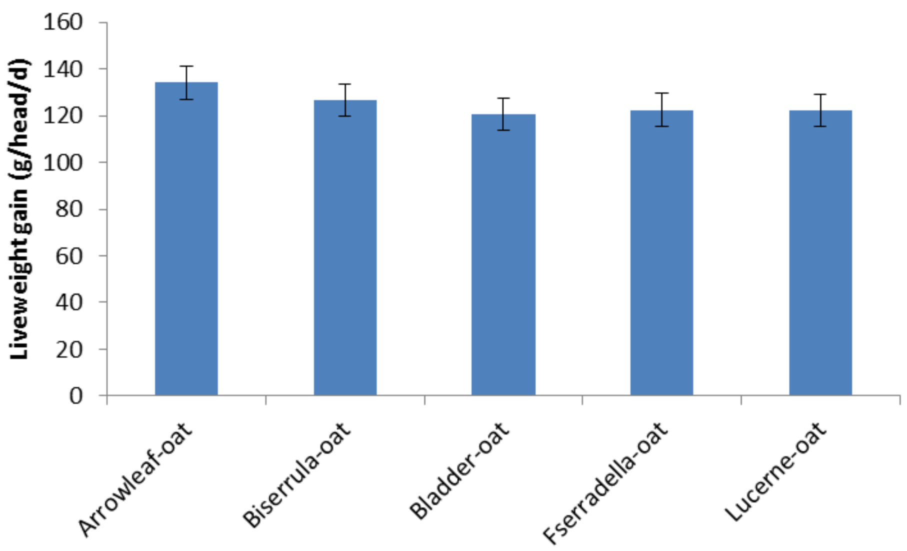


Fig. 31 Liveweight gain of crossbred lambs grazing oat-cereal mixtures over a 42 day period in winter 2016 at Wagga Wagga, NSW.

While there was no significant difference in weight gain between treatments, there were significant differences in feed quality (Fig. 32), particularly in terms of digestibility with the lucerne-oat mix having a lower digestibility through the majority of the grazing period. This suggests that animals were selecting a diet of higher quality than indicated by the pluck samples sent for feed testing.

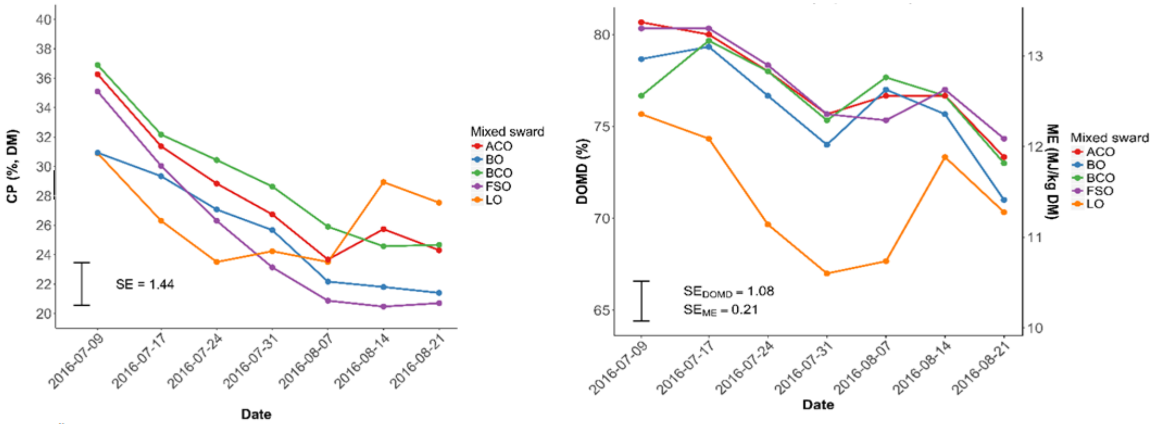


Fig. 32 The crude protein (CP%) and digestibility (DOMD%) of various annual legume – oat mixtures arrowleaf clover-oats (AC0), biserrula-oats (BO), bladder clover-oats(BCO), French serradells-oats (FSO)and lucerne-oats (LO) pastures at Wagga Wagga during a six week grazing experiment in winter 2016.

b) Grazing trials – spring

In spring 2015, a six week grazing trial was undertaken at the same site as the winter grazing experiment using both Merino and crossbred lambs at stocking rates of 25-35 DSE/ha depending on feed on offer. Liveweight gain on arrowleaf and bladder clover was significantly higher than on lucerne or lucerne-phalaris mixtures while weight gains were significantly higher on biserrula than on lucerne-phalaris mixture (Fig. 33). No photosensitisation was observed on biserrula plots following formation of initial seed pods and those animals mildly affected by photosensitisation self-resolved on the plots.

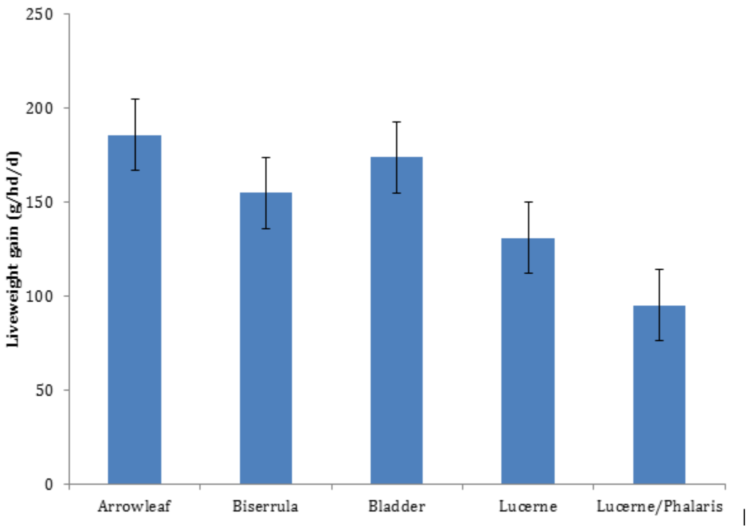


Fig. 33 Liveweight gain (g/hd/d) of lambs grazing a range of legume or legume/perennial grass pastures at Wagga Wagga in spring 2015.

Differences in feed quality explain some of the differences recorded in liveweight gain. Digestibility and/or crude protein of arrowleaf clover was higher than for lucerne through considerable portions of the grazing experiment while the quality of the lucerne-phalaris mixture was lower, particularly toward the end of the experimental period than the better performing species (Fig. 34). Interestingly, bladder clover, while comparatively lower in protein and digestibility than other pure legume treatments throughout much of the experimental period, achieved weight gain equivalent to arrowleaf clover, again indicating that sheep may be selecting a diet of higher quality than indicated by the feed test results.

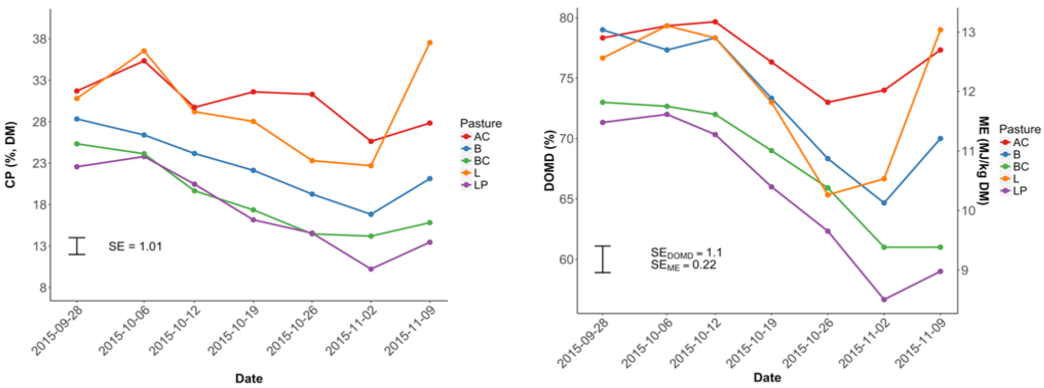


Fig. 34 The crude protein (CP%) and digestibility (DOMD%) of arrowleaf clover (AC), biserrula (B), bladder clover (BC), lucerne (L) and lucerne-phalaris pastures at Wagga Wagga during a six week grazing experiment in spring 2015.

4.4.3 Use of alternative legumes as a conserved feed

To determine the potential of alternative legumes as a conserved fodder, an animal study was undertaken as Charles Sturt University. Merino wether lambs 9-10 months of age (34 kg) were used in this experiment with eight lambs allocated per diet. Arrowleaf clover (cv, Cefalu), bladder clover (cv. Bartolo), subterranean clover (cv. Mt Barker) hay were sourced from local Riverina farms for the experiment. The arrowleaf clover was approximately 70 cm high at the time of cutting and in the late vegetative stage of growth. The bladder and subterranean clover was approximately 40 cm high and at approximately 40% flowering. The lucerne-oaten clover was fed as a chaffed mix compromising 50% of each component. Chaffing of this ration was done to minimise selectivity where two feeds of significantly different feed quality are fed. Animals were fed ad lib on the diet for 51 days following a one-week adaptation period. The feed quality of the diets are shown in Table 4.

Table 4. The metabolisable energy (ME MJ/kg DM), digestibility (DOMD%) and crude protein (CP%) of several hays fed to merino weaners in an animal house study at Charles Sturt University NSW in 2015

	Arrowleaf clover	Bladder clover	Subterranean clover	Lucerne/oat mix

ME (MJ/kg DM)	10^{b}	10.5^{b}	10.0^{b}	9.1^{a}
DOMD (%)	64.4^{b}	66.6^{b}	63.8^{b}	59.6^{a}
CP	14.6^{b}	19.7^{c}	15.9^{b}	11.2^{a}

Liveweight gain achieved on bladder clover hay was significantly higher than for all other species fed (Table 5) while weight gain on arrowleaf clover was higher than for either subterranean clover and lucerne-oat mix. The differences in weight gain are partially attributable to differences in feed quality, particularly digestibility which influences the rate of passage through the animal and their ability to ingest feed offered. In this experiment, intake was lowest for lucerne-oat mix (1.26 kg/d) and highest for bladder clover (1.50 kg/d). It is interesting that bladder clover had feed quality (DOMD) with 40% of plants flowering at time of cut 2% higher than arrowleaf which was cut in the vegetative stage of growth. This indicates that bladder clover retains relatively high feed quality even in reproductive stages of growth.

Table 5. Liveweight gain of Merino wether lambs fed an ad-lib diet of several legume of legume-cereal hays in a 51 day animal house study at Charles Sturt University NSW in 2015

	Liveweight gain (kg)	Liveweight gain (g/head/d)
Arrowleaf clover	10.40b	204
Bladder clover	12.65c	248
Subterranean clover	9.55a	187
Lucerne/oaten	9.31a	183
LSD	0.70	

Using the results from Table 5 and the Grazfeed model, the potential return from the various forages on a per hectare basis was calculated assuming 4 t DM/ha of each feed could be conserved (Table 6). This analysis showed that bladder clover could give an additional return of more than $1200 /ha of feed conserved compared to traditional subterranean clover or lucerne-oat hay mixtures. Arrowleaf clover at the quality fed in this experiment could also provide an additional $140-$180/ha conserved fodder compared to traditional hays.

Table 6. Predicted liveweight gain and value of liveweight gain per hectare of conserved forage for a range of legume or legume/cereal hays. Value of liveweight gain assumes value of $2.80/kg liveweight

	Harvested material (t/ha)	Start weight (kg)	End weight (kg)	Days to target	Intake plus wastage (kg/head)*	Intake total (kg/hd)	Sheep supported /ha conserved	Liveweight gain (kg/ha conserved)	Value liveweight gain ($/ha conserved)
Arrowleaf	4	30	42	59	1.33	78.4	51	612	1714
Bladder	4	30	42	48	1.33	64.4	62	744	2806
Subclover	4	30	42	64	1.33	85.4	47	562	1575

Lucerne/oats	4	30	42	65	1.33	87.6	46	548	1535

*For the purposes of this calculation, intake plus wastage has been estimated at 3.5% of average liveweight over the period of feeding. This may underestimate wastage on some of the forage.

The value of serradella pods fed to sheep was evaluated in a joint study with Dr Hayley Norman. Yellow serradella pod contains approximately 33% seed, whilst French serradella pod contains approximately 50% seed. This difference accounts for the decreased protein in the pods of yellow serradella relative to the French species, which has a very high protein content of 26.3% (Appendix 9.3). Notably, this is greater than the protein content of most legumes when in the vegetative phase. A further notable difference is the high content of crude fats in the French serradella pod. The fat content of French serradella pods exceeds that of lupin seed, which makes it a very valuable source of summer nutrition for grazing animals. The other remarkable fat in this study was the very high protein content of seeds of bladder clover – in excess of 17%. The collective data show the potentially massive value of french serradella pod to the over-summer nutrition available to grazing sheep. Given the high biomass produced by French serradella, this has the potential to greatly reduce the summer-autumn feedgap in southern Australia.

The full data may be accessed in Appendix 9.3 of this report.

4.4.3.2 Management of the seedbank under grazing to ensure regeneration

A number of studies were undertaken to determine the effect of grazing management in spring and summer on seed production and plant regeneration in subsequent years across a number of species.

Summer residue management and the effect of grazing on following autumn regeneration

The effect of grazing on senesced forages was studied on-farm at Beckom, NSW in summer 2015/2016 on biserrula, bladder clover and French serradella. A similar study was also undertaken on replicated (n=4) biserrula plots at Charles Sturt University (CSU site), which had been used for 2014 spring grazing experiments reported in milestone 6. Transects were established in each paddock with a minimum of 50 fixed sampling locations in the farm paddocks and 35 fixed sampling locations per plot at the CSU site. Herbage mass at each sampling location was determined at the end of the spring grazing period at each sampling location. Average summer grazing pressure at Beckom was 6 DSE/ha over a six-week period. At CSU, the plots were grazed at 18 DSE/ha for two weeks during summer. Each sampling site was revisited approximately five weeks after opening autumn rain in 2015 to determine plant density.

Strong relationships were found between residual spring herbage and plant density in the following autumn (Fig. 35). Higher quantities of residual spring herbage were required for bladder clover and French serradella in order to reach an acceptable plant density (500 plants/m^2) for subsequent herbage production. Both bladder clover and French serradella have larger seed than biserrula and hence a higher proportion of ingested seed of these species will be digested by grazing livestock in comparison to biserrula. Even at the high summer grazing pressure used at CSU, biserrula was still able to achieve high plant regeneration counts across the range of spring residual herbage levels recorded.

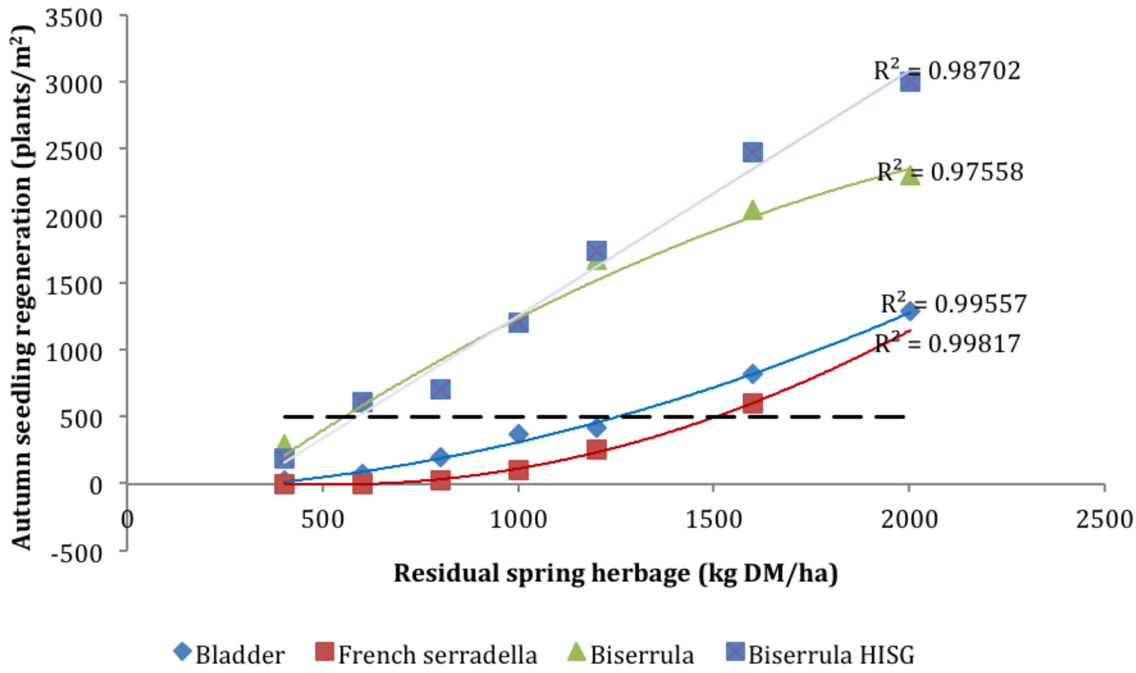


Fig. 35. The relationship between residual spring herbage (kg DM/ha) and the following autumn seedling regeneration (plants/m2) for bladder clover, French serradella and biserrula at Beckom NSW with summer grazing at 6 DSE/ha and for biserrula at Charles Sturt University, Wagga Wagga where residue was grazed at high intensity over the summer period (HISG).

Interpretation

- Biserrula is more resistant to summer grazing of senesced residue with respect to grazing impacting autumn regeneration compared to other species. This is likely to be due to a higher proportion of biserrula seed surviving ingestion as a result of its smaller seed size.
- Greater care may be required in the establishment year with respect to grazing of bladder clover and French serradella over the first summer period to ensure an adequate seedbank is formed for regeneration in subsequent years and to avoid cost of resowing pastures

Grazing impacts during Spring on seed production of hardseeded annual legumes.

Two experiments, both replicated (n=3), were undertaken to determine the effect of grazing on seed production. The first of these sites was located at Wagga Wagga where Spring grazing was completed in mid November 2015. Stocking rates throughout the growing season varied from 18 DSE/ha in winter to 30 DSE/ha in spring. Comparison at the Wagga site was made between biserrula, bladder clover and arrowleaf clover. A second site at Temora had grazing completed by mid December. Comparison was made between biserrula, bladder/gland clover mix and subterranean clover at this site.

Wagga Wagga

Transects were established within each plot with a minimum of 35 matched permanent sampling location per plot. Seed was collected from one of the matched area per sampling location in the bladder clover plots and seed yield determined as these plots had fully senesced. As a result of November rain, biserrula recommenced active growth resulting in a second generation of seed production and therefore results presented for biserrula are estimates only. Arrowleaf cover only commenced flowering very late in the season and therefore no estimate of seed production was made at this time.

A strong relationship was found between end of spring herbage level and seed yield for bladder clover (Fig. 36). The capacity of bladder clover to produce high quantities of seed even at low forage levels is evident from these results. Historic research on subterranean clover by Dr Phil Cocks suggested seedbanks of approximately 150 kg seed/ha for strong regenerating subterranean clover based pastures. If we use this as a guideline, it can be seen that bladder clover reaches this level of seed production at approximately 750 kg DM/ha residual spring herbage.

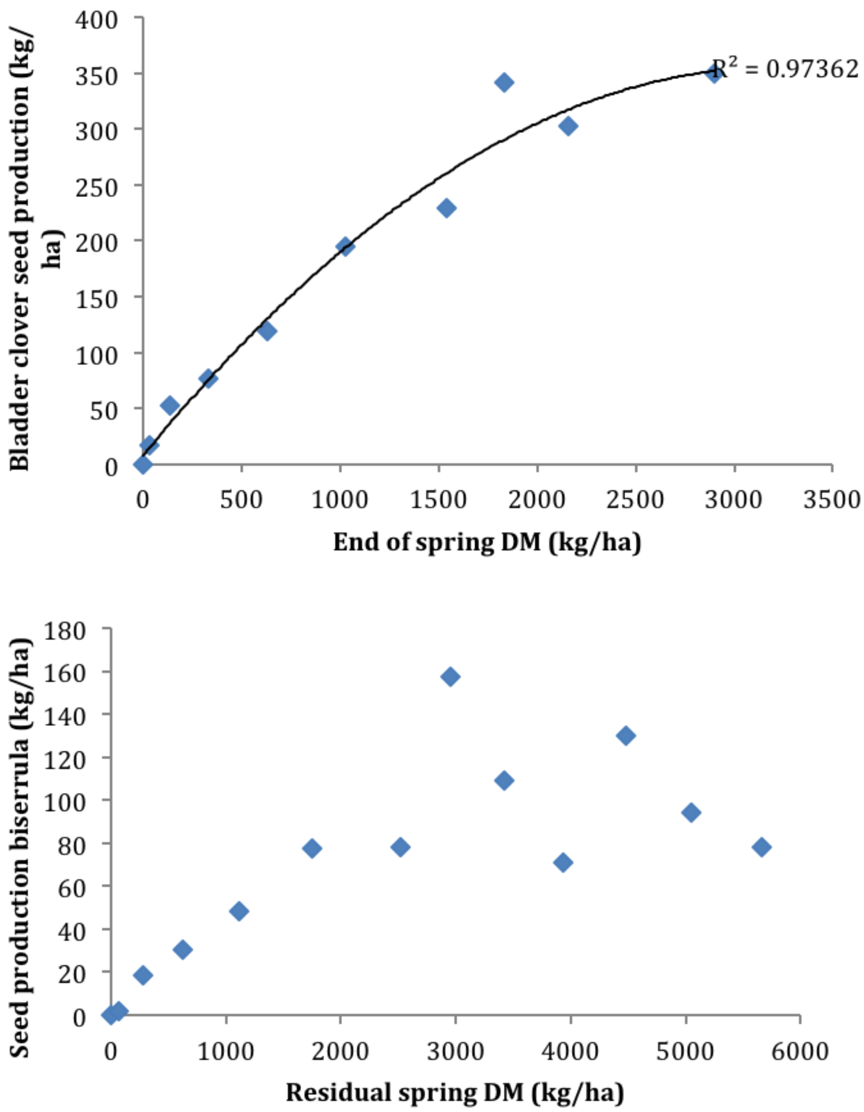


Fig. 36. The relationship between end of Spring herbage (kg DM/ha) and seed production for bladder clover (top) and preliminary relationship between spring residual herbage and seed production for biserrula at Wagga Wagga NSW.

The final result for biserrula seed production (which responded to late rains by re-flowering) reached 150 kg/ha seed production at approximately 1600 kg DM/ha (Fig. 37). However, given the small seed size of biserrula, it could be argued that 150 kg seed/ha is not required for this species to provide adequate regeneration. Biserrula seed is approximately one-third the size of subterranean clover. Arrowleaf clover was much more sensitive to residual herbage level in determining seed production with higher residue levels required to achieve 150 kg seed/ha than either of the other two species (Fig. 37). Anecdotally, producers have reported greater difficultly in maintaining arrowleaf in longer term pastures and have attributed this to the prominent seed heads that are produced near the top of the canopy as compared to a species such as biserrula where seed pods are afforded some protection due to its growth habit. As with biserrula however, arrowleaf clover is again about one-third the size of subterranean clover and thus whether a seedbank of 150 kg/ha is required is debatable. Regeneration counts to assess this were undertaken following the autumn break in 2016.

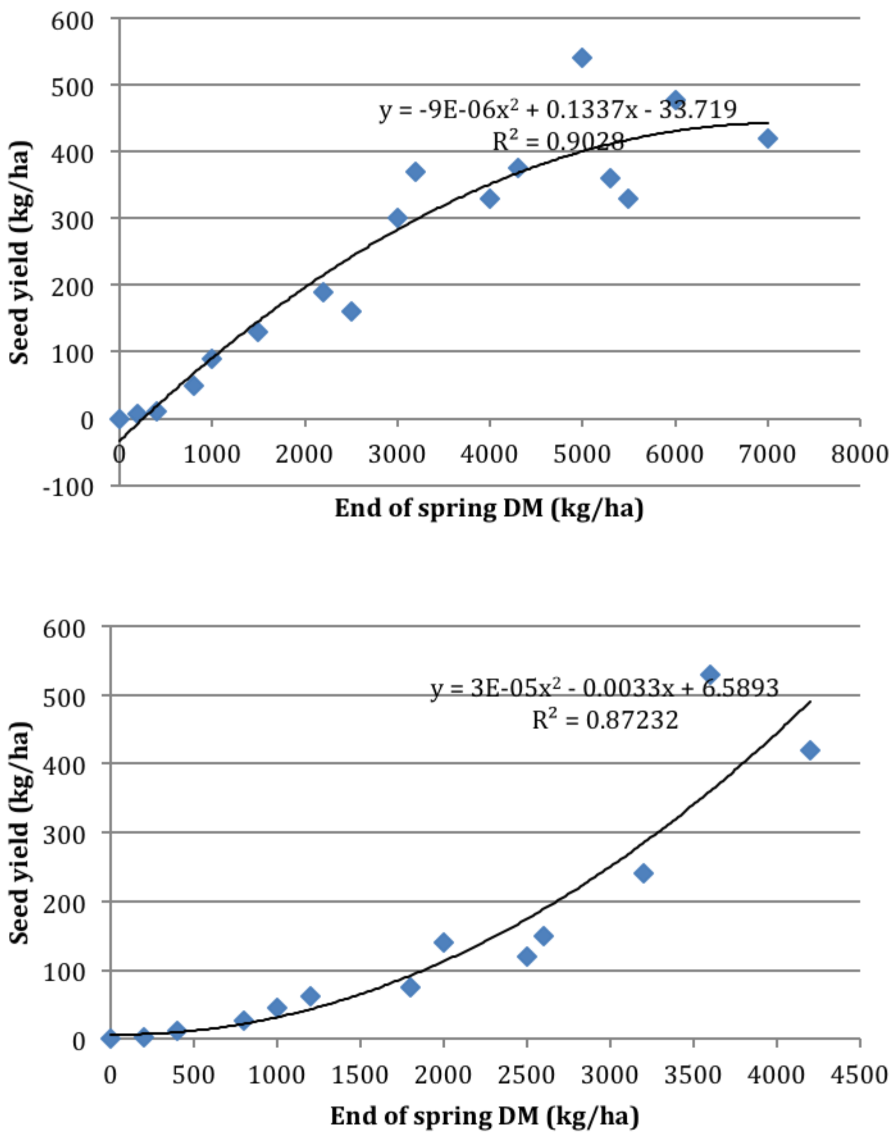


Fig. 37. The relationship between end of Spring dry matter (kg/ha) and seed yield (kg/ha) for biserrula (top) and arrowleaf clover (bottom)

The data indicates that by leaving approximately 2 tonnes of dry matter on the ground at the end of Spring will ensure an adequate seed production of these species. This is not an unreasonable target, as most farmers have an excess of forage at this time in the season, where plant growth has been maximal through this warm, moist period.

4.5 Extension activities

With the assistance of the Grower Groups, the project has had a very large outreach and extension footprint, in both NSW and WA, through both farmer groups, Local Land Services and the consultant / banking industries.

4.5.1 Extension activities focussed on consultants through workshops in 10 agricultural regions

As an example, 28 extension activities were held in NSW during 2016 (Table 7) and seven in WA (Table 8). Our extension activities consistently throughout this project have simultaneously targeted producers and consultants. There is sound reasoning for this. At the commencement of the Pastures Australia project ‘Agronomy and management of new annual legumes’ of which MLA was a co-funder, an extensive survey of 300 producers and 35 industry advisors was undertaken to assess the knowledge needs for adoption of new annual legume species (Hackney et al. 2008). The needs of both groups was essentially the same with requirements to see legumes growing in their region before they would feel comfortable growing or recommending the species, access and access to information on how to grow and manage the species. However, there was considerable disparity between what constituted ‘success’ in terms of pastures fulfilling production goals. More than 80% of advisors considered their recommendations with respect to selection and management of pasture species was highly successful (i.e. production goals met on more than 90% of occasions). However, only 30% of producers indicated their pastures were highly successful in achieving production goals. Further questioning of advisors revealed that success was considered the client took their advice not necessarily that it was implemented or worked. Because of this, we have made a conscious effort to have open forums where both producers and advisors can attend as the level of knowledge on how to grow and manage these species was not significantly different between the two groups. Further, producers have always been very eager to engage in this project and its predecessors whereas there was a general reluctance amongst consultants initially. It has been the pressure from producers on their individual consultants for information on the selection and management of these species that has seen an increase in consultant participation in extension activities throughout this project.

It should be noted that there has been considerable demand from some sectors of the industry advisory sector during the 2016 season. As a result of the legume nodulation workshops in June 2016, Incitec-Pivot requested participation in their extension activities at Ballarat and Albury. This industry demand increased in 2017 with requests to present this information at the Grassland Society of NSW Conference, The Red Meat Updates in Tasmania and the Australian Agronomy Conference. The results of the legume survey have now been delivered to more than 1500 farmers and advisers. We are now receiving feedback from leading industry consultants that they are changing their recommendations with respect to target soil pH (for pasture and grain legumes) along with review of the use of residual herbicides that may impact on legume root growth and rhizobia survival.

Further, the Agriwest and AgNVet rural agribusiness have requested inclusion hardseeded legume and nodulation research to be delivered in their pre-season grower and staff meetings. Similarly, Auswest Seeds have requested and had delivered for their staff, training on factors affecting nodulation and the use of hardseeded legumes in rotations.

Table 7. Extension activities in NSW in 2016 showing location, total attendance and number of consultants attending

Location	Date	Topics	Total attendance	Number of consultants attending
Nyngan	4/2/2016	Pre-season: Hardseeded legumes in rotation	83	10
Wagga	16/2/2016	Pre-season: Summer sowing/nodulation	21	21
Wirrinya	3/3/2016	Pre-season: Legume selection for pasture-crop rotation with Agriwest Forbes	27	4
Parkes	9/3/2016	Pre-season: Nodulation and N-fixation	42	15
Forbes	15/3/2016	Auswest seeds – legume fit in rotations	12	12
Barellan	17/3/2016	Barellan GRDC update – summer sowing	48	14
Alectown	19/4/2016	Pre-season: Legume selection for pasture-crop rotation with Agriwest Forbes and Parkes	29	5
Junee, Wallendbeen, Forbes, Bathurst, Dunedoo, Mendooran	14-17/6/2016	Legume nodulation survey results and factors affecting N-fixation workshops	162	21

Forbes	28/6/2016	Legume nodulation survey results presentation for Auswest seeds	11	11
Ballarat	26/7/2016	Legume nodulation survey results with Incitec Pivot	57	16
Albury	26/7/2016	Legume nodulation survey results with Incitec Pivot	48	15
Beckom	5/8/2016	Farmer/advisor bus trip to Beckom – Mike O'Hare summer sowing, crop-pasture rotation	53	12
Coonabarabran	12/9/2016	Field day – hardseeded legumes and tropical grasses	98	17
Tooraweenah	15/9/2016	Field day – legume selection and nodulation	27	5
Trangie	15/9/2016	Field day – legume selection and nodulation	13	2
Cooks Myall	17/9/2016	Field day – legume selection and nodulation	17	4
Yeoval	10/10/2016	Field day – legume selection and nodulation	26	6
Manildra	13/10/2016	MLA Pasture Updates – legume survey results	32	?
West Wyalong	18/10/2016	Legume survey nodulation results	15	13
Cookamidgera	19/10/2016	Field day – legume selection and nodulation	15	3
Boggabri	21/10/2016	Field day – hardseeded legumes and tropical grasses	79	13
Greenethorpe	27/10/2016	Field day – legume selection and nodulation	62	9
Tamworth	28/10/2016	Field day – legume selection and nodulation	120	?
		Total	1097	>228

Table 8. Extension activities in WA during 2016 showing location, total attendance and number of consultants attending

Location	Date	Topics	Total attendance	Number of consultants attending
Lake King	4/2/2016	Pre-season: Hardseeded legumes in rotation, summer sowing, new breeding program targets at Murdoch Uni	20	2
Corrigin	26/7/2016	Weed control options, Legume selection for pasture-crop rotations free N farming, MALDI-ID	33	3
Esperance	10/9/2016	Weed control options, Legume selection for pasture-crop rotations free N farming	59	4
Arthur River	9/8/2016	Nodulation and N-fixation, recovery of poor sub clover pasture through remedial inoculation, MALDI-ID	42	4
Merredin Pasture Updates	15/8/2016	Pasture updates -summer sowing, new cultivars, new legume breeding at Murdoch	12	5
Esperance Pasture Updates	14/9/2016	Pasture updates -summer sowing, new cultivars, new legume breeding program at Murdoch	22	6
Serpentine	19/9/2016	Appropriate legume pastures for controlled fat beef production on deep sands	4	2

In February 2017 nine pre-season meetings for producers and advisors were held at Eugowra, Bathurst, Dunedoo, Tooraweenah, Forbes, Condobolin, West Wyalong and Temora involving more than 280 producers and advisers. A comprehensive survey was undertaken at each workshop to determine the issues producers are facing with respect to their feedbase and livestock production. The results of this survey have been reported in Hackney et al (2017b). Briefly, the survey found feedbase was limiting ability to sell or maintain livestock at the weight or condition score they desired 50% of the time. Producers most wanted assistance on pasture species selection and management and had a strong preference for face-to-face delivery of this information and regional on-ground evaluation in order to change practices which re-iterated the results of the 2008 survey (Hackney et al. 2008). It is critical that appropriate information is made available and delivered in the formats producers find most relevant particularly at a time where many producers are increasing their investment in livestock.

In WA there was substantial expansion of pink serradella cv. Margurita into adjacent regions in 2017, as depicted in Fig. 38. It is interesting that the hard seeded pink serradella dominates adoption. This is primarily because of its high seed yield, ease of harvest and adaptation to sandy soils. It reinforces our focus upon only breeding species that can produce large quantities of inexpensive seed. To do otherwise invites substantial failure in the current investment climate.

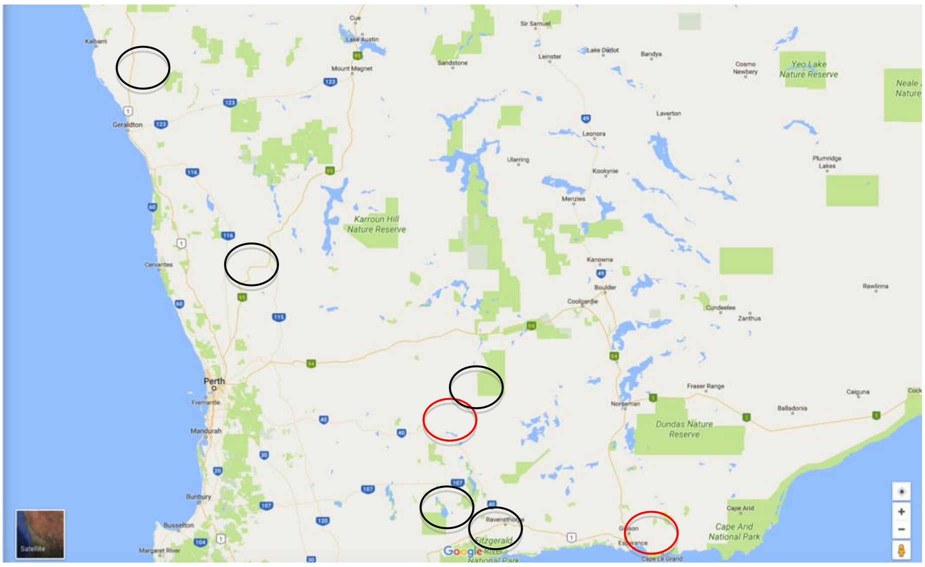


Fig. 38 The core trial sites at the beginning of the project (red circles) in WA, at Corrigin and Esperance, and the expansion sites in 2017 at Binnu, Bruce Rock, Dandaragan, Lake King and Ravensthorpe (black circles).

4.5.2 Extension documents relating to the impact of legume nutrition on red meat and wool production systems

Three short videos, two of which have been loaded onto Youtube, cover the key topics and the golden rules for success: paddock selection, cultivar selection, inoculation, weed control, and monitoring N fixation.
These are available on the CRS web site, or at:
Free 'n' Farming - https://youtu.be/h5fekWCPJrI
Summer Sowing Legume Pastures in WA - https://youtu.be/pZKMtDvJZA4

4.5.3 Seed cleaning figures as a guide to uptake

During the summers of 2016 and 2017, one major cleaning shed in the Great Southern of WA processed the following quantities of seed:

Pink serradella - 311 tonnes
Yellow serradella - 398 tonnes
Bladder clover - 140 tonnes
Biserrula - 53 tonnes

Our estimate is that this seed/pod would be sufficient to be sown over 100,000 ha, which is reflective of major uptake. These figures are not captured by seed sales data, as the material is mainly for private on-farm re-sowing.

4.5.4 Ongoing extension opportunities

New sites were established in 2018 in both WA and in NSW to provide an ongoing source of material for extension purposes.

In Western Australia, new experimental and demonstration sites were implemented in 2018 north of Corrigin near Babakin and in the northern region of Esperance near Cascade

Small plot rotational trial at Ardath (Corrigin, WA) on low pH sandy/duplex soil using hard-seeded French serradella cv. Margurita).

The site at Ardath, on a mixed farm owned by Phil Foss, has an annual rainfall average of 300mm. The treatments describing the rotations for the trial are presented below. The trial was summer sown to hard-seeded French serradella cv. Margurita pod on the 19th February 2018 at 20kg/ha, combined with 80kg/ha of 3P:2K fertiliser. The summer sown plots were sprayed with 100ml/ha Spinnaker® post sowing pre emergence (PSPE). A knockdown (glyphosate 1.5 l/ha) was applied on the 6th June before sowing the scarified Margurita seed and cereal the day after. All new sowings included 80kg/ha of 3P:2K fertiliser while the Barley cv. Spartacus, sown at 70kg/ha, had 50 units of DAP applied over the area. Additionally, the new sowings were sprayed with 100ml/ha Spinnaker® post sowing pre emergence (PSPE) and then the whole trial sprayed with 120ml/ha Talstar® insecticide. Soil samples were taken on the 4th June for PredictB and 27th June for soil water holding capacity.

Treatments	1	2	3	4	5
2017 (Prev.)	W (Mace)	W (Mace)	W (Mace)	W (Mace)	W (Mace)
2018	SS M	SS M	NS M	CF	B (Spartacus)
2019	W (Chief)	W (Chief)	W (Chief)	W (Chief)	W (Chief)

2020	B (Spartacus)	LR	LR	B (Spartacus)	B (Spartacus)
2021	W	W	W	W	W

Key: SS M = Summer sowing Serradella cv. Margurita, NS M = Normal sowing Serradella cv. Margurita, CF = Chemical fallow, LR = Legume regeneration cv. Margurita, W = Wheat cv. Mace or Chief, B = Barley cv. Spartacus

<u>Small plot rotational trial at Cascade (Esperance, WA) on high pH loam/clay soil using hard-seeded bladder clover cv. Bartolo</u>

A suitable paddock was selected at Cascade, on a mixed farm owned by Mark Walters, with an annual rainfall average of 300mm. The treatments describing the rotations for the trial are presented below. The trial was summer sown to hard-seeded French serradella cv. Margurita pod on the 1st March 2018 at 20kg/ha, combined with 80kg/ha of 3P:2K fertiliser. The summer sown plots were sprayed with 100ml/ha Spinnaker® post sowing pre emergence (PSPE) on the 17th April 2018. A knockdown (glyphosate 1.5 l/ha) was applied on the 19th June before sowing the scarified Margurita seed and cereal the day after. All new sowings included 80kg/ha of 3P:2K fertiliser while the Barley cv. Spartacus, sown at 70kg/ha, had 50 units of DAP applied over the area. Additionally, the new sowings were sprayed with 100ml/ha Spinnaker® post sowing pre emergence (PSPE) and then the whole trial sprayed with 120ml/ha Talstar® insecticide. Soil samples were taken on the 6th June for PredictB and 19th June for soil water holding capacity.

Rotation treatments that will be used over the 4 years at Cascade on the small plot long term site.

Treatments	1	2	3	4	5
2017 (Prev.)	B (Spartacus)	B (Spartacus)	B (Spartacus)	B (Spartacus)	B (Spartacus)
2018	SS M	SS M	NS M	CF	W (Chief)
2019	W (Chief)	W (Chief)	W (Chief)	W (Chief)	B (Spartacus)
2020	B (Spartacus)	LR	LR	B (Spartacus)	W (Chief)
2021	W (Chief)	W (Chief)	W (Chief)	W (Chief)	B (Spartacus)

Key: SS M = Summer sowing Serradella cv. Margurita, NS M = Normal sowing Serradella cv. Margurita, CF = Chemical fallow, LR = Legume regeneration cv. Margurita, W = Wheat cv. Chief, B = Barley cv. Spartacus

These trials will continue for four years and provide a long term opportunity for demonstration of the principles developed for pasture establishment in this project.

In NSW, the ongoing sites were at Condobolin and Ungarie

At Condobolin – the farmer has sown 150 ha of serradella (mix of Santorini and Margurita) suited to summer sowing in NSW. This arose from small nurseries which were sown on-farm in 2016 where the serradellas, biserrula and gland clover were best performing. The farmer has chosen French and yellow serradella as he feels they fit best with his livestock production system. Traditionally he has grown lucerne and medics but experienced large annual losses due to bloat. The soil is a red chromosol (red-brown earth)

At Ungarie –the farmer has sown 20 ha of Cefalu and 20 ha of gland clover. Gland was chosen on basis of widespread adaptation as demonstrated in previous NSW feedbase trials and the sowing area has a mix of soil types, some not well drained. Arrowleaf clover was chosen as the farmer wanted a higher production alternative. Both were sown at 7 kg/ha with scarified seed.

Additionally, at both sites we chose again to demonstrate the findings of this project with a time of sowing trial. Biserrula, bladder, gland, French serradella, arrowleaf all went is as summer sown, compared with an early tos with scarified seed (April), and a late sown comparison (late May) along with Cavalier medic and Dalkeith sub (early and late sow). Here we are demonstrating time of sowing x species in lower rainfall areas as instigated in this MLA/AWI feedbase. Three replicates of each treatment were sown, plots 3 m x 10 m. Summer sowing serradella 30 kg pod/ha, unscarified seed 15 kg/ha; April and May scarified sowing (sub, medic and bladder, serradella 10 kg/ha; gland, biserrula, gland, arrowleaf 7 kg/ha).

Further a rotation trial was implemented at Ungarie only - treatments are:

1. Trifolium mix sown in summer (15kg/ha; 5 kg of each unscarified), or late autumn (10 kg/ha, equal quantities of each)

2. French serradella (summer sown 30 kg/ha) or late autumn sown (10 kg/ha)

3. Sub clover (10 kg/ha late May only)

4. Cavalier medic (10 kg/ha late May only)

5. Continuous crop control

Two plots of each treatment per rep and four reps. In year 2 all plots will be cropped; year three half plots will be cropped, half left to regenerate, while in year four all plots will be cropped. This MLA/AWI project provided information on hard seed breakdown and spp. compatibility (e.g trifolium mix) which allowed development of the rotation trial to determine livestock and crop benefits arising from use of hardseeded legumes in the rotation.

5 Discussion

5.1 Outcomes of the project

Prior to this project we had sufficient information on the performance of the new alternative legumes, over a number of years, regions and soil types, to be confident that they could improve the feedbase. Whilst we had generated routine agronomic packages for their establishment and management, our intuition was that the major barrier to adoption of the alternative pasture legumes was the time available for mixed farmers to focus on sowing pastures. This seems to have been an accurate premise, given the adoption rates of summer sowing by the end of the project. However, we were also unsure of whether the hard seed breakdown patterns of the material developed in WA would marry with the climate in NSW. The project was able to determine that, in fact, many more legume species had the ideal hard seed break-down pattern for summer sowing when grown in NSW, than we found in WA. This provided a major impetus for broader evaluation of the summer sowing package in NSW. It eventuated that twin sowing, strategic dry sowing and summer sowing all had niches in that State.

The success of out-of-season sowing also hinged on delivering the specific rhizobial inoculants alive – a difficult task when soil temperatures can reach 65°C. In particular, gland, arrowleaf and bladder clover, and biserrula, have very specific rhizobia, and it is essential that the right strains are available for nodulation when these legumes germinate. We needed to be able to determine which rhizobia were forming nodules on these plants – the inoculants or other (less effective) strains residing in the soil. Because isolation of bacteria from nodules is a very time-consuming process, we took the opportunity with 1 .5 yrs of post doctoral salary in the Centre for Rhizobium Studies at Murdoch University to investigate modern technologies for possible application to identification of nodule occupants. Out of this research has come the diagnostic approach of MALDI-ID. Whilst it remains under development, there is exciting potential in the use of mass spectrometry and next gen sequencing to accurately, cheaply, and quickly determine if inoculation has been successful.

Going forward, we see the opportunity for expanding the summer sowing approach to lower rainfall regions, and finer textured soils. We see the need to examine the cause of phytosensitivity in sheep grazing biserrula, because this new legume has a valuable role in the feedbase on soils in WA and NSW where sub-clover has become unreliable as the main legume in the feedbase. We also need to develop a biserrula cultivar with a hard seed breakdown pattern suitable for summer sowing in WA. Because farmers are already reducing the application of N to crops grown after these legumes (particularly where low available sheep numbers allow underutilisation of the N rich biomass) we need to better understand the dynamics of N mineralisation from the legume residues to better inform crop agronomy decisions. Whilst this may seem a focus on the crop phase of the rotation, it is likely that the benefits of these new legumes are so great for the feedbase that producers will widen their adoption onto cropping soils, particularly those prone to frost.

The major improvement we would have liked to see from this project was greater quantification of the benefits of the improved feedbase to animal production and profitability. The budget of the project was cut shortly before contracting and this did not allow for the complex and large field – based animal experiments required to achieve this outcome. Instead we relied to a great extent on pen trials and farmer observations and testimonies to gauge impact on animals.

6 Conclusions/recommendations

6.1 Conclusions

The alternative annual pasture legumes are capable of delivering substantial productivity gains to producers in mixed farming systems on soils where, for a complex range of reasons, sub-clover has declined. Summer sowing in WA, as well as Twin sowing in NSW, offers an attractive approach to implementation of these legumes which minimises the cost of the operation and maximises production. By adoption of these practices, we feel producers will have been given the opportunity to increase stocking rates because of increased confidence in the carrying capacity of their feedbase.

Ley-farming with this “second generation” of annual legumes has evolved to include crop:pasture sequences of 1:1; 2:1, 3:1 and even 4:1 made possible by the development of very hard-seeded, deep rooted and early maturing cultivars of *Ornithopus* spp. and *Biserrula pelecinus* (Loi et al., 2005) (Nutt, 2012). Juxtaposed against the traditional ley-farming described by Donald (1965), where the pasture phase regenerated from dormant seed and typically lasted for 1-3 years, we now have up to a three-fold decrease in the frequency of the pasture years in rotation (from 2:3, to 4:1), with concomitantly increased tillage, and thus reduced sustainability factors offered to the agro-ecosytem. However, the new annual pasture legumes evaluated and demonstrated in this feedbase project are reliable in these intensively cropped systems, and we contend they will continue to deliver some of the sustainability and yield benefits of traditional ley-farming systems.

6.1.1 Future R & D

This feedbase project has been pivotal to overcoming adoption barriers for alternative feedbase legumes in NSW and WA. The ideas that were researched have recently been captured going forward into the new R&D4P project. We suggest MLA should take the opportunity to invest in a breeding program for *Biserrula pelecinus.* This legume is relatively new to agriculture and has proven itself to be very valuable to producers, but there has never been a breeding program since its early domestication (Howieson et al 1995). Targets for the breeding program would be early maturing cultivars with reduced phyto-sensitivity, and hard seed breakdown patterns that suit them for broad summer-sowing.

Further, during this project producers began experimenting with the alternative clovers such as bladder, arrowleaf and gland on soils of finer texture than we had originally targetted, in areas of the farm suited to cropping, but where frost sensitivity was recognised as a risk to crop profitability. It would be valuable, therefore, to more fully assess whether the alternative pasture legumes could be implemented on the soils of better quality (which deliver a consistently high crop seed yield), where protein levels in grains (and profit) might be maximized with the N produced by the pasture legumes, whilst providing expanded feedbase opportunities for animals.

7 Key messages

7.1 Alternative annual legumes improve the feedbase

7.1.1 Hardseeded annual pasture legumes – pasture establishment and grazing guidelines for persistent pastures

- Hardseeded annual pasture legumes such as serradella, arrowleaf clover, biserrula, bladder clover and gland clover can be summer and twin-sown for ease of establishment
- They must be inoculated with rhizobia protected from heat in the summer sowing operation to be successful and allow N fixation
- If established well, the alternative legumes increase animal weight gain and carrying capacity on suitable soils
- These legumes are resilient in the face of intensification of the cropping activities, but care must be taken with herbicide application in the crop phase
- Most of the alternative legumes have much smaller seed than subterranean clover and annual medics
- Smaller seed size enables a higher proportion of ingested seed to escape digestion
- As with any new pasture sowing, first year focus should be on establishing a large and resilient seedbank for subsequent years regeneration
- Low weed pressure is essential in the establishment year
- Sowing time can have a significant impact on seed production and hence the project recommends sowing as early as possible in the season to take advantage of warm, wet conditions
- Plant growth habit has an impact on seed production under grazing, but under normal stocking rates this is not a threat to long term persistence of the feedbase
- Plant residues need to be grazed to encourage hardseed breakdown
- Producers should aim to allow moderate seed set for seed bank replenishment at least every three years
- The new pastures are deeper rooted than clovers and medics and provide some protection against false breaks and dry periods in the winter months

- The legume residues also provide an excellent source of dry feed over summer, or excess feed can be conserved as hay or silage to assist in closing the feedgap
- Notable is the potentially massive value of French serradella pod (high in fats and protein) to the over-summer nutrition available to grazing sheep. Given the high biomass produced by French serradella, this has the potential to greatly reduce the summer-autumn feedgap in southern Australia.
- Well grown stands of the alternative legumes provide excellent N nutrition for any rotational crop

It is clear that the contemporary pressures on pasture legumes and rhizobia, such as the intensification of cropping and variable rainfall, will remain into the foreseeable future. This is impacting the production from sub-clover in some regions. Alternative legumes with resilient rhizobia, that can tolerate these stresses, must be made available for producers to have confidence in their pasture feedbase, in ley-farming systems. The various RDCs which invest in pasture research and breeding will need to decide whether the legumes that inspired Donald (1965) can be improved to meet contemporary challenges in pasture production, or whether new pasture legumes to take the animal industries forward in southern Australia for the next 100 years, such as those reported here, must be supported.

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9 Appendices

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9.2 Farmnote

Hardseeded annual pasture legumes – pasture establishment and grazing guidelines for persistent pastures

Key points

- Hardseeded annual pasture legumes such as arrowleaf clover, biserrula, bladder clover and gland clover have much smaller seed than subterranean clover and annual medics
- Smaller seed size enables a higher proportion of ingested seed to escape digestion
- As with any new pasture sowing, first year focus should be on establishing a large and resilient seedbank for subsequent years regeneration
- Low weed pressure is essential in the establishment year
- Sowing time can have a significant impact on seed production
- Plant growth habit has an impact on seed production under grazing
- Plant residues need to be grazed to encourage hardseed breakdown
- Producers should aim to allow moderate seed set for seed bank replenishment at least every three years

Introduction

Hardseeded annual pasture legumes such as arrowleaf clover (*Trifolium vesiculosum*), biserrula (*Biserrula pelecinus*), bladder clover (*T. spumosum*), gland clover (*T. glanduliferum*), French serradella (*Ornithopus sativus*) and yellow serradella (*O. compressus*) have survived and thrived throughout their native range in Europe and north Africa for many thousands of years. There is little control of grazing throughout this region and much of the area may be considered over grazed by Australian standards.

These species set seed above ground as opposed to subterranean clover (*T. subterraneum*) which buries a large proportion of its seed below the ground or on the soil surface. The aerial seeding nature of the hardseeded legume species has, over time, raised questions about how to best manage grazing to ensure establishment and maintenance of an adequate seedbank for reliable regeneration.

This factsheet presents guidelines for the establishment and maintenance of resilient legume seedbanks and guidelines for grazing to ensure adequate seedbank replenishment.

Sowing time and seed production

Sowing time has a significant impact on both herbage and seed production. Sowing hardseeded annual legume-based pastures early in the growing season allows establishing plants to utilise higher autumn temperatures and produce higher quantities of herbage prior to the onset of low winter temperatures. However these advantages need to be balanced against the need for weed control and if high weed numbers are expected it is usually better to wait for weed germination and control before sowing..

The hardseeded annual legumes referred to in this publication can be harvested on-farm with a conventional header and the seed can then be sown in summer-early autumn (without scarification). Seed softening over the late summer-early autumn period then enables seedling emergence on the opening autumn rains and therefore increased opportunity for early autumn growth. Similarly, where producers are purchasing scarified seed of the deeper-rooted annual legume species (arrowleaf clover, biserrula, French and yellow serradella), strategic dry sowing ahead of the autumn break can also enable increased herbage production compared to late autumn sowing. Subterranean clover is not suited to summer sowing due to its susceptibility to false breaks as a consequence of lower hard seed levels and care is needed in dry autumn sowing of this species due to potential for seedling loss due to its shallow root system.

Research in both Western Australia and New South Wales over the past decade has shown summer sowing and strategic dry sowing of hardseeded annual legumes can significantly increase herbage production throughout the growing season. (Figure 1).

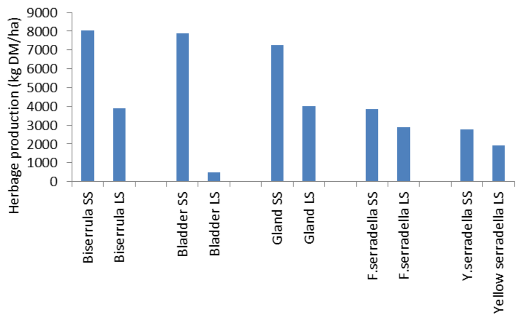


Figure 1. Herbage production (kg DM/ha) for biserrula, bladder clover, gland clover, French serradella and yellow serradella either sown unscarified/in-pod summer (Febrauary) sowing

(SS) or as scarified seed in late May (NS) at Greenethope, NSW

Both early and strategic dry sowing has also generally resulted in higher seed yields compared to late autumn sowings (Figure 2).

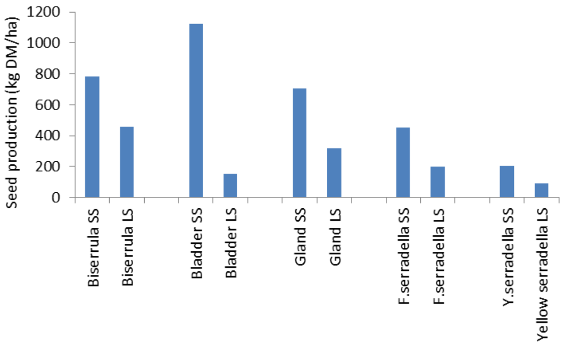


Figure 2. Seed production (kg/ha) for biserrula, bladder clover, gland clover, French serradella and yellow serradella either sown unscarified/in-pod summer (Febrauary) sowing (SS) or as scarified seed in late May (NS) at Greenethope, NSW

For traditional pasture species such as subterranean clover, it is considered a seedbank of 100-150 kg/ha will be adequate to ensure reliable regeneration. Generally, summer sowing or strategic dry sowing of hardseeded annual legumes has easily exceeded this benchmark in Western Australian and New South Wales, but late autumn sowing has generally resulted in much reduced ability in attaining this benchmark with both hardseeded annual legumes and also subterranean clover.

Sowing rates and sowing techniques

Several options are available to establish hardseeded legume pastures. These are:

Summer sowing – where unscarified or in-pod seed is sown in mid to late summer to allow seed to soften over the late summer early autumn period enabling establishment on the opening autumn rain. Sowing rates for bare seeded species (arrowleaf, bladder clover, biserrula and gland clover) are 12-15 kg/ha and 20-30 kg/ha for pod of French and yellow serradella. A long-life granular inoculant must be used at 10 kg/ha (for each inoculant group if sowing a mixture of different legume species).

Important note: It is critical to seek advice on species/varieties suitable for use in summer sowing in your region. Not all varieties/species are suitable for this purpose.

Some details are available in these short videos:

Free 'n' Farming - https://youtu.be/h5fekWCPJrI

Summer Sowing Legume Pastures in WA - https://youtu.be/pZKMtDvJZA4

Scarified seed sowing

Scarified seed sowing is suitable for all species. Sowing rates of 5-10 kg/ha are suggested for monoculture sowing with rates of 3-6 kg/ha suggested in mixtures with grasses or lucerne.

Stand alone sowing of pasture is preferred to undersowing as the cover crop, even sown at low rates has been shown to have a detrimental effect on both seed yield and seed size. This also applies to traditional species such as subterranean clover.

Grazing hardseeded legumes

The establishment year

As with any annual legume-based pasture, the primary objective in the establishment year is to maximise seed set to ensure a large and resilient seedbank is established for subsequent years regeneration.

Legumes sown early (either as hard seed summer sown or strategic dry-sown) can produce very high quantities of herbage during the establishment year with 5-10 t DM/ha commonly recorded. Where early growing season conditions are favourable, light to moderate short duration grazing in late winter and early spring may assist in controlling overburden of growth without compromising seed production. Generally, pastures sown in late autumn and early winter will not produce sufficient bulk of feed for grazing in the establishment year. The ability to graze first year stands of hardseeded annual legumes will be driven by growing conditions. In very dry years it is prudent to leave establishing pastures ungrazed during flowering and seed production to maximise seed production.

Residual spring herbage and effect on seed production

The extent to which hardseeded legumes can be grazed during the establishment phase is to some degree species specific. It can be affected by growth habit of the plant as well as presence or absence of protective mechanisms for the seed, specifically the seed pod and also the palatability of the plant. The nature of growth of the plant also affects seed production capability with species of indeterminate nature have a greater capacity to produce seed later into the growing season. **Plant growth habit**

Hardseeded legumes with upright growth habit and where the inflorescence is located near the top of the canopy are more exposed to predation of the seed head and specifically the maturing seed held within the seedhead. Greater care is needed in grazing of these species during the flowering period as heavy grazing at this time can result in removal of many of the seedheads prior to completion of seed formation. Arrowleaf clover can be particularly sensitive to overgrazing during reproductive stages of growth as it has both an upright growth habit and its seed head located at or near the top of the canopy.

In contrast, trailing plants such as biserrula which produce seed pods that are not prominent in the canopy and are found along the length of the runners have increased protection from grazing animals.

Mechanisms of seed protection and dispersal

Where seed is produced in pods and where pods are not readily ingested there is increased protection from predation by grazing animals for the developing seed. The serrated nature of biserrula pod and their relative toughness with increasing aging make them less attractive to livestock and therefore they are often not preferentially ingested until feed supply becomes more limiting by which time seed has fully formed.

Both gland clover and French serradella readily shed their seed as grazing animals move through the pasture. However bladder clover and yellow serradella are often sought out for their high feed value. Passage through a grazing animal, particularly in bladder clover maybe an important form of dispersal.

Grazing dry pasture over summer at Corrigin showed a distinct preference in sheep for grazing the seed heads/pods of bladder clover > yellow serradella > French serradella > Biserrula > gland clover. The amount and type of seed found in manure over the commonly grazed plots is shown in table 1

Table 1. Seed number and germination of pasture and weed species found in sheep manure when grazing different species in common (sampled April 2014).

Species	No./m^2	Germi-ation %	Hardseed %
Yellow serr.	53	81	19
French serr.	1		
Biserrula	11	13	77
Bladder clover	222	44	56
Woolly ball clover	345	47	53

Subclover	3	3	0
Capeweed	99	1	0
Paddy melon	12	0	0

Seed in the sheep manure showed a high level of germinable seed than expected and this may indicate more favourable conditions for dormancy breakdown.

Even though the plots of the various legume were grazed to low levels of dry matter over the summer (>1000 kg/ha), the long term persistence of most of the species was not compromised (Table 2). The exceptions were bladder clover and yellow serradella. However given the disproportional amount of bladder clover represented in the manure, on a whole paddock basis, passage through the grazing animal may not be as severe as the plot experiment suggests.

Of note is the increase in plant establishment density after cropping and the incorporation of seed into the soil.

Table 2. Seed yield at senescence in 2013 and corresponding germination in the next year (2014) after heavy grazing (which was sprayed out for cropping) and in 2015.

	Seed yield (kg/ha)	2014 plants/ m^2	2015 plants/ m^2
French serr.	218	1930	3960
Bladder clover	745	110	800
Biserrula	354	60	9820
Subclover	497	1870	3600
Gland clover	276	4540	14200
Yellow serr.	465	110	820

Indeterminant growth habit and impact on seed production

Both biserrula and serradella are indeterminant plants meaning they will continue to grow and produce seed whilst moisture is available. This characteristic is very valuable in allowing seed production later in the season following a grazing event or where highly variable spring moisture conditions may impede seed production of species with a more determinant pattern of growth (e.g. subterranean clover). The value of indeterminate growth in seed production is shown in Table 3. Following hot, dry early spring growing conditions, full senescence occurred on bladder clover, gland clover and subterranean clover. The biserrula also appear predominately senesced at this time. Seed yield of all species was recorded at this time. Fifty millimetres of rain was subsequently received in early November enabling the biserrula to recommence growth and produce more seed. In this instance, biserrula produced more than double the amount of seed of the next highest yielding species.

Table 3. Seed production (seeds/m2) of biserrula, gland clover, bladder clover and subterranean clover at Temora, NSW in 2015.

	Early spring seeds/m^2	Late spring seeds/m^2	Total seeds/m^2
Biserrula	2715	3258	5973
Gland clover	2046	0	2046
Bladder clover	760	0	760
Subclover	250	0	250

Grazing residues over summer

Hardseeded annual legumes can produce significant quantities of herbage. Frequently, this cannot be utilised in the growing season due to inadequate numbers of livestock. The dry residue of these pastures offer considerable grazing potential over the summer period.

The proportion of ingested seed that is digested by grazing livestock is affected by seed size. Species with smaller seed are better able to escape mastication (grinding) and also have more rapid passage through the gut resulting in generally a higher levels of excretion in viable condition. Arrowleaf clover, biserrula and gland clover have very small seed (more than 1 000 000 seeds/kg). Bladder clover has approximately 500 000 seeds/kg. French and yellow serradella generally contain 200 000 – 350 000 seeds/kg.

A study at Wagga Wagga in southern NSW has shown the effect of residual spring herbage (post grazing) on seed production of some hardseeded annual legumes of differing growth habit and seed size. In this study, the residual herbage required to achieve seed production of 150 kg/ha was determined for arrowleaf clover, biserrula and bladder clover (Table 4) where plots had been grazed for a period of eight weeks up to early-mid November. ***It is critical to note here that plants were still actively growing at the time of removal of sheep from plots.*** Following completion of seed set, plots were grazed over summer for 4 weeks at 8-10 DSE/ha. Seedlings emerging were then counted in autumn.

Table 4. The residual herbage in spring (kg DM/ha) required to achieve seed yield of 150 kg/ha for biserrula, arrowleaf clover and bladder clover and the number of seedlings emerging in the following autumn following grazing of residues over summer at 8-10 DSE/ha for four weeks

	Residual herbage (kg DM/ha)	Seed yield (kg/ha)	Seedlings (plants/m^2)
Biserrula	1600	150	650
Arrowleaf clover	2200	150	660
Bladder clover	750	150	310

Bladder clover, a highly prolific, relatively short stature plant was able to produce the required benchmark of seed at a lower residual herbage level than other species. Arrowleaf clover, with a more upright growth habit and more exposed seed heads required a higher residual herbage mass to achieve the same level of seed production.

In terms of seedling regeneration, the effect of seed size and interaction with digestion can clearly be seen. Both smaller seeded species (arrowleaf and biserrula) were better able to produce higher seedling numbers following summer grazing of residues while grazing the larger seeded bladder clover has reduced seedling density presumably as a result of increased seed digestion. Seedling densities of 500 plants/m^2 will produce regenerating stands of more than adequate density.

As a result of these studies, the authors would recommend grazing to no less than 1500 kg DM/ha for biserrula and 2000 kg DM/ha for arrowleaf clover in newly sown stands. To allow for digestion of bladder clover seed by grazing animals, results of this study indicate residual herbage mass of 1700 kg DM/ha would be the minimum to ensure adequate seed production in an establishing stand.

In seasons with poor growing conditions, it is recommended to forego grazing to ensure maximum seed production.

Managing seed production in established stands

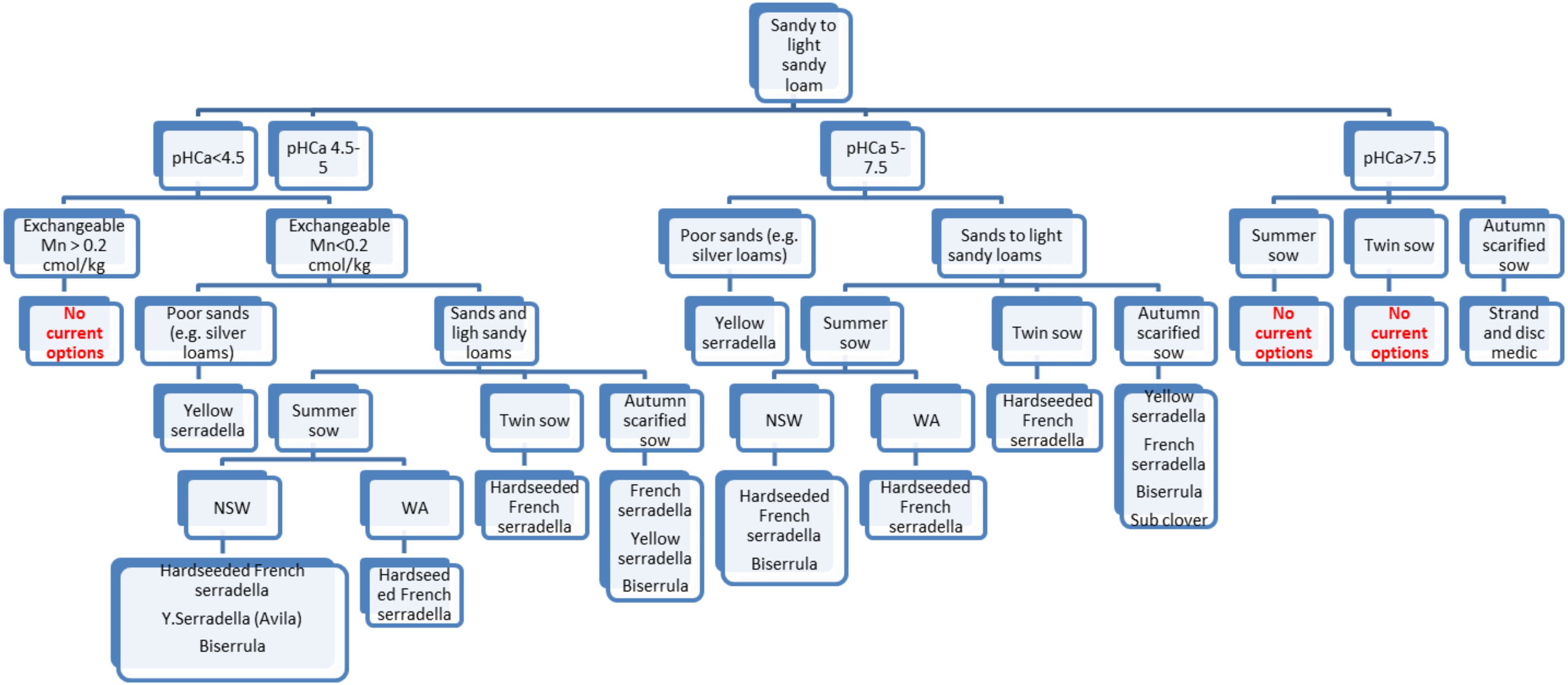
The hardseed level of legumes discussed in this publication are higher than for traditional species such as subterranean clover. Therefore, their longevity in the seedbank is greater. In addition, the smaller seed size, particularly of arrowleaf clover, biserrula and gland clover mean more than 50% of ingested seed remains viable following excretion. Under most conditions and providing there has been establishment of a seedbank in the order of 150 kg seed/ha in the establishment year, grazing no lower than the minimum targets mentioned above every two to three years should result in maintenance of an adequate seedbank.

Cropping frequency in crop:pasture leys

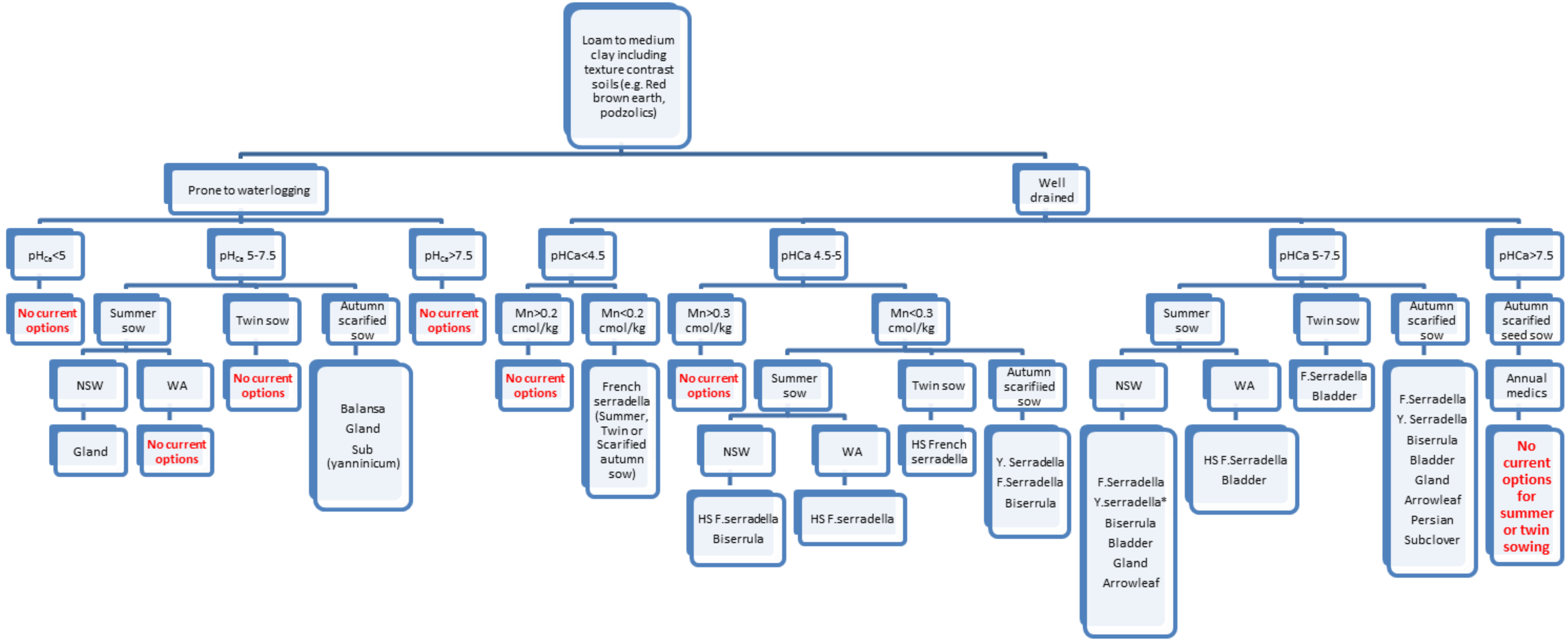
The hardseeded legumes that are the focus of this farmnote respond very strongly to cropping. It is recommended that for serradellas, biserrula, arrowleaf clover, gland clover and bladder clover, the year after establishment becomes a cropping year. Cropping with minimum tillage machinery will ensure pods and seeds produced on the surface are buried. If a reasonable seed production has been achieved in the establishment year (>100 kg / ha) then regeneration will be ensured in year three. There will be no necessity to limit grazing pressures in this regenerating season under normal circumstances to ensure the seedbank is preserved. For the very hard seeded legumes such as biserrula and yellow serradella we recommend allowing regeneration in year 3, but following a normal season a sufficient seedbank will be developed to allow 3 or 4 successive crops.

For the aerial seeding clovers we recommend only a single year of crop before allowing a return to pasture.

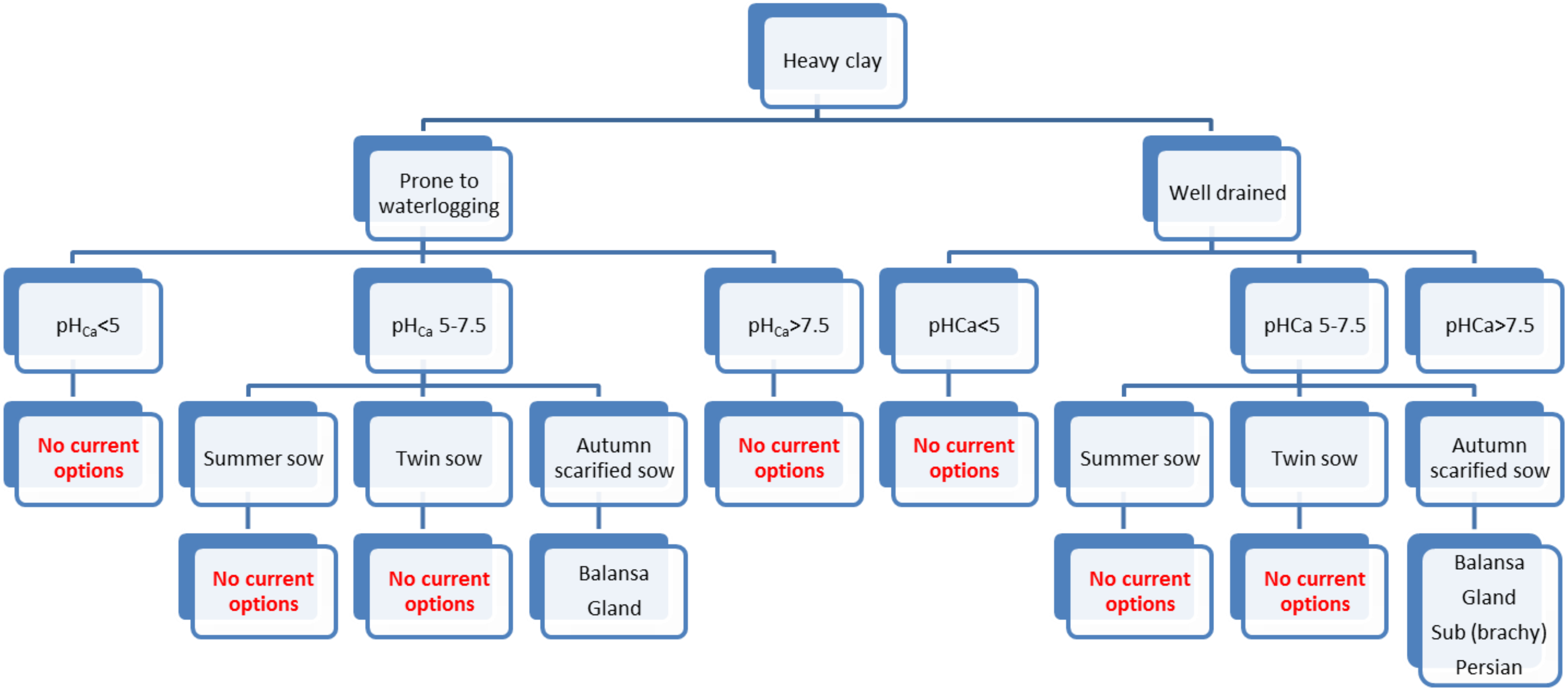
Appendix 2


Sandy to light sandy loam
pHCa<4.5
pHCa 4.5-5
pHCa 5-7.5
pHCa>7.5
Exchangeable Mn > 0.2 cmol/kg
Exchangeable Mn<0.2 cmol/kg
Poor sands (e.g. silver loams)
Sands to light sandy loams
Summer sow
Twin sow
Autumn scarified sow
No current options
Poor sands (e.g. silver loams)
Sands and ligh sandy loams
Yellow serradella
Summer sow
Twin sow
Autumn scarified sow
No current options
No current options
Strand and disc medic
Yellow serradella
Summer sow
Twin sow
Autumn scarified sow
NSW
WA
Hardseeded French serradella
Yellow serradella
French serradella
Biserrula
Sub clover
NSW
WA
Hardseeded French serradella
French serradella
Yellow serradella
Biserrula
Hardseeded French serradella
Biserrula
Hardseeded French serradella
Hardseeded French serradella
Y.Serradella (Avila)
Biserrula
Hardseeded French serradella

Appendix 2a. The impact of soil chemical and physical parameters and establishment methodology on legume suitability for pastures on sandy to light sandy loam soils



Appendix 2b. The impact of soil chemical and physical parameters and establishment methodology on legume suitability for pastures on loam to medium clay soils


Heavy clay
Prone to waterlogging
Well drained
pHCa<5
pHCa 5-7.5
pHCa>7.5
pHCa<5
pHCa 5-7.5
pHCa>7.5
No current options
Summer sow
Twin sow
Autumn scarified sow
No current options
No current options
Summer sow
Twin sow
Autumn scarified sow
No current options
No current options
Balansa
Gland
No current options
No current options
Balansa
Gland
Sub (brachy)
Persian

Appendix 2c. The impact of soil chemical and physical parameters and establishment methodology on legume suitability for pastures on heavy clay soil

9.3. Nutritional value of serradella pods (Data courtesy of Dr Hayley Norman, CSIRO, Floreat)

In vitro Laboratory data

Species	Type	NDF (%DM)	Hemicellulose	ADF (%DM)	DMD (%)	ME (MJ/kg DM)	OM (%DM)	Ash (%DM)	Crude protein	Crude fat %
Yellow serradella	vegetative	30.8	9.4	21.3	68.7	10.1	90.0	10.0	23.7	
French serradella	vegetative	30.4	9.1	21.3	67.6	9.9	86.8	13.2	23.0	
Biserrula	vegetative	28.8	9.8	19.0	69.1	10.2	88.8	11.2	23.4	
Bladder clover	vegetative	30.9	8.7	22.2	72.6	10.8	86.6	13.4	25.8	
Yellow serradella	flowering/podding	39.4	10.6	28.9	61.1	8.8	92.4	7.6	17.3	
Yellow serradella	flowering/podding	45.2	11.8	33.4	60.7	8.7	84.2	15.8	15.1	
French serradella	flowering/podding	34.8	9.4	25.4	64.2	9.3	91.2	8.8	17.7	
Biserrula	flowering/podding	33.7	10.9	22.8	68.0	10.0	90.9	9.1	17.4	
Bladder clover	flowering/podding	33.4	11.8	21.5	71.3	10.6	92.3	7.7	15.2	
Yellow serradella	mature seed	61.5	15.6	45.9	46.1	6.2	94.8	5.2	11.1	
French serradella	mature seed	57.9	14.4	43.5	50.4	7.0	93.2	6.8	10.2	
Biserrula	mature seed	55.5	18.8	36.6	56.1	7.9	92.7	7.3	13.6	
Bladder clover	mature seed	50.8	21.5	29.3	64.7	9.4	93.1	6.9	17.0	
French serradella	hay frozen	37.1	11.0	26.0	63.6	9.2	91.2	8.8	18.1	
French serradella	hay frozen + rain	49.6	14.8	34.8	54.5	7.7	92.1	7.9	18.1	
Oats	hay frozen	45.4	21.3	24.1	66.0	9.7	94.6	5.4	5.8	
Oats	hay frozen + rain	51.5	25.8	25.7	57.8	8.2	96.4	3.6	5.5	

French serradella	hay	47.4	9.5	37.8	59.6	8.5	93.4	6.6	14.2	
French serradella	hay	44.7	9.9	34.8	58.1	8.3	92.0	8.0		
Biserrula	hay	40.6	10.6	30.1	67.3	9.9	89.1	10.9	15.9	
Ryegrass	hay	63.0	23.2	39.8	52.9	7.4	90.7	9.3	9.0	
French serradella	Straw	62.7	17.0	45.8	44.0	5.9	93.1	6.9	10.3	
French serradella	Straw	65.4	18.9	46.6	46.1	6.2	94.7	5.3	11.9	
French serradella	Straw	63.9	17.8	46.0	44.6	6.0	95.8	4.2	10.6	
French serradella	Straw	64.0	17.9	46.1	44.9	6.0	94.5	5.5	10.9	
French serradella	whole pods	39.0	13.0	26.0	64.0	11.5	97.0	3.0	26.3	8.8
Yellow serradella	whole pods	61.0	18.0	43.0	52.0	9.2	97.0	3.0	16.9	4.3
Burr medic	whole pods	54.0	19.0	35.0	58.0	9.5	94.0	6.0	20.9	2.9
Lupin	Seed	23.0	9.0	14.0	76.0	13.0	93.0	7.0	33.6	6.5

*M/D - MJ of metabolisable energy at the maintenance level of feeding. Estimated using SCA (1990) roughage and roughage (majority of samples) with high proetin and fat (for pod samples) 1.4

** Total nitrogen x 6.25

*** Hay was rained on after cutting. Nutritional value may have declined.