final report

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Prepared by: Richard Simpson
CSIRO Agriculture and Food

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Phosphorus Efficient Pastures: delivering high nitrogen and water use efficiency, and reducing cost of production across southern Australia.

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Abstract

This project aimed to reduce the phosphorus (P)-fertiliser dependence of Australian pastures.

Critical soil test P (STP) benchmarks for clover-based pasture production were confirmed. Their application to target P-fertiliser use achieves effective use of P with a declining P cost of production. New (lower) STP benchmarks apply to serradella pastures. However, in soils with very low P retention it is also important to recognise and use “legacy” P at depth in the soil profile.

The research focussed on enabling wider use of serradella to reduce P-fertiliser costs. This will also diversify the legume base of pastures and capture other beneficial serradella characteristics. Serradellas have a wider remit than light, or acid-infertile soils. Their use has expanded rapidly in phase farming, but uptake in permanent grasslands (~6M ha) is stalled by poor persistence. This was associated with cultivars with unstable flowering dates that confound maturity-type selections, softseeded varieties, and non-ideal seed softening patterns among hardseeded serradellas. A short list of cultivars for further research to improve persistence in permanent pastures was identified.

P efficiency among subterranean clovers was examined but no varieties could emulate the high P efficiency of serradellas. However, a two-fold range in yield in P-deficient soil (i.e. variation in P efficiency) was observed in controlled-environment studies. P efficiency was associated with roots that explored soil for P more effectively. Important traits were proliferation of nutrient foraging root length, long specific root length, long root hairs. Short root hairs was the major factor limiting P-efficiency. Differences among varieties could not be verified in the field because of drought. Current STP benchmarks should continue to be followed for subterranean clovers.

Executive summary

Phosphorus efficient pastures

This project aimed to deliver transformational change that can reduce the P-dependence of Australian agriculture. Phosphorus (P) is the primary nutrient input that drives legume nitrogen capture for pasture and crop growth in Australia. It underpins high productivity and profitability. However, P-fertiliser costs have doubled since 2000, increasing the fertiliser cost of production. More effective management of P fertilisers and the use of more P-efficient legumes in pastures achieves multiple benefits - fertilised pastures develop better ground cover with reduced risks of erosion, they grow faster and have more extensive root systems that allow them to use rainfall (higher water-use-efficiency) and nutrients (higher nitrogen-use-efficiency) more effectively. This delivers greater production per hectare of land (higher land-use-efficiency) and helps to ensure a profitable and resilient farm sector.

Opportunities to reduce P fertiliser costs by using serradella pastures: Continued analysis of field experiment data from a preceding project demonstrated that the critical P requirement of serradellas (i.e. the soil test P [STP] level necessary for maximum growth) was significantly lower than that of subterranean clover, Australia's most widely-used pasture legume. These data and other published data underpin estimates that a pasture based on serradella will have an annual P maintenance cost for high production that is up to ~30% less than the amount of P fertiliser needed to maintain a productive subterranean clover-based pasture[1]. The key to achieving this is to fertilise serradella pasture to its lower critical STP requirement, as this is expected to slow the accumulation of P in soil (i.e. P "fixation").

Opportunities to reduce P fertiliser costs by improved soil P management: The project has delivered knowledge via a revised "Five Easy Steps" P management decision tool to enable farmers to target appropriately the use of P fertilisers to the legume species they use in their pasture system. Datasets analysed for revision of the P management tool, indicated that farmers who manage their P fertiliser inputs to achieve STP targets for high pasture production, will also benefit from a steadily reducing P fertiliser requirement. Initial reductions in annual fertiliser inputs (achieved after 5-10 years) were about 40-50% when a shift from building soil P to maintaining the target P fertility level was made. Importantly, the annual maintenance-P costs continued to decline and were a further ~20% lower after 20 or 25 years of sustained, targeted fertiliser practice. These gains in P efficiency are driven by better management and should be possible on many farms, irrespective of the pasture legume being used.

Research addressing differing needs in areas where serradella is and is not used: A combination of "participatory research" conducted by farm groups and their consultants, and "core research" by a team of scientists was used to explore more effective use of P fertiliser in areas where serradella pastures are already used. In areas where serradella is not currently used, the integrated participatory and core research focussed on serradella growth and persistence (the ability to regenerate year-after-year) in permanent pastures.

New ways to assess soil P fertility and capture benefits from "legacy" P in soil profiles: Severe droughts affected almost all field experiments during the project. Nevertheless, it was shown that in deep sandy soils where serradella is grown in southern WA, the usual practice of surface soil P testing (0-10 cm depth) was not a reliable indicator of the P available to a serradella pasture. Substantial reserves of "legacy P" from earlier fertiliser applications were available deeper in the soil

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[1] Simpson et al. (2014)

profile. Farmers who are aware of this P can potentially reduce their P fertiliser inputs. Attempts to prove the merits of deeper soil testing (0-60 cm) were commenced but not completed before the end of the project; one farm group began testing soils to depth in all of their other research activities.

Changing ideas about how much P needs to be applied: In light soils in northern NSW, it was demonstrated that P was retained in surface soil layers and standard soil testing continued. Even in drought conditions, the improved P efficiency of serradella was indicated. The farm groups now expect that they can fertilise these pastures to lower concentrations of STP than was previously promoted, although this was not able to be proven due to persistent drought.

Evaluation of serradella cultivars in areas where they are not presently used: Serradella pastures were sown, and current cultivars were assessed often for the first time, in southern NSW locations and in central Victoria. In the Southern Tableland and Monaro regions of NSW, serradellas yielded comparably or better than subterranean clover when growing at a similar legume density. The serradellas were especially resistant to difficult drought conditions when regenerating, and demonstrated superior seed yields under dry spring conditions. In some instances, serradella was able to establish while subterranean clover failed in very dry seasonal conditions.

Lack of serradella persistence in permanent pastures constrains adoption: It became clear that maintenance of legume density and persistence of most serradella cultivars beyond the first few years after establishment was potentially problematic in these permanent pasture locations.

The issues identified as potentially contributing to persistence problems were:
Unstable flowering dates: In pasture systems, germination dates vary widely and seasonally depending on when breaking rainfall occurs. Annual pasture plants must accommodate variable starts to the growing season and still flower reliably in spring after the risk of frost has diminished to ensure seed production and persistence. However, unlike the subterranean clover cultivars that were grown as controls, many serradellas exhibited unstable flowering dates when sown early. French serradella cultivars were generally less stable (very premature flowering) than yellow serradellas. This issue has not been previously recognised.

Hardseededness and seed softening patterns: Hardseededness describes seeds that have a water-impermeable seed coat. This enforces seed dormancy and protects against premature germination on a false break to the growing season; it also ensures some seeds survive serious droughts. Impermeability slowly breaks down over summer and autumn (seed softening) allowing a proportion of the seeds to germinate. It is a critical attribute for persistence by annual pasture species in variable rainfed environments. A number of the French serradella varieties do not produce hard seeds and they do not persist in permanent pastures, especially during drought years (this was confirmed in the project). Among the hardseeded cultivars of serradella, there was a large diversity in the rates and patterns of seed softening. This has not been reported previously; it explained why some hardseeded cultivars have been reported as softseeded. Few serradella cultivars emulated the seed softening pattern of well-adapted and persistent subterranean clover cultivars. Most serradellas had very high levels of hardseededness with very slow seed softening. This may not be an overly detrimental trait once seed banks of serradella pastures are established, but it was associated with a low regeneration of seedlings in the year after pastures were sown. In a permanent pasture, this provides an undesirable opportunity for weeds to invade newly sown pastures and may contribute to poor pasture establishment and persistence.
Low tolerance of waterlogging: Serradellas are known to be intolerant of persistent waterlogging. This limits their use to soils with reasonable drainage. The research showed that serradellas failed to recover quickly after a waterlogging event. The results did not explain the apparent tolerance of

poorly drained soils by slender serradella (cv. Jebala), which is often included in a serradella pasture mix to cover waterlogged areas of paddocks. This serradella species grows slowly during winter and has a large flush of late spring growth, suggesting its “tolerance” may instead be due to avoidance of waterlogging stress.

The serradellas grew well in very acid soils in the field experiments, although a surprising result was that there appeared to be large differences in tolerance of aluminium toxicity (an issue associated with very acid soils) among the cultivars of serradella when grown in hydroponic culture. Further work is underway to verify these differences in aluminium toxic soil.

Farm group participatory research experiments highlighted the problem of limited herbicide options for controlling weeds within serradella pastures.

Standout serradella cultivars indicate a way forward: Among the varieties that could be tested, two hardseeded yellow serradella cultivars (Avila and King) were notable because they exhibited good persistence. Cultivar Avila achieved comparable densities to subterranean clover cv. Leura in an experiment that had been sown prior to the project and was, therefore, monitored for the longest period (up to 6 years after sowing). The regeneration densities of Avila seedlings was also outstanding in the shorter-term experiments in which it was sown. It is a late maturing variety suitable for high rainfall areas and was one of the few serradellas that exhibited appropriate and stable flowering dates in its target environment in southern NSW. Cultivar King is an early maturing variety that was also noted for high seed production and regeneration in the short-term experiments. It has proven persistence and yield in permanent pastures in its target environment (northern NSW) where it also exhibited stable flowering.

When the relatively good persistence of Avila and King was considered together with the results from all of the physiology/agronomy experiments, it was concluded that poor persistence in permanent pastures is most probably an issue of cultivar suitability (i.e. most current cultivars sold to farmers in permanent pasture areas are not adequately adapted to these areas [non-ideal flowering times, seed softening rates]). The issue can be solved by improved cultivar development. Nevertheless, some current cultivars are being used successfully in crop-pasture rotations where growing seasons are shorter and long-term persistence in a mixed pasture is less of an issue. In fact, adoption of serradella in the crop-pasture zone is increasing.

Knowledge and experience barriers: Farmers and researchers found that the research results indicated that serradella pastures have sufficient merit to warrant further investigations into their use. Farmers indicated they were keen to diversify their pasture base and were concerned about their heavy reliance on only subterranean clover, but they needed longer-term evidence of serradella persistence before they would invest in new serradella pastures. The cost of a failed, or non-persistent pasture is too great.

It was concluded that issues of serradella persistence were a significant barrier to adoption; this may indicate why uptake of serradellas in permanent pasture areas has been stalled at a time when uptake in crop-pasture systems was increasing.

Areas where it is unclear whether serradella will have a major role: In the central range’s region of Victoria, failure to establish serradellas was a recurring problem. Various issues were involved: the timeliness of sowing was crucial in a short window before cold, wet and sometimes waterlogged conditions occurred; slower serradella germination and weed and grass competition added to the problem, and the available cultivars of serradella were, most probably, not well-enough adapted to the region (as found in southern NSW). In contrast, the farm group participants found subterranean clover was relatively easy to establish under the same conditions. Nevertheless, serradella was established at the last of the three experiment sites to be sown and has regenerated well in 2020. It is possible that successful use of serradella in this region may prove to be very site-specific.

More P-efficient cultivars of subterranean clover: The ability of subterranean clover cultivars to grow in P-deficient soils was examined in controlled-environment studies. The growth response of clovers to P-deficient soil was complex, but the experiments indicated the most P-efficient genotypes could achieve almost twice the growth of the least P efficient genotypes. Current cultivars featured amongst the most and the least P-efficient genotypes. However, no subterranean clover cultivars could match the high P-efficiency of the serradellas. In theory, P-efficiency differences among clover cultivars could be used (by choosing the right cultivar) to improve production in paddocks that cannot be easily fertilised to achieve optimum P fertility. However, the differentiation in P-efficiency among the subterranean clover cultivars that had been demonstrated in controlled-environment experiments, could not be validated in the field due to the impacts of persistent drought. Presently, it is recommended that farmers continue to fertilise all subterranean clover cultivars to the current critical STP recommendations for clover-based pastures.

Root characteristics confer better P efficiency: Differences among subterranean clover cultivars in P efficiency was attributed to differences in their abilities to develop a large root-soil interface for P uptake. The best cultivars achieve high P uptake from moderately P-deficient soil by growing more root length in soil patches where P resources were relatively concentrated. Some clover cultivars were better at proliferating root length and cultivars that produced more root length per unit root mass had an advantage. The clovers generally had short root hairs (hair-like projections that increase the surface area for P uptake) and this was the major reason they were unable to match the P efficiency of serradellas which all have much longer root hairs.

***Can we breed clovers that can match the P efficiency of the serradellas*?** Understanding how root characteristics deliver improved P uptake from low-P soils indicated how it may be possible to develop new clover cultivars that approach the high P efficiency of the serradellas. A “core collection” of clover lines representing ~80% of the variation in the subterranean clover genome and other more-diverse *Trifolium* species (different clover species that can potentially be inter-crossed with subterranean clover) were examined for P-efficient root characteristics that could match those found among serradellas. The variation in root characteristics across this diverse collection was large (i.e. 2-fold differences between the smallest and largest root traits), longer root hairs were found in two of the allied *Trifolium* species, but none of the clover root attributes matched those of the highly P-efficient serradellas. A Genome-Wide Association Study found single-nucleotide polymorphisms (i.e. variations in the genetic sequence of subterranean clover; aka SNPs) that were associated with P-efficient traits including: yield indices that indicate consistently higher yield in low-P soil, root mass and length, root diameter, and the plants ability to shift growth towards roots in low P soil. However, SNPs were not associated with other traits considered important for P efficiency such as root hair length (RHL) and root length per unit root mass. This may mean that these traits are not controlled by a simple set of genes or that there was just not enough variation in the genome for these traits to enable correlation with genomic variation. Nevertheless, the results indicate that it may potentially be possible to use genetic markers to breed for some aspects of improved P efficiency in subterranean clover, however, the restricted magnitude of key root traits (such as RHL) measured within 80% of the genome, suggests that the opportunity to breed for a “step-change” in clover P efficiency by conventional breeding methods may be limited.

Training and on-going relationships: The project was used as a training vehicle for two PhD scholars (University of Western Australia; University of New England), an Honours (by research) student (Charles Sturt University) and two undergraduate students (CSIRO Vacation Scholars) from the

University of New England and University of Western Australia. Scientists visiting CSIRO (Canberra) and the University of Western Australia (Perth) were funded by INRA (Institut National de la Recherche Agronomique, France), Rothamsted Research (UK), University of Buenos Aires (Argentina), , Gansu Agricultural University (China) and an Endeavour Fellowship (Iran) to participate in critical experiments. New relationships were forged internationally, and between researchers and farmer groups. Many of these national and international relationships will continue beyond the timeframe of the project.

Project key messages and recommendations

Lowering P costs of serradella systems (Activity B4)

- Analysis of field benchmarking data concerning the critical P requirements of pasture legumes was completed and published:
  (i) confirming the validity of STP benchmarks for clover-based pastures in southern Australia,
  (ii) extending benchmarks for soil P management to nine alternative pasture legumes,
  (iii) flagging the very high P requirement of lucerne, and
  (iv) setting new lower STP benchmarks for pastures based on three forage crop legumes (*Trifolium incarnatum, T. purpureum*, *T. vesiculosum*) and two pasture legumes (*Ornithopus compressus* [yellow serradella], *O. sativus* [French serradella])

- Farmers adopting a targeted approach to P fertiliser use can achieve highly effective use of fertiliser and a steadily declining fertiliser cost of production.

- Despite severe drought experiments have provided further (indirect) evidence that the serradellas are more P efficient that subterranean clover.

- The movement of P in soil profiles should routinely be checked when surface soil Phosphorus Buffering Index (Burkitt *et al.* 2008) test values are <35. The research demonstrated that:
  (i) standard surface soil P tests (10 cm depth) was misleading in soils where P leached to depth,
  (ii) P applied as fertiliser moved readily to depth within the soil profile, and
  (iii) “legacy P” (from previous P fertiliser applications) at depth was sometimes sufficient to eliminate the need for P fertiliser applications in soil incorrectly diagnosed as P deficient using surface soil testing.

- The fastest way to improve P efficiency in soils types with very low P buffering (low P retention) will be to recognise and use legacy P as part of the farm’s soil P management plan. This will also reduce the potential for loss of P to the wider environment where it can be a significant pollutant.

- Alternative soil sampling/testing protocols need to be developed for use in soil types with low P retention.

Extending the adaptation range of serradella systems (Activity B5)

- This project was focussed on wider use of serradella pastures to reduce the **P-cost pf pasture production.** However, wider use will also **diversify the legume base of pastures** (risk mitigation), capture **“low-bloat”** characteristics of serradellas, **extend growing season** in years with late rainfall, improve **early- and late-season drought tolerance** as a consequence of faster root growth and/or deeper root systems, and **reduce lost production due to clover diseases**.

- Serradellas should no longer be promoted primarily for use in light, sandy, or acid and infertile soils; however, they are unsuited to neutral-alkaline soil types and will not tolerate soils that are subjected to persistent waterlogging.

- The use serradella of in southern Australia is expanding rapidly in phase farming systems but uptake in permanent pastures areas (~6M ha) is stalled. The major problem is poor persistence.

- Factors considered to be contributing to poor serradella persistence include:
  (i) promotion of cultivars with inappropriate flowering dates,
  (ii) unstable flowering dates that confound maturity-type selections,
  (iii) continued promotion of softseeded varieties to farmers,
  (iv) non-ideal seed softening patterns among hardseeded serradella varieties.

- The work has informed development of a short list of serradella cultivars for research to improve persistence in permanent pasture areas where serradellas are not adopted.

Identifying P efficient subterranean clover cultivars (Activity B6)

- The project has not identified any subterranean clover genotypes that can emulate the high P efficiency of the serradellas.

- However, a two-fold range in subterranean clover yield in P-deficient soil (i.e. P efficiency) was observed consistently in controlled-environment studies. The yields were similar among subterranean clover cultivars and among core-collection genotypes.

- P efficiency was associated with root systems that explored soil for P more effectively. Three root traits were most important: preferential allocation of plant mass to roots enabling proliferation of root length for nutrient foraging, long root length per unit root mass, long root hairs. The importance of these root attributes was not changed by the colonisation of clover roots by arbuscular mycorrhizal fungi.

- Differences among clover varieties in P efficiency could not be verified in the field because of persistent droughts. Without field proof, it is recommended that the current benchmarks for fertilising subterranean clover be followed (e.g. Gourley *et al.* 2019).

- Short root hairs on subterranean clover was the major factor limiting achievement of a step-change in P-efficiency of this species:
  (i) In theory, ~80% of the genetic variation for RHL in the subterranean clover genome has been explored in the present experiments by examining RHL among the core collection of subterranean clover lines. However, this leaves about ~20% of the variation in the subterranean clover genome as yet unexplored. Accessions that have evolved under very low soil P conditions need to be identified and assessed.
  (ii) The RHL of a number of plant species has now been doubled or tripled using molecular genetic technologies. Subterranean clover is an obvious target for application of this technology.

- The current experiments did not provide hope that genetic markers can be used to select for P-efficient root traits in subterranean clover. However, SNP’s were associated with yield indices that reflected consistently better yield in moderately P-deficient soil. Further work is required to validate this result. Taken at face value, it may be feasible to develop genetic markers for yield indices to eliminate low P-efficiency lines during early-stage subterranean clover breeding.

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1 Background

1.1 Reducing the phosphorus (P)-fertiliser costs of Australian agriculture

1.1.1 The importance of P fertiliser for agricultural production

Phosphorus (P) is one of the handful of nutrients used in the fertilisers that are now essential for global food security (Stewart *et al.* 2005, Sutton *et al.* 2013, Sattari *et al.* 2014). Agriculture is the largest user of P. Indeed, ~93% of the P used in Australia is applied as fertiliser in agriculture (Cordell and White 2009). Globally, only about half of the total P inputs to soil are recovered by crops. Roughly half of the unrecovered P is accumulated in fertilised soils, and half becomes dispersed in the natural environment (e.g. Vaccari *et al.* 2019, Lun *et al.* 2018, Bouwman *et al.* 2013, MacDonald *et al.* 2011, Cordell *et al.* 2009). For Australia, total recovery of P in agricultural products is less than this and is about 25% or less of the P applied as fertiliser (McLaughlin *et al.* 1992, Cordell and White 2009). Most mineral P fertiliser is manufactured from phosphate rock. Because such a large proportion of the P applied as fertiliser is ultimately accumulated in soil or dispersed in the environment, the world's phosphate rock reserves are considered to be a non-renewable resource.

Global estimates of P flows, such as these, clearly have their uses but they mask the diversity of farming systems across the world and, consequently, the opportunities for more sustainable use of the world's finite P reserves. There could not be a wider divergence in the P status of the soils that are used globally for agriculture. At one extreme are soils that are naturally P fertile or have been fertilised for so long (e.g. Vitousek *et al.* 2009, Sattari *et al.* 2012, Barrow and Debnath 2014) that maximum yields can be achieved without continuous application of P fertiliser. Applications of P fertiliser to soils such as these, often exacerbate P losses to aquatic systems and contribute significantly to ecosystem damage (Jarvie *et al.* 2013, Rowe *et al.* 2016). In contrast, vast areas of agricultural soil are P deficient for optimum crop growth, and production per hectare is either low relative to the potential of the agricultural production system (e.g. large areas of Africa), or relies on the use of P fertiliser to achieve high yields (e.g. large areas of southern Australia) (Stewart *et al.* 2005, Vitousek *et al.* 2009, Lynch 2011).

Grassland farming is largely conducted on P-deficient soils in the temperate zone of southern Australia. Nitrogen (N) fertilisers are generally not used except in irrigated and high-rainfall, high-return dairy pastures, or occasionally as a strategic application to boost dryland pasture growth rates in winter. Instead, N deficiency is corrected by growing pasture legumes that are sown with grass in mixed pastures or are oversown or encouraged to invade in natural grasslands. The most important pasture legumes are annual *Medicago* spp. (annual medics; low rainfall, neutral to alkaline soils), *Trifolium subterraneum* L. (subterranean clover; 300 mm - >900 mm annual rainfall, acid to neutral soils), *T. repens* L. (white clover; >900 mm rainfall, acid to neutral soils), and *Medicago sativa* L. (lucerne) which is used to a lesser extent as both a pasture and forage species provided soils are not subject to waterlogging or extreme acidity (Nichols *et al.* 2012). Of these key legumes, subterranean clover is the most widely used (~29.3 million hectares of dryland pasture; Hill and Donald 1998).

Rainfall is the key limiting resource in the southern Australian grassland system. It determines total potential production and financial return per hectare; indeed, farms are often compared on the basis of production per 100 mm rainfall received (e.g. McEachern and Brown 2010). In these environments, pastures are re-sown to improved varieties relatively infrequently. The high risk of a re-sown pasture failing to establish due to drought or because of low pasture renovation skill, combined with long periods (5-9 years) to repay the capital cost of pasture improvement are major disincentives (Lewis *et al.* 2012, Jackson and Malcolm 2018). As a result, pastures in non-arable areas are essentially permanent and are anticipated to remain in production for up to 20 years (Malcolm *et al.* 2014). New pasture varieties are selected for production combined with persistence reflecting this reality (e.g. Culvenor and Simpson 2016).

Low soil fertility limits the efficiency of rainfall use (Mills *et al.* 2006). Consequently, application of P fertiliser is the easiest and most economic way to achieve high N-fixation by the legume component of pastures, high land-use efficiency and to realise the productive potential of most farms (Mills *et al.* 2006).

Unfortunately, the Phosphorus Balance Efficiency (PBE[2]) of sheep and beef production systems is very low. Farmers need to apply 5- to 9-fold more P as fertiliser than they remove in animal products (median PBE = 9-19%; Weaver and Wong 2011). This is due mainly to the moderate to high P-sorbing capacity of most Australian soils which accumulate large proportions of the P that is applied as fertiliser (e.g. McLaughlin *et al.* 2011, Weaver and Wong 2011, Simpson *et al.* 2015).

Phosphorus fertiliser is, consequently, a significant production cost for dryland grazing farms. It typically accounts for 20-25% of annual variable costs and is often the largest cost after labour and debt-servicing (McEachern and Brown 2010).

1.1.2 Previous attempts to reduce P-fertiliser costs

Research to reduce P-fertiliser use in agriculture has been a global priority for many years driven both by a need to improve the efficiency of use of a scarce resource and the pollution of the aquatic environment when P is lost from agriculture. Most effort has focussed on mobilising the large bank of sparingly-available (“fixed”) phosphate or organic P in soil using plants (Vance *et al.* 2003; Richardson *et al.* 2007) or micro-organisms (Khan *et al.* 2010) that exude organic acids and/or phosphatases from their roots. Success has been achieved with crop plants such as *Lupinus albus* that exude citrate from specialised root structures (cluster roots), but there are few examples of crops that can dose this and attempts to mimic P mobilisation by organic anion exudation from roots of common crop species by breeding or genetic engineering have not been successful (Richardson *et al.* 2011, Ryan *et al.* 2014). Microbial inoculants for crops have had minimal or no reliable impact (Karamanos *et al.* 2010). Consequently, P-efficiency in Australian agriculture has not improved over the last 40 years, at least (cf. McLaughlin 1982 with Weaver and Wong 2011). In contrast, in low-input, subsistence agriculture, where P fertiliser use is minimal, crops with an improved ability to explore topsoil for scarce P resources have been used successfully to increase crops yields (e.g. Burridge *et al.* 2019), albeit at a low level compared with what may be achieved by applying fertiliser.

1.1.3 A new approach to reducing P-fertiliser costs in fertilised agriculture

Because the rate at which P is released from soil by weathering reactions is very slow, the amount of P fertiliser required for agricultural production is effectively the sum of P removed in agricultural products, P loss to the wider environment and P accumulations in paddocks. In a system where plant-available P is being maintained at a stable concentration, this may be summarised as:

$$P_{\text{fertiliser}} = P_{\text{export}} + P_{\text{erosion/leaching}} + P_{\text{waste dispersal}} + P_{\text{soil accum}}$$

Where: P_{export} = removal of P in products; $P_{\text{erosion/leaching}}$ = P lost by leaching, runoff or soil movement; $P_{\text{waste dispersal}}$ = net accumulation of P in small areas of farms as a result of uneven dispersal of animal excreta; $P_{\text{soil accum}}$ = P accumulating as sparingly-available phosphate or organic P compounds that are only slowly mineralised.

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[2] PBE is a measure of the efficiency with which fertiliser is used on farms and is calculated as:
PBE (%) = 100*(P output in agricultural products / P inputs in fertiliser and imported feed).

In most cases, low efficiency of P use in Australian agriculture is due to P accumulation in soil when pastures or crops are fertilised (McLaughlin *et al.* 2011, Simpson *et al.* 2014). However, there are also clear examples, particularly in deep sandy soils, of inefficiency that also involves loss of P by leaching (e.g. Ozanne *et al.* 1961, Lewis *et al.* 1987).

Laboratory experiments show that the rate of phosphate sorption ("fixation") in soil depends on the concentration of phosphate in soil solution and the duration of phosphate-soil contact (Barrow 1980a, Barrow 1980b). P accumulation in pastures is more complex and also involves accumulation of organic P in soil, and P accumulation in excreta in stock camps. However, Simpson *et al.* (2015) demonstrated that the rate at which P accumulates in fertilised pasture paddocks is also directly related to the soil test P (STP) concentration of the soil. On this basis, they argued that managing STP concentrations of pastures and using pasture legumes with lower "critical" P requirements than subterranean clover (i.e. legumes that achieve equivalent high yields at lower STP concentrations) would reduce P fertiliser costs by slowing the rate of P accumulation in soil (Simpson *et al.* 2014).

At the commencement of this project, the critical[3] STP requirements of a number of alternative pasture legumes had been determined in a series of field experiments. A small number of legumes with substantially lower critical P requirements than subterranean clover (*Trifolium subterraneum*) were identified; the most promising pasture legumes were species of serradella (*Ornithopus compressus* [yellow serradella] and *O. sativus* [French (aka pink) serradella]; Sandral *et al.* 2019). In experiments conducted near Yass and in the NSW Riverina, subterranean clover required a Colwell STP concentration of ~33 mg/kg (Olsen P ~13 mg/kg) for near-maximum yield, whereas French serradella required ~21 mg/kg for the same yield (Olsen P ~8 mg/kg). This was achieved while roots of both species were highly colonised by mycorrhiza. The lower P requirement of the serradella is due to its long fine roots, low specific root length and long root hairs which assist soil exploration and efficient P uptake at low STP concentrations (Haling *et al.* 2016a, Haling *et al.* 2016b, Yang *et al.* 2017).

It was subsequently estimated, using empirically measured rates of P accumulation in grazed fields (Simpson *et al.* 2015), that the fertiliser cost of maintaining a productive subterranean clover pasture (critical Olsen P concentration ~15 mg/kg) would be reduced by up to 30% if subterranean clover could be replaced by serradella (critical Olsen P concentration ~10 mg/kg) (Simpson *et al.* 2014).

This is a conceptually different strategy to all previous attempts to reduce P fertiliser costs when the objective has been to mobilise the phosphate or organic P that is accumulated in soil after applying fertiliser. By using plants with lower critical P requirements, the aim is to slow P accumulation in the soil, rather than to reverse it. The recent determination of critical STP benchmarks for a range of alternative pasture legumes (Sandral *et al.* 2019) indicated that this could potentially be achieved using existing pasture species (i.e. the serradellas), or perhaps by breeding novel variants of successful pasture legumes (e.g. subterranean clover) with a more P-efficient root morphology and a lower critical P requirement (Lynch 2007, Yang *et al.* 2017, Haling *et al.* 2016b).

1.2 Research questions and project activities

1.2.1 Can serradella pastures be managed at lower soil test P concentrations than a subterranean clover-based pasture, without loss in productivity?

Serradellas are grown with soil test P concentrations that are up to twice that indicated as necessary by recent research (e.g. cf. Freebairn 2013 with Sandral *et al.* 2019). For the predicted P-efficiency

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[3] The critical STP requirement of a pasture plant is defined for southern Australia the STP concentration that can support 95% of maximum yield (Gourley et al. 2019).

gains to be captured, it will be necessary for soil P management to be targeted at the (putative) low critical STP requirement of the serradellas (e.g. Sandral *et al.* 2019). Participatory research with farming groups in areas of WA and NSW, where serradellas are already used, was established to provide proof-of-concept that serradella pastures can achieve high yields at lower soil test P levels. This research was designed to build on the increasing role of farming groups in successful adoption through on-farm experimentation and regional demonstrations and was backed by peer-support among farming groups, agricultural advisors and collaborating research scientists.

1.2.2 What are the seed production, plant persistence, climatic and edaphic constraints to wider use of serradella(s) across southern Australia?

Yellow serradella is largely used in acid, sandy soils (e.g. WA, Coonabarabran, NSW) and for a long time, this soil type was regarded as the niche environment to which serradella was adapted (Bolland and Gladstones 1987, Loi *et al.* 2005, Freebairn 1996). However, recent development of hardseeded French serradella cultivars (e.g. Nutt 2004a, Nutt 2004b) has underpinned a dramatic expansion of serradella use in ley-farming systems (e.g. WA, NSW Riverina) and on other soil types (Nichols *et al.* 2012, Hackney *et al.* 2013). It is easily forgotten that it has taken nearly a century of development to define and refine edaphic and climatic boundaries within which subterranean clover is used. The first 20-30 years of cultivar release was focussed on development of varieties with a range of maturity types to enable wider adoption of subterranean clover (Donald 1970). Subsequently, cultivars have been developed to resolved issues of significant toxicity to animals, plant disease, insect attack, edaphic constraints and yield improvement. By comparison, the use of serradella is still in its infancy with cultivar development akin to the situation for subterranean clover in the 1920s-30s when inadequate cultivar options limited the districts in which it could be grown across southern Australia.

This project used field and glasshouse-based research to define the maturity type of serradella cultivars, flowering physiology, seed production, hardseededness and seed softening patterns, and seedling survival; characteristics considered essential for use of serradella cultivars in permanent pastures outside the existing areas of use. Tolerance of serradella species to cold temperature and waterlogged, acid and dry soils was also examined to inform wider use and cultivar development. Participatory research with farming groups in areas of Vic and southern NSW, where serradellas are not used, was undertaken to test the yield and persistence of current cultivars of serradella in farm landscapes. This research was designed to raise awareness of the attributes of serradellas and to encourage adoption through on-farm experimentation and regional demonstrations should the serradellas prove useful in comparisons with locally-adapted subterranean clover cultivars.

1.2.3 Can producers increase the productivity of subterranean clover pastures in low P paddocks by choosing the right cultivar?

Most farms have paddocks that are not fertilised to optimal STP concentrations; in some cases (e.g. adverse topography) it is not feasible to achieve optimal P fertility. Field and glasshouse-based plant growth and root morphology experiments will test observations that there is a significant range in root foraging capability among the existing subterranean clovers and that this can deliver substantially better yield in moderately P-deficient soil.

1.2.4 Can the nutrient foraging traits of subterranean clover be modified to achieve P-efficiency that is equal to that of the serradellas?

Many root traits are implicated in efficient P acquisition, but the ideal combinations of traits vary among species. Recent research identifies: root proliferation in nutrient patches as an asset, and low specific root length and low root hair length (RHL) as detrimental for P acquisition by subterranean clover. Research will examine trade-offs in breeding for these traits and trait-combinations, and sources of genetic variation that may be used to lift the P-efficiency of subterranean clover closer to that of the serradellas.

1.2.5 Can rapid, early root phenotype- and genomic-screening protocols for P efficiency be incorporated routinely into subterranean clover breeding?

Can rapid, early root phenotype- and genomic-screening protocols for P efficiency be incorporated routinely into subterranean clover breeding? Roots traits are hard to modify in plant breeding programs because they are the unseen half of the plant and difficult to measure. This project will examine the potential to apply genomic screening to clover root traits with the aim of enabling rapid screening for root phenes at the seedling-stage.

1.2.6 Assisting adoption of improved P management strategies.

Irrespective of the legume(s) on which a pasture is based, efficient use of P fertiliser can only be achieved by informed use of soil testing, and the development of targets for soil P management that are guided by the critical STP requirement for pasture production. The project results, and other advances in soil P management strategies that have evolved over the last decade were used to update the "Five Easy Steps" P management tool and technical booklet. This tool was first released in 2009 and remains in the top ten tools accessed regularly on the MLA website. Feedback from consultants and other users of the tool was also used to improve the way information is presented to farmers in the updated version.

2 Project objectives

1. Development of low-P pasture systems based on highly P-efficient legumes, the serradellas (*Ornithopus* spp.), using a combination of farmer-participatory and traditional research. Benefit: ~30% reduction in P-fertiliser costs without loss in production; achieved over an immediate/short-term timeframe.
2. Identification and then promotion of the most P-efficient cultivars of subterranean clover (*Trifolium subterranean*). Benefit: potential to double the production of subterranean clover pastures that are grown in situations where optimal soil P fertility cannot be achieved or maintained (short/medium-term timeframe).
3. Development of the knowledge and genetic/agronomic protocols necessary for development of subterranean clover cultivars with P-efficient root traits analogous to those of serradella species. Benefit: future cultivars that require substantially less P-fertiliser (up to 30% less than current cultivars) and 2 to 4-fold better growth in low-P soils (medium/long-term timeframe).
4. Updates to the industry decision support tool ("Five Easy Steps" soil-P management tool) to include the principles and management that underpin the achievement of highly productive, P-efficient pasture systems. This tool applies to pastures grown on acid soils across southern Australia (~35M ha) and is already in use in many farming districts.
5. At least 240 producers directly equipped with knowledge to improve their P efficiency in their farm context; 800 producers associated with farming system groups informed of the potential benefits, and a further >3000 aware and motivated to gain more information.

3 Methodology

3.1 Overview of methods

More detailed descriptions of the Materials and Methods for each experiment may be found in the Final Internal Milestone reports that are appended to this Overview Report. What follows is a brief description of the Methods used in the key experiments that addressed the Project Outputs. This is intended to provide context for the Results, Discussion and Conclusions in this report.

3.1.1 Output 4(a) – Conduct participatory plot scale experiments in areas of WA and NSW where serradella is already grown to assess serradella production in response to P fertiliser application and quantify the critical P level required by the legume.

Experiments in which the yield response of legume monoculture to the application of P were established with 6 P treatments (applied as triple superphosphate), 3 legumes treatments (subterranean clover, yellow serradella and French serradella), and 4 replicates (randomised complete block design). Background nutrients (K, S and micronutrients) were applied in an attempt to ensure that P and N were the only limiting nutrients. The legumes were inoculated with appropriate rhizobium strains at sowing. The intention was to measure pasture yield during spring so that the critical soil test P (STP) requirements of each legume could be determined. The critical P requirement of pasture is defined in southern Australia as the soil P level at which 95% of maximum yield is achieved during the period of fastest pasture growth (typically spring) (Gourley *et al.* 2019).

P-response experiments were established by research team members in areas associated with the participating farmer groups in WA and northern NSW where serradellas are already used. Sites in WA were able to be located adjacent to farm group participatory experiments; data from the adjacent P response experiments has been invaluable in interpreting the participatory experiments and *vice versa*. In northern NSW, it was also necessary to locate some experiments on similar soil types at separate sites. Cross-comparison of results from the co-located and separated experiments, with those being run by the farm groups, also proved to be valuable.

Further details: Appendix 9 and 12.

3.1.2 Output 4(b) – Conduct participatory paddock scale experiments with farmer groups in areas of WA and NSW where serradella is already grown to improve knowledge of the P-fertiliser and general management of serradella-based pasture systems.

Participatory research experiments were established by farm groups at four locations in southern WA and at two in northern NSW, in areas where serradella is widely used. The experiments were located at: Katanning (Fig. 3.1 – 1) and Broomehill, WA (Southern Dirt farm group), Grass Patch and Neridup, WA (ASHEEP farm group) and Boggabri (Boggabri Grazing Group) and Purlewaugh (Purlewaugh NSW Farmers group) in northern NSW.

A similar base experiment was planned for each of the sites. The sites were selected by the farm groups as P deficient on the basis of surface soil (0-10 cm) tests. It was permitted to select sites with an established serradella pasture or to sow a locally-adapted serradella variety with or without grass. Soil P treatments were applied using a randomised block design (n=3): low P, medium P and high P with the aim being to have a P deficient soil, a soil that was close to the optimum for serradella, and a supra-optimal P treatment to check whether higher soil P fertility would deliver improved pasture yields. Yellow serradella (cv. Avila) was sown at the ASHEEP sites and yellow serradella (cv. King) regenerated along with the spring-through-autumn growing perennial Consol lovegrass (*Eragrostis curvula*) at Purlewaugh or red grass (*Bothriochloa macra*)-wiregrass (*Aristida ramosa*) pasture at Boggabri. An equal mix of French serradella (cv. Margurita), yellow serradella (cv. Avila) and subterranean clover (cv. Seaton Park) was sown at the Southern Dirt sites. However, the sown clover did not survive the dry conditions that followed sowing. Subterranean clover often volunteered in these pastures usually at comparatively low densities. Plant establishment was assessed each year and the frequency of major species was determined during along with herbage mass.

All sites were impacted by severe and persistent drought conditions and, as more detailed soil analyses became available, the aims of the work expanded to understanding how to more effectively manage soil P for serradella pastures. Deep soil cores (up to a metre depth at 10 cm intervals and/or 0-60 cm depth samples) were taken in the subsequent years of the experiments to understand the distribution of P in the soil profiles.

Figure 3.1 – 1 Dr Brad Nutt talking with farmers at the Katanning participatory research site during the Southern Dirt Spring Field Day in 2018. Participatory and core research sites provided foci for field days and farmwalk activities across the whole project.

Further details: Appendix 3, 4 and 8.

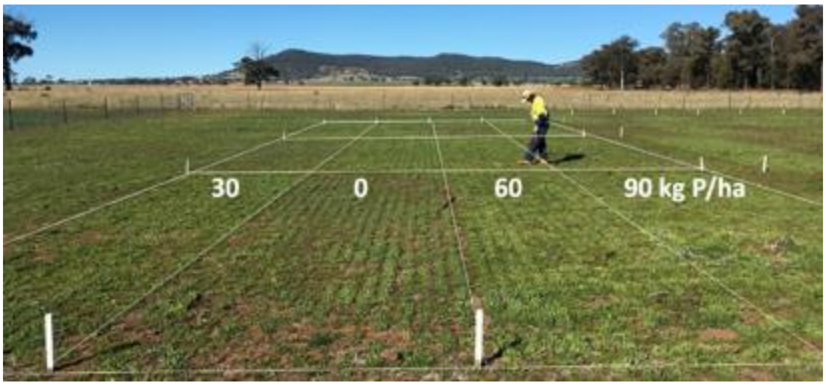
3.1.3 Output 4(c) – Development of guidelines for managing soil P fertility in crop-pasture transitions involving serradella pastures.

The P efficiency benefits of using serradella-based pastures can only be realised if farmers manage their soil P fertility to the lower critical soil test P (STP) benchmarks that are appropriate for serradella (Simpson *et al.* 2014). Equipping farmers to do this is a major objective of this project and is addressed by several Outputs including 4(c). A number of experiments addressing Output 4(c) were conducted with the objective of understanding management of soil P fertility to achieve target levels, with a particular emphasis on monitoring and changing soil fertility between phases in farm operations.

3.1.3.1 Rate of P rundown when fertiliser is withheld

Experiments were conducted at Cowra, southern NSW, Purlewaugh, northern NSW and Spring Ridge (Fig. 3.1 – 2), northern NSW. In each case, P-fertiliser was applied to sites with a relatively modest level of P fertility to establish four initial surface soil (0-10 cm) concentrations of soil P that ranged from ~15 and ~65 mg Colwell P/kg soil that were subsequently monitored at 6 or 12-month intervals to determine the rates at which STP concentrations decline when fertiliser is withheld.

Figure 3.1 - 2
Pasture establishment in the Spring Ridge P-rundown experiment in 2017. Colwell P in P0 treatment was ~10 mg/kg soil. Growth response to P fertiliser already evident.



3.1.3.2 Legacy of drill rows on spatial variability of extractable nutrients

Archived soil samples from an experiment conducted at Wagga Wagga (Hayes *et al.* 2017) were analysed for Colwell extractable P, Colwell extractable potassium (K) and KCl40-extractable sulfur (S) to assess the impact of pasture drill rows on the variability of nutrients in paddocks. Soil was sampled at the end of a pasture phase (Sep 2014), prior to sowing a wheat crop (Apr 2015) on the pasture drill row and between drill rows. Soil cores were collected and analysed at intervals to 0.6 m depth. The objective was to understand whether drill rows resulted in legacy impacts on the variability of extractable P, K and S concentrations across paddocks or within soil profiles.

3.1.3.3 Spatial variability of extractable P in pasture paddock soils

Uniform areas of pasture paddocks were grid sampled for Colwell P (0-10 cm) to assess the spatial variability of STP and the consequences for the soil sampling intensity needed to obtain a representative soil test in paddocks that do not present obvious topographical challenges for sampling soil.

3.1.3.4 Spatial variogram analysis of extractable P in cropping soils

Archived soil samples from a study of soil carbon (C) and nitrogen (N) in cropping paddocks at sites near Wagga Wagga and Yerong Creek were assessed for topsoil Colwell P to provide information about the sampling intensity needed to estimate soil P fertility when transitioning from cropping to pasture production. The soils from the two sites had been analysed previously for a range of soil parameters that were analysed using four variogram models: linear, spherical, Gaussian and exponential. The first paper from the study reported the variance in organic C and N (Conyers *et al.* 2018). A biometric analysis of the soil P data is continuing.

Further details: Appendix 9

3.1.4 Output 5(a) - Conduct participatory research experiments with farmer groups in areas serradella is not used to examine productivity, persistence and potential use.

Participatory research experiments were established at seven diverse locations in southern NSW and central Victoria with farm groups in areas where serradella is not used. The experiments were located: at Redcliff (via Bombala) and Glenfinnan (via Cooma) on the eastern and western sides of the Monaro plateau, NSW with the Monaro Farming Systems group; at Bigga (Tableland Farming Systems; Fig. 3.1 - 3) and Yass (Bookham Agricultural Bureau) on the Southern Tablelands, NSW; and at Baynton, Sidonia and Pastoria (via Kyneton) in the central ranges of Victoria (Central Ranges Grasslands Society).

A similar base experiment was planned for each site with French serradella, yellow serradella and subterranean clover sown with phalaris in most experiments. The cultivar of subterranean clover was determined by local recommendations, the French serradella cultivar was Margurita (a mid-season variety; the only hardseeded cultivar for which seed was commercially available), and the yellow serradella was either Santorini (early-mid season maturity) or Avila (later maturity), both of which are hardseeeded. In NSW, two experiments were sown at each site to explore the effect of farm landscape variability. In Victoria, experiments were also sown on different farms (soil types). The objective set for soil P fertility management was to maintain soil close to the critical STP requirement for serradella (Olsen P = 10 mg/kg; Colwell P = 20 mg/kg for soils with PBI 40-80; Sandral *et al.* 2019). This was not always possible and in these circumstances the aim was to manage soil P towards the critical STP concentration. Variants of the base experiment were also sown: the two cultivars of yellow serradella were compared along with subterranean clover in experiments on the Monaro; at Baynton the legume-grass mixtures were sown along a paddock transect that included low-lying areas.

The objective of all experiments was to gather data on pasture establishment, relative growth and persistence of the alternative legumes, and their potential suitability for use in pastures at these locations. The sites also served as a focus for field days.

Figure 3.1 – 3. Field day at the Tableland Farming Systems participatory research site (Bigga, NSW) on 4 December 2017. At this time, subterranean clover was finishing but the serradellas were still green and responding to late season showers.

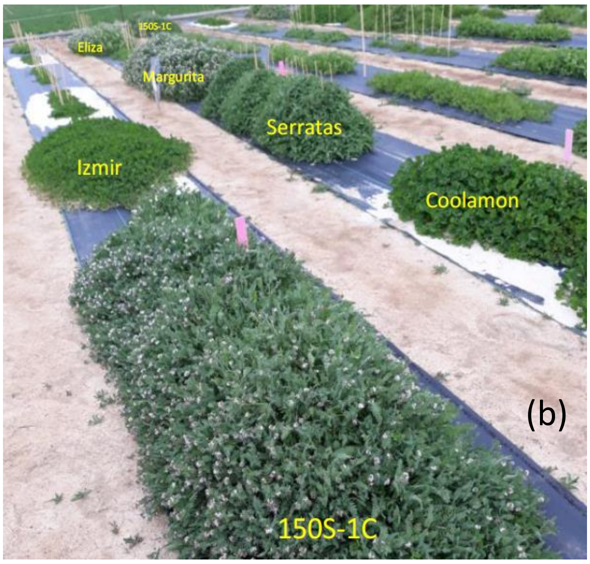
Further details: Appendix 5, 6 and 7

3.1.5 Output 5(b) - Conduct field experiments with serradella to quantify the range in serradella flowering time, seed production, hardseededness/ breakdown, persistence, seedling recruitment and survival traits that underpin cultivar development.

3.1.5.1 Flowering time and flowering date stability

The initial objective of these experiments was to characterise serradella cultivars according to their maturity type. To this end, a national experiment was commenced to determine serradella flowering dates (maturity types) by sowing a common collection of yellow serradellas (*O. compressus* - King, GEH72.1A, Santorini, Yellotas, Avila), French serradellas (*O. sativus* - Cadiz, Margurita, Erica, Serratas) and slender serradella (*O. pinnatus* - Jebala) alongside subterranean clover controls (*T. subterreaneum* – Izmir, Seaton Park, Coolamon, Goulburn, Leura) at approximately the same date in 2017 (late April-mid May) in Perth, Cowra, Canberra and Tamworth. The subterranean clover control cultivars represented early to late maturity types. Sites were prepared similarly with weed mat (black, 0.91 m width) laid in parallel lengths about 1.1 m apart (e.g. Fig. 3.1 - 4). Plots were

Figure 3.1 - 4. Views from the national flowering time experiment in 2018: (a) mid-spring at the UWA Shenton Park Research Station, Perth, WA and (b) the equivalent experiment at CSIRO's Ginninderra Experiment Station, Canberra, ACT where examples of French serradella cultivars and subterranean clover control cultivars can be seen. Cultivar Eliza is an earlier flowering French serradella, cv. Margurita and breeder's line 150S-1C are of mid-season maturity and cv. Serratas is among the latest maturing varieties available presently.



established by burning 20 holes (0.05 cm diam.) in the weed mat in two parallel rows (0.1 m apart), with holes offset and spaced at 0.2 m along each 2 m row. Plots were spaced 1.0 m apart along the length of the weed mat. At the appropriate sowing time, up to four germinable seeds were sown in each hole with the objective of establishing 20 small groups of each cultivar in each plot. When the legumes were established, sand was placed on the weed mat under the subterranean clover cultivars to aid burr burial and seed development. All sites were irrigated after sowing if required to ensure germination and emergence, and throughout the experiment as required to ensure low soil moisture did not affect plant growth rates, flowering times and seed production.

Various observations in 2017 indicated that some serradella cultivars may not flower consistently. The national experiment was replanned and expanded in 2018 with two sowing dates to enable assessment of serradella flowering dates (maturity type) and the stability of flowering dates.

Eighteen pasture legumes (six yellow serradella cultivars, six French serradella cultivars, slender serradella and five subterranean clover controls (e.g. Fig. 6.2 - 4) were sown as above at most sites in the weeks commencing 19 March and 1 May 2018, (and at Cowra on 4 April and 7 May) in a randomised block design (n=3) that was common to all sites.

Additional serradella lines were also assessed for flowering time at the Perth, Cowra and Canberra sites. These data (see Internal Milestone Reports) have been used to inform further potential research and arenot presented here, as it was not part of the national experiment.

The genotypes were typically assessed about every 2-4 days to monitor the commencement of flowering. Individual plants were not distinguished, and once a flower was observed within a planting group (i.e. in each weed mat hole), the plant group was recorded as having flowered. Assessments continued until all planting positions contained at least one plant with a flower.

Box plots of flowering time were formed using all data (max. n=60) for each cultivar within a site. Average median flowering dates (i.e. when 50% of plant groups had produced at least one flower) and statistical treatment of the data is based on the replicate structure of the experiment.

Further details: Appendix 9, 10 and 11.

3.1.5.2 Serradella seed production

Seed of many serradella varieties was in short supply. Seed production was assessed, therefore, using microswards at Canberra and Cowra. These data were supplemented by measuring the development of soil seed reserves and subsequent seedling establishment in experiments set up to assess serradella persistence or production.

Seed yields in microswards: Seed yield of eight serradella cultivars was measured in experiments at Cowra and Canberra. Because the quantities of seed of some cultivars was very limited, five varieties of yellow serradella (cvs. Avila, GEH72.1A, King, Santorini, Yellotas) and four varieties of French serradella (cvs. Cadiz, Erica, Margurita, Serratas) were sown in circular microswards surrounded by reflective sleeves to approximate light conditions in a pasture sward (e.g. Fig. 3.1 - 5).

The microswards were established on weed mat with a grid of small holes in it to facilitate sowing and to assist the harvesting of seed pods that are shed readily by the serradellas when the pods mature. The serradellas were inoculated with Group S *Rhizobium* strain WSM 471; *T. subterraneum* plants were inoculated with Group C *Rhizobium* strain WSM 1325, (NewEdge Microbials, Albury, NSW)). Three replicate microswards were established for each cultivar in a randomised block design.

Pods were harvested when the plants had died at the end of the growing season. Subsamples of the pods were opened, and seeds collected to estimate seed number and seed weight per pod and these data were used with pod yield information to determine seed yield per hectare.

Figure 3.1 - 5. Serradella varieties growing in micro-swards at Cowra, NSW for seed production measurements.

Seed reserves and seedling establishment in a soil fertility experiment: Soil seed reserves were assessed in ungrazed swards of French serradella cv. Margurita, yellow serradella cv. Avila and subterranean clover cv. Leura established at Gunning in 2017 with at high (43.8 mg/kg Colwell P/kg, November 2018) and low (21.1 mg/kg) soil P fertility levels, with and without 3.5 t lime/ha (n=4 replicates). The PBI at Gunning was 120 and predicted critical Colwell P for clover pasture was 34 mg/kg. Consequently, the P treatments in the experiment represented sufficient P supply for both serradella and subterranean clover (high P treatment) and a near-critical P supply for serradella, but deficient for subterranean clover (low P treatment). Lime raised pH_{CaCl2} in the surface soil (0-10 cm) from 4.2 to 4.5 and reduced exchangeable Al from 17.0% to 7.7% of cation exchange capacity ($P<0.05$).

In these experiments, a strip of soil (1 m long x 0.1 m wide x 0.02 m deep) was excavated from the middle of each plot. The soil was sieved, and residual plant herbage was threshed to collect all legume seeds. Seeds were separated from organic matter and soil using sieves of differing size. Clean seed samples were weighed and counted. Seeds of subterranean clover and French serradella were removed from their burrs/pods. Yellow serradella seeds could not be easily de-hulled. All data are, therefore, reported in terms of numbers of seeds/m^2.

Seed production in serradella persistence experiments: Soil seed reserves were quantified at the end of the first growing season in five field experiments established on the Monaro and Southern Tablelands of NSW. The primary focus of the sites which are located in areas where serradella is not used currently, was to examine serradella persistence relative to locally-adapted subterranean clover cultivars. The experimental period at each of the “persistence experiments” sites was generally characterised by substantial rainfall in early autumn followed by drier than usual winters and very poor spring conditions. Much of the rainfall received annually at each of the sites occurred on the margins of the winter-spring growing season reducing the potential for utilisation by the winter-growing annual legume species. Seasonal conditions became progressively drier with time with annual totals in 2019 at Goulburn, Bombala, Bigga, Gunning and Yass tallying only 57%, 62%, 62%, 62% and 64% of the long-term median annual rainfall at the sites, respectively.
Seed reserves were harvested (by the method described above) in late January-early February 2018 (end of year 1) from persistence experiments that had been sown at Bigga, Goulburn and Bombala in 2017. Two further serradella persistence experiments sown in 2018 near Yass and Gunning, NSW, were sampled in early February 2019. Early persistence data from the other experiments and the limited availability of viable seed for some varieties dictated that the Gunning experiment be restricted to the most promising serradella cultivars (**for further experiment details see Section 3.1.5.4** and Figs 6.2 - 11 and 6.2 - 12).

Further details: Appendix 9 and 10

3.1.5.3 Hardseededness and seed softening

Seed softening experiments were conducted in parallel in the contrasting climates at Canberra and Cowra. Serradella pods and subterranean clover burrs were collected in late-summer from flowering time experiments at each site and the initial proportions of hard seeds were determined. Pod fragments containing seeds were placed in plastic coated micromesh (1.25 mm “fly wire”) bags that were pinned to bare soil (Fig. 3.1 – 3). At ~4 or ~6-week intervals, commencing in January or February, mesh bags with seeds from each pasture legume (n=3) were collected from the field over a ~6 month period and the seeds subjected to a germination test. A final seed germination test was conducted on pods/seeds that had been pinned to the soil until near the end of the growing season. Subterranean clovers collected in the same way were tested using bare seeds that had been rubbed gently from their burrs. In these experiments, seed theft by ants was a major potential problem. Unexpectedly, ants were found to be capable of creating holes in the micromesh bags. Once inside they consumed softened seeds preferentially. To minimise this problem, the experiments were

Figure 3.1 – 3. Micromesh pouches each containing 100 seeds of a serradella or a subterranean clover cultivar pinned to the bare soil surface at Cowra, NSW to examine the rate of seed softening. The pouches and surrounding soil were treated with insecticide to protect against seed theft by ants.

treated with insecticide. Seed germination tests were conducted by placing pod fragments/seeds on moistened filter paper in petri dishes maintained at 15°C in the dark for two weeks.

Further details: Appendix 9 and 10

3.1.5.4 Serradella persistence

Persistence in established legume monocultures

Three field experiments from a previous project (B.PUE.0104) were revisited in the current project to monitor persistence over a longer timeframe than possible in any of the newly-sown experiments. A range of alternative pasture legume treatments had been established as monocultures in 2013 (Goulburn), 2014 (Burrinjuck) and between 2012-2014 (Yass) at 6 levels of soil P fertility. The legumes included yellow serradella, French serradella and subterranean clover. In the experiment at Yass each legume treatment was over-sown each year with a high seedling rate. The legume monocultures were maintained mainly by mowing (intermittent grazing at Goulburn) but were not mown or grazed during seed production in spring. Invasion of the treatments by grasses and weeds was permitted after 2013 (Goulburn) and after 2014 (Burrinjuck and Yass). Frequency of eight annual legumes grown with 6 levels of soil P fertility (range: deficient to supra-optimal P; n=3) was monitored in 2016 at each site. Frequency was then monitored again in 2017 and 2018 at Yass. More details of the initial experiment and its design at Goulburn are provided in Hayes *et al.* (2015) and at Burrinjuck and Yass in Sandral *et al.* (2019).

Frequency of the sown legumes (a measure of ground occupancy) was assessed by placing a 0.5 x 0.5 m quadrat that was divided into 100 (50 x 50 mm) squares and counting the number of squares containing the base of a sown legume. This measurement was repeated at three randomly chosen locations per plot in August 2016 and August 2017.

Total herbage mass was also estimated by visually scoring ten 0.1 m^2 quadrats per plot and calibrating visual scores with ten representative quadrat cuts taken from across the site. The species composition of each plot was assessed using the dry-weight rank method ('t Mannetje and Haydock 1963) at 10 locations along a transect of each plot.

Persistence in newly-sown experiments

A network of five field experiments was established to compare the persistence of a wider range of serradella cultivars with locally-adapted subterranean clover cultivars across the Monaro and Southern Tablelands of NSW where serradella is not used currently. Four of the experiments were co-located with the farmer participatory sites near Bombala, Yass, Goulburn and Bigga, NSW and were sown in April and May 2017 (n=3) and consisted of ~7 yellow serradella cultivars, ~6 French serradella cultivars and ~10 subterranean clover cultivars (see Figs 6.2 - 11 and 6.2 - 12). These sites also had ~5 perennial legumes sown as part of another study (P.PSH.1030). The objective was to compare essentially the same legume treatments across different environments. However, due to low seed availability some cultivars could not be tested at all sites. All serradella and subterranean clover cultivars were sown with 10 kg/ha germinable seed. The exception was cultivar Cadiz which was sown in pod at a rate of 30 kg/ha. Only data from the annual legume treatments are reported here.

As reported above, these sites experienced very early rainfall at the end of summer/early autumn, but this was followed by very dry winter and spring conditions. Conditions became progressively drier with time with annual totals in 2019 at Goulburn, Bombala, Bigga, Gunning and Yass tallying only 57%, 62%, 62%, 62% and 64% of the long-term median annual rainfall at the sites, respectively.

The experiment at Yass failed to establish successfully due to drought and was re-sown in May 2018. A further experiment was sown near Gunning also in May 2018 using a subset of 10 of the more promising cultivars from the three species.

All data were checked for normal distributions and log-transformed where appropriate prior to conducting an analysis of variance.

Further details: Appendix 9

3.1.6 Output 5(c) Conduct a ‘watch and act’ regime for occurrence of any serradella leaf disease at all field sites to assess whether leaf disease is likely to occur when serradella pastures are used.

In a previous project, small patches of collapsed, necrotic serradella herbage were observed in most years during spring in experiments with ungrazed monocultures at Yass, NSW. *Sclerotinia* spp. was tentatively identified on necrotic shoot material. These disease patches were always treated with fungicide in the previous experiment to ensure the objectives of the experiment were not compromised. The extent to which disease may have become a problem could not be assessed.

Sclerotinia disease outbreaks are not considered a significant issue in serradella-growing areas of WA (B. Nutt, *pers. commun*.). However, it was unclear because of the observation of disease patches in the NSW experiment, whether leaf disease will prove to be an issue when serradella is used more widely in permanent pastures of the high rainfall zone.

Four approaches were planned to assess whether there were disease risks associated with the wider use of serradellas:

1. the pre-existing field experiment near Yass, where disease symptoms were previously observed, was monitored for disease incidence and as a potential source of disease inoculum.
2. a new disease-screening nursery of diverse serradella germplasm was sown adjacent to the pre-existing experiment at Yass.

3. serradella growers in WA were asked to alert the project team of the presence of any foliar disease in paddocks during the growing season.
4. varietal responses to *Sclerotinia* disease were to be examined in a screenhouse nursery in Perth that is commonly used to screen canola germplasm for leaf diseases.

This last objective was initially postponed because there were no disease outbreaks (i.e. no inoculum sources) identified in WA in 2017 and, subsequently, had to be abandoned because no disease on serradella was reported in WA at all during the project.

In fact, as is reported below, there was little if any foliar disease observed on serradella across the whole of the project network from 2016-2019. This combined with the impact of continuing drought on the 'disease-screening' experiments led to the objectives of this work being modified to a 'watch and act' regime for occurrence of any serradella leaf disease at all field sites.

Further details: Appendix 9

3.1.7 Output 5(d) - Conduct glasshouse/controlled-environment research to identify and quantify the suspected soil and environment constraints to the serradella adaptation range.

A series of controlled-environment/ glasshouse experiments were conducted to quantify the susceptibility to, or tolerance of aluminium (Al) toxicity, manganese (Mn) toxicity (common problems in very acid soils), waterlogging, and cool (winter) growth temperatures by a range of serradella cultivars.

3.1.7.1 Tolerance of aluminium and manganese toxicity by serradellas

Various cultivars of yellow, French and slender serradella and a hybrid (yellow x French) serradella were subjected to a range of Al and Mn concentrations in hydroponic culture, following methods commonly used to assess Al (acid soil) tolerance by crop and pasture genotypes (e.g. Culvenor *et al.* 1986). Control species with known tolerance or sensitivity were included to benchmark the responses to the potential toxins. Germinated seedlings were grown in eight tubs of varying concentrations of Al (0 to 370 µM) and Mn (18 to 1821 µM) concentration and root and shoot growth assessed relative to the control species to rank sensitivity.

Additional experiments in Al-toxic and Mn-toxic soils were conducted to verify the results from hydroponic culture (see Appendix 11). An additional and definitive assessment of the validity of the hydroponic studies of Al tolerance was also conducted and is reported in the **Supplementary Final Report**.

3.1.7.2 Sensitivity to waterlogging by serradellas

The impacts of waterlogged soil on the growth and recovery of serradellas was assessed for a range of cultivars/ accessions of French, yellow and slender serradellas. Cultivars of balansa clover, a highly waterlogging-tolerant pasture legume, were included as controls. Waterlogged conditions were simulated by growing the plants for 21 d in aerated solution culture followed by 14 d in stagnant (deoxygenated) agar. Plants were then returned to an aerated nutrient solution for a further 14 d to measure their rate of recovery from waterlogging. Control plants were grown in aerated nutrient solution for the duration of the experiment. The impact of waterlogging was assessed by measuring differences in root and shoot growth responses, root porosity and tissue potassium (K^+) concentrations.

3.1.7.3 Tolerance of serradellas to cold temperatures

A series of experiments were used to assess the cold tolerance of serradellas. Initial experiments in petri dishes and pots of soil were used to assess the effect of four temperature regimes (10/5, 15/10, 20/15 and 25/20; max/min °C) on the germination or emergence of a range of cultivars of French, yellow and slender serradellas. *Trifolium* and *Medicago* species were included as controls. Calendar and degree days to achieve 50% germination or seedlings emerged was assessed.
The relative growth rate of serradella cultivars was compared at "cool" and "ideal" growing temperatures. The anecdotal view that some serradella cultivars and, most notably, yellow serradellas grow slowly during cold winter months was tested. Growth assessments were benchmarked against a winter-active lucerne (cv. SARDI 10) and a subterranean clover also known for its good winter vigour (cv. Woogenellup). Plants were grown at 23/18°C for three weeks to allow establishment before a cold treatment (15/10°C) was applied. Control treatments continued to grow at 23/18°C. Shoot and root relative growth rates were assessed weekly until 8 weeks of growth. Variation in photosynthetic rates were also measured using the LI-6400 portable gas-exchange system (Li-COR, Lincoln, NE, USA).

Further details: Appendix 11

3.1.8 Output 5(e) - Describe the soil and climatic zones where P efficient serradellas could be utilised and any key traits to be developed in varieties for better adaptation to these new areas of use.

The areas across southern Australia in which serradellas are anticipated to be adapted were mapped. Initially, the climatic adaptation zone for serradella was defined using the predictions of Hill (1996). Experiences of producers, key consultants, researchers and published information was gathered and used to further refine the climatic (mainly rainfall) and edaphic (mainly soil pH) zones suitable for serradellas and the areas in which there is current "successful" use of serradella. The districts in which serradellas are currently used were superimposed on the potential adaptation zone to define areas into which serradellas use may potentially be expanded.

Knowledge gained in the project was used: (i) to identify potential "best-bet" cultivars from those that are available currently or are "near-market" for further testing in environments where serradellas are not presently used, and (ii) to define key phenological traits considered necessary to ensure serradella production and persistence in the permanent pasture area of south-eastern Australia where adoption of serradella pasture has been very slow.

Further details: Appendix 9

3.1.9 Output 6 (a) – Conduct field and glasshouse experiments to rank commercially-available subterranean clover cultivars for P-efficiency and to quantify the yield advantages of using them in pastures on moderately P-deficient soils.

A wide range of current, recent and "historic" cultivars of subterranean clover were screened for P-efficiency in a series of controlled environment experiments. Plants were grown in micro-swards with the spread of the leaf canopy constrained to the width of the pot by a reflective sleeve to approximate light conditions in a pasture sward. The cultivars were either sown with 50 mg of seed per pot (3 to 12 plants/pot depending upon seed mass), an approach originally intended to ensure fast canopy closure, or were sown at a common low plant density (3 plants/pot; 505 plants/m^2). Phosphorus was applied to a defined topsoil layer to mimic the stratification of P that occurs in the field with surface spreading of fertiliser. These conditions were designed to reflect the conditions experienced by plants during spring growth in pasture swards in the field. Plant growth was typically assessed at two levels of soil P availability: in a moderately P-deficient soil (where differences in growth rate among the genotypes was considered indicative of P-efficiency), and at a high level of soil P availability (sufficient for maximum yield). Plants were harvested after 5 weeks growth and shoot dry mass was determined. Cultivars were ranked for P-efficiency based on their shoot yield in the moderately P-deficient soil.

A subset of cultivars was selected to validate the P-efficiency rankings under field conditions. Cultivars were selected to represent the range in P-efficiency rankings, but was also determined by the availability of seed (i.e. sufficient seed for field experiments was only available for current and some recent cultivars). The experiment was sown in late winter/ early spring to allow the cultivars to be compared during vegetative growth under warm, moist (spring) conditions. This was intended to ensure that the confounding influence of maturity type on growth rate was avoided. Plants were grown at two P treatments (“intermediate” and “high” soil P availability). The experiment was attempted in three consecutive years (2017-19) but failed in all three years (despite irrigation in 2018 and 2019) due to the impacts of disease and persistent droughts.

Further details: Appendix 10

3.1.10 Output 6 (b) - Conduct experiments to identify the root traits and trait combinations that confer P-efficiency in subterranean clover and the diversity of these root traits to underpin identification of variation (genes) useful for plant improvement.

Cultivars of subterranean clover, lines of phylogenetically-related clover species and serradellas were grown using a range of methods to characterise how root morphology and root acclimation to soil P availability assisted P acquisition in low P soil. Plants were grown in controlled-environment experiments in either pots or root boxes in which P was applied to the “topsoil” of a low P soil “profile”. In a majority of the experiments, plants were grown as microswards in conditions that mimicked plant growth during spiring in the field (as described above). Various treatments were applied to understand the expression and relative value of root traits for P acquisition efficiency. The treatments included: soil P supply, canopy constraints, planting density and mycorrhizal colonisation.

Roots were harvested by gently washing from soil. Root length and root diameter were determined by scanning roots using a flatbed scanning and these traits were automatically analysed using the root analysis program WinRHIZO^{TM} (Regent Instruments, Canada). Roots were subsequently dried and specific root length (i.e. root length per unit root dry mass) was calculated. Roots were typically characterised in both the P-amended “topsoil” layer and the low-P “subsoil” layer to determine dry mass allocations to the P-enriched topsoil (i.e. the nutrient foraging roots). A subsample of roots was typically archived at 4^{o}C in 70% ethanol for microscopic analysis of RHL and, in some cases, mycorrhizal colonisation of the roots.

Extensive screening experiments were conducted to characterise the diversity of the root traits known to be associated with P-acquisition efficiency in subterranean clover. As part of this, the core collection of subterranean clovers (97 diverse lines estimated to represent approximately 80% of the genetic diversity of the species) and a set of 28 cultivars were screened for shoot yield and root traits in moderately P-deficient soil and with sufficient (i.e. surplus to requirement) soil-P availability in the micro-sward system. This data was contributed to the Genome Wide Association Study reported on in Output 6c.

Further details: Appendix 10

3.1.11 Output 6 (c) – Develop molecular tools for breeding nutrient-efficient subterranean clover cultivars.

This activity aimed to integrate genotypic and phenotypic root trait information on the subterranean clover core collection from this, and related projects to develop molecular markers for root phenotypic traits related to P uptake in subterranean clover. To achieve this, a 124-member diversity panel, including the 97 subterranean clover core collection lines and 27 of the set of 28 diverse Australian cultivars, was assembled. Whole-genome sequencing was then completed for all 124 lines/cultivars to develop a Haplotype Map (HapMap).

Data on the root traits of the same set of lines that had been generated in a number of previously reported experiments were compiled. A P-limited yield index was also calculated using combinations of shoot dry mass data from three of these experiments. A Genome-Wide Association Study (GWAS) was used to identify possible candidate genes for root traits and the P-limited yield index.

Further details: Appendix 10 and 11

3.1.12 Output 6 (d) – Examine the diversity of P-efficient root traits in species of *Trifolium* that are genetically-allied to subterranean clover and investigate the potential for introducing genes by inter-specific hybridisation.

Root traits of phylogenetically-allied clovers: The shoot yield, root morphology and root acclimation to P-stress of representative genotype of subterranean clover, French serradella and each of the seven *Trifolium* species from the *Trifolium* Section, *Tricosephalum* which also contains subterranean clover (Ellison *et al.* 1978) was determined in response to soil P. A few of these phylogenetically-allied clover species have been interspecifically-crossed to subterranean clover using traditional crosses (Katznelson 1967) and it was, therefore, anticipated that this assembly of genotypes may deliver a wider diversity in root traits and P efficiency than exists within the subterranean clover genome alone.

Plants were grown in a controlled-environment facility using the micro-sward system outlined above. Root morphology and acclimation traits were also characterised as outlined previously.

Subsequently, further assessment was made of the diversity in RHL among a wider range of accessions for two of the phyllogenetically-allied clover species; the plant growth system was similar to that reported previously.

A model to explore root trait interactions: An empirically-derived model was developed in Microsoft Excel to assess how P acquisition is influenced by different configurations of the root morphology traits that had been observed in subterranean clover and the allied *Trifolium* species. The model was parameterised using data for subterranean clover (cv. Leura) grown for 5 weeks under the micro-sward conditions described previously. The model therefore reflects the growth of plants under these strict experimental conditions and in the absence of arbuscular mycorrhizal fungi. The effect of changes in root length proliferation, specific root length and RHL on the effective surface area of the root for P acquisition was assessed in order to determine the magnitude of root traits that would be required to significantly improve the P-acquisition efficiency of subterranean clover.

Further details: Appendix 10

3.1.13 Output 7 (a) Review and revise P fertiliser rate calculations and spreadsheets for on-farm fertiliser management planning and update the “Five Easy Steps” P application tool; Output 7 (b) - Revise targets for soil P fertility management of pastures in the “Five Easy Steps” P application tool and Output 7 (c) - Release updated version of the “Five Easy Steps’ P management tool.

Desktop studies using existing data, new information in the scientific literature, consultations with users of the current P-management booklet and computer tool, some experiments to test the P tool calculations, and a large effort to publish the data on which the tool and its revisions are based, were used to review and revise the P fertiliser rates and targets for soil P fertility used in the “Five Easy Steps” tool. Feedback from consultants was used to improve the clarity of text and messages in the tool. The revised version of the “Five Easy Steps” was developed as an upgrade to the existing P-tool on the MLA website.

Further details: Appendix 10

4 Results and Discussion – (Activity B4) Lowering P costs of serradella systems

4.1 Results: Output 4(a) – Conduct participatory plot scale experiments in areas of WA and NSW where serradella is already grown to assess serradella production in response of P fertiliser application and quantify the critical P level required by the legume.

4.1.1 Site and seasonal conditions

All experiments experienced severe drought conditions for the duration of the project (Table 1). The sequential droughts in northern NSW were considered among the worst conditions in a century. The critical STP requirement of pasture is defined by growth in average to good spring conditions when temperatures and soil moisture are expected to be near-optimal for pasture growth (e.g. Gourley *et al.* 2019, Sandral *et al.* 2019). It cannot be determined in dry soil conditions because the water-filled path for diffusion of phosphate to roots becomes attenuated or broken in dry soil; this increases the apparent amount of P required for maximum growth rate.

Although all sites continued to be managed to enable the determination of the critical P requirement of the pastures, the on-going dry conditions dictated that the emphasis of data collection and analysis should shift to understanding how to better manage soil P for pasture production. This became an especially important objective for both of the WA sites where soil testing showed that the soils at these sites had extremely low Phosphorus Buffering Index values (Table 1).

4.1.2 Herbage yields

Essentially equivalent herbage yields were achieved by French serradella (cv. Margurita), yellow serradella (cv. Avila) and subterranean clover (cv. Seaton Park) at both WA sites, except at Grass Patch in 2017, when the subterranean clover failed to establish as a result of a prolonged dry period immediately after sowing. In contrast, both serradellas were able to establish despite the dry conditions and went on to yield 3-4 t DM/ha.

In general, the yields of the yellow and French serradellas growing in the deep sandy soils at the WA sites were low reflecting the dry seasonal conditions. Yields were also not significantly improved by application of P (Fig. 4.1 - 1). This could potentially have been a result of poor growth in dry conditions, but the lack of response to P application was observed even in seasons where more reasonable pasture growth (e.g. >3 t DM/ha) was possible. Subterranean clover was also generally unresponsive to application of P, except in 2018 at Katanning and Neridup. However, in both instances the yield of subterranean clover was still increasing at STP concentrations of 75 and 100 mg Colwell P/kg soil. This is well above the expected critical P requirement of subterranean clover pasture on low PBI soils (~15 mg Colwell P/kg; Gourley *et al.* 2019).

In northern NSW, pastures either failed to establish or established and failed to grow in the driest growing seasons (2017 Purlewaugh; 2018 Spring Ridge) and there was no spring pasture to harvest (Fig. 4.1 – 2). In seasons with enough moisture for some pasture growth, subterranean clover grew relatively poorly compared to that of the serradellas. French serradella (cv. Margurita) often yielded

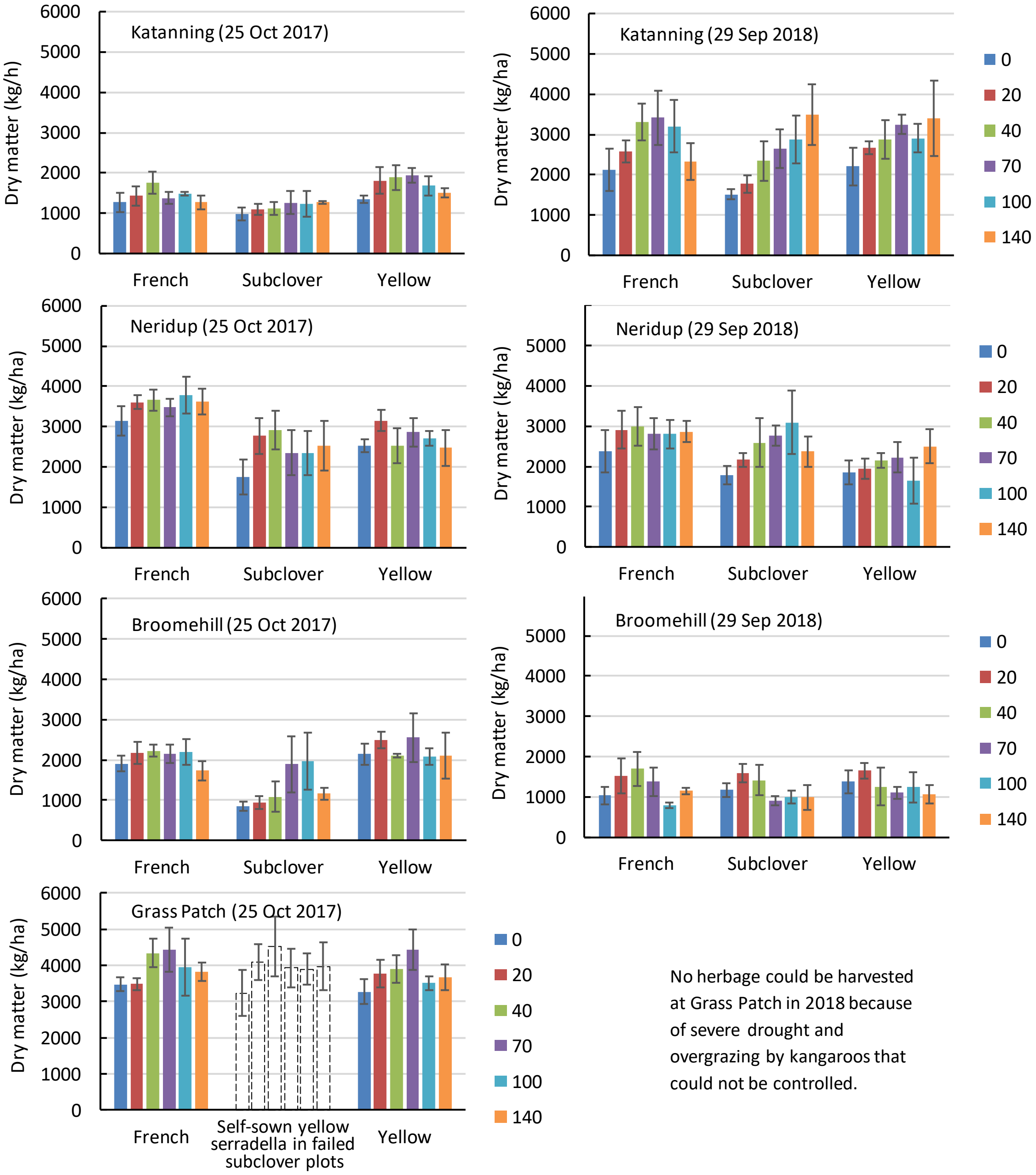
Table 4.1 - 1. Brief site details and growing season conditions for the P response experiments in WA and northern NSW.

Location (associated farm group)	Soil description	Species and cultivar sown	Prevailing weather conditions	Phosphorus Buffering Index (0-10 cm)[#]	Topsoil (0-10 cm) STP range resulting from P treatments. (mg Colwell P/mg soil)*
Katanning, WA (Southern Dirt)	Deep grey sand Topsoil pH_{Ca} 4.8	Subterranean clover (cv. Seaton Park) Yellow serradella (cv. Avila) French serradella (cv. Margurita)	Below average growing season rainfall (May-Oct): 267, 248, 222 mm, ex totals of 406, 352, 299 for 2017, 2018, 2019, respectively.	19	10 – 100
Broomehill, WA (Southern Dirt)	Non-wetting deep grey sand Topsoil pH_{Ca} 5.1		Below average growing season rainfall (May-Oct): 242, 256, 205 mm, ex totals of 439, 320, 258 for 2017, 2018, 2019, respectively.	14	10 – 50
Grass Patch, WA (ASHEEP)	Deep non-wetting sand Topsoil pH_{Ca} 5.2		Below average growing season rainfall (May-Oct): 193, 155, 156 mm, ex totals of 422, 375, 194 for 2017, 2018, 2019, respectively.	5	12 – 55
Neridup, WA (ASHEEP)	Deep sand, clayed in 2015 Topsoil pH_{Ca} 4.6		Below average growing season rainfall (May-Oct): 294, 289, 252 mm, ex totals of 601, 447, 295 for 2017, 2018, 2019, respectively.	13	5 – 100
Purlewaugh (via Coonabarabran), NSW (Purlewaugh NSW Farmers)	Brown chromosol Topsoil pH_{Ca} 4.3	Subterranean clover (cv. Dalkeith) Yellow serradella (cv. King) French serradella (cv. Margurita)	Well below average (51-64% of long-term average total rainfall received). Growing season rainfall (May-Sep): 18, 80 and 78 mm for 2017, 2018, 2019, respectively.	16**	17 – 50
Spring Ridge (via Gunnedah), NSW (Boggabri Grazing Group)	Brown chromosol Topsoil pH_{Ca} 5.1		Well below average (59-83% of long-term average total rainfall received). Growing season rainfall (May-Sep): 84, 59 and 68 mm for 2017, 2018, 2019, respectively.	52	11 – 48

[#] Burkitt *et al.* (2008)

* Colwell (1963)

**PBI value of 33 also measured in initial sample from this site



No herbage could be harvested at Grass Patch in 2018 because of severe drought and overgrazing by kangaroos that could not be controlled.

Figure 4.1 - 1. Herbage dry matter (kg/ha) harvested in spring 2017 and 2018 from three pasture species treatments (French serradella cv. Margurita, subterranean clover cv. Seaton Park, yellow serradella cv. Avila) grown with 6 rates of triple superphosphate application at the WA P-response experiment sites. Differences in growth periods and seasonal conditions do not permit direct yield comparisons between years. At Grass Patch in 2017, there was very little rainfall until 10 weeks after sowing and subterranean clover failed to establish. The yields shown (dashed lines) were for yellow serradella that subsequently volunteered from the seed bank. Significant (*P*<0.05) yield responses to P application were only observed for subterranean clover in 2018 at Katanning and Neridup.

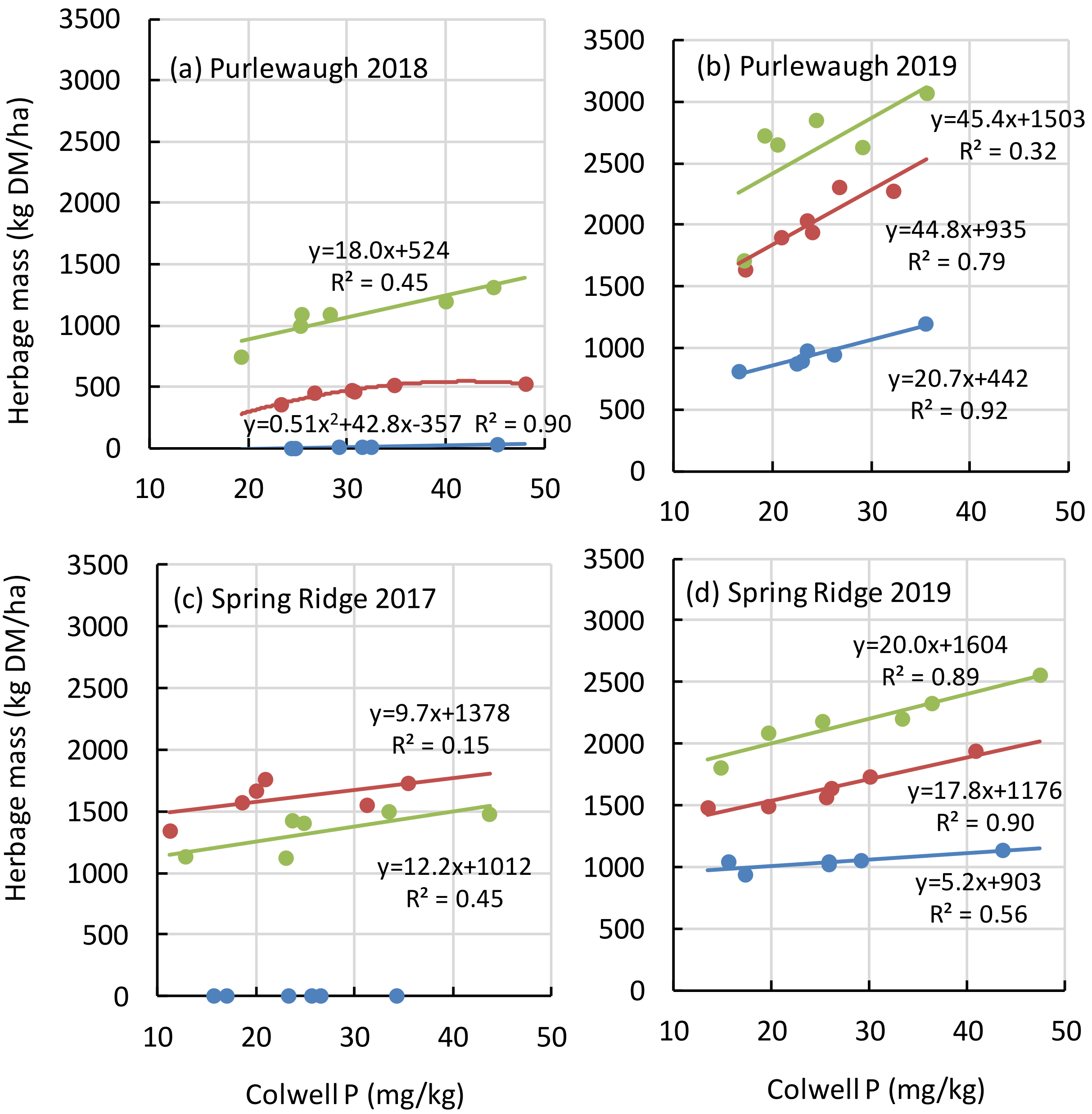


Figure 4.1 - 2. Peak spring herbage production of subterranean clover cv. Dalkeith (●), yellow serradella cv. King (●) and French serradella cv. Margurita (●) in 2017, 2018 and/or 2019 at Purlewaugh (a, b) and Spring Ridge (c, d) with increasing levels of soil P (Colwell P, 0-10 cm). There was insufficient herbage for harvesting in the years where no results are shown.

20-25% more herbage than yellow serradella (cv. King), except at Spring Ridge in 2017 when their herbage yields were comparable.

In the years where pasture growth was observed, spring pasture yields were improved by (typically) up to ~50% with high rates of P application (one exception). Yield increases in response to P were linear with little evidence (one possible exception) that a critical level of P for maximum yield had been achieved. Indeed, the herbage yields were increased by STP concentrations that were well above the expected critical P requirements for maximum yields of serradellas and of subterranean clover (Sandral *et al.* 2019).

Despite the adverse impact of dry soils on P uptake by the legumes, the rate of yield increase per increase in Colwell P (i.e. the gradient of the yield-STP relationships) is indicative of the relative P efficiency of the alternative legumes. In 2019, when subterranean clover did not fail to establish and grow, the gradients of the serradella yield-STP relationships were always greater (~≥2-fold higher) than that of the subterranean clover. This suggests that the serradellas were able to grow at least twice the rate of the subterranean clover in the P-deficient soil.

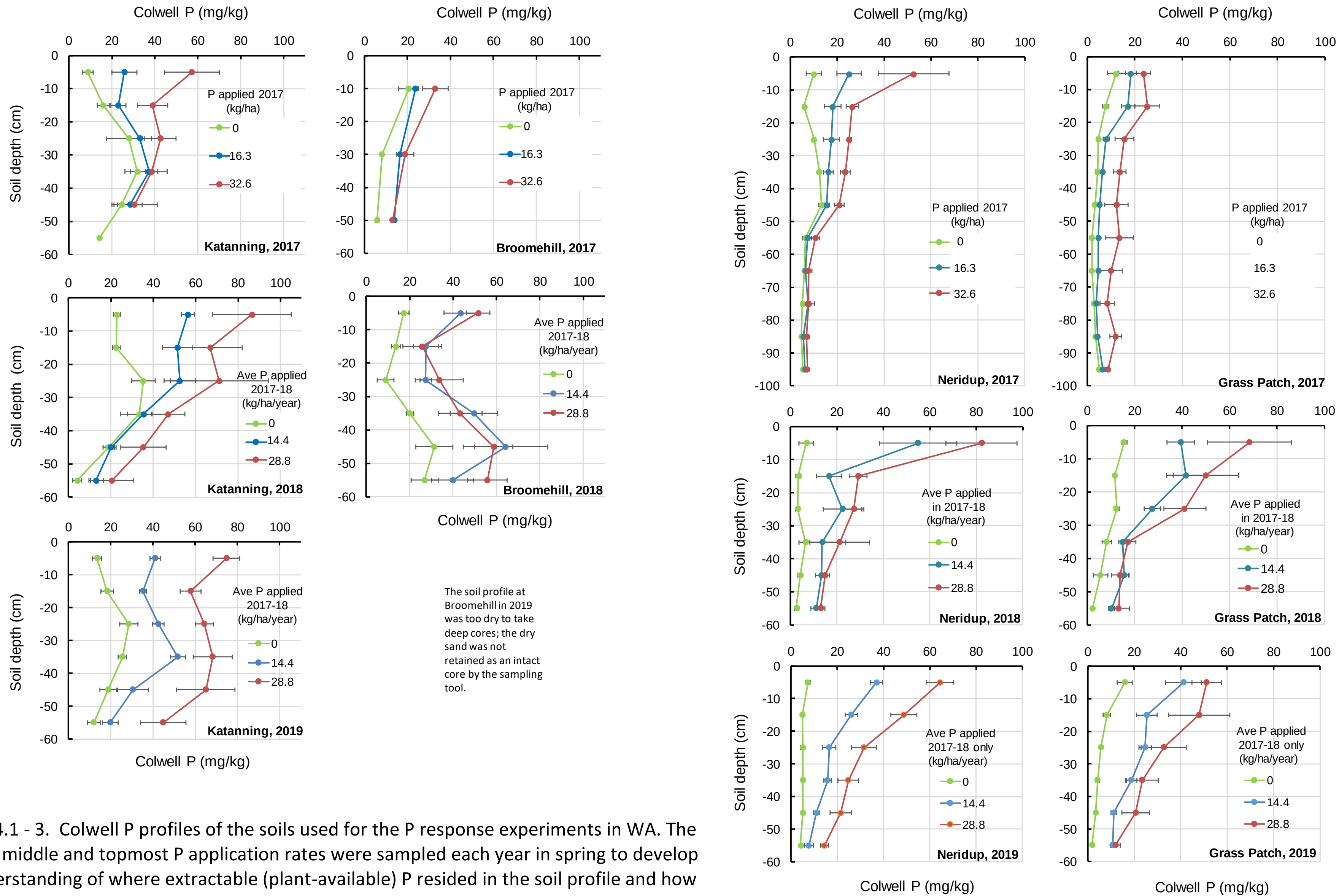
4.1.3 Soil test P profiles

The first series of soil test results indicated that the topsoil (0-10 cm) PBI results were extremely low (PBI = 1-12) and low (PBI = 30-52) at the experiment sites in WA and Northern NSW, respectively. This immediately invoked a series of previously unplanned, deep soil-core assessments of the plant available P profiles of soil at sites in each district because very low PBI results (e.g. <35) can be indicative of soils that may allow P to leach to depth in the soil profile. The initial investigations were conducted at the farm-group participatory experiments in northern NSW which, although not adjacent to the P-response experiments had returned similar PBI results, and in key P treatments of the P-response experiments in WA which were adjacent to the farm-group participatory experiments.

The Colwell P profiles from all WA sites indicated very clearly that P applied to the surface of the soil moved quickly to depth in the soil profile (Figs. 4.1 - 3). The topsoil Colwell P concentration of the unfertilised soil profiles was often similar to, or less than the Colwell P concentration of subsoil layers indicating that these soils can contain “legacy” P (i.e. P that had accumulated at depth as a result of P leaching from the upper soil layers with or without P fertiliser application). This was most clearly demonstrated by the Colwell P profile at Katanning which contained a clear bulge of available P at 20-50 cm depth. The subsoil reserve of plant-available P was always accentuated when P fertiliser was applied because P moved quickly into all of these soil profiles. In some cases (e.g. at Grass Patch), P moved very deep (~90 cm) within the soil profile and may even have moved below a metre depth within 6 months after it had been applied a fertiliser.

In contrast, the Colwell P profiles to one metre depth at the Spring Ridge (PBI 30) and Purlewaugh (PBI 33) farm group participatory research sites in northern NSW gave no indication of P movement below ~20 cm depth (see Figs 4.3 - 2 and 4.5 - 1).

In the revised “Five Easy Steps” P management tool, it is now recommended that farmers seek further local advice, check whether P from previous fertiliser applications has moved to depth in the soil profile and to consider the merits of using a less soluble form of P fertiliser when the PBI of the soil is <35 as there is an increased risk that P may be lost in runoff and/or leaching.



The soil profile at Broomehill in 2019 was too dry to take deep cores; the dry sand was not retained as an intact core by the sampling tool.

Figure 4.1 - 3. Colwell P profiles of the soils used for the P response experiments in WA. The lowest, middle and topmost P application rates were sampled each year in spring to develop an understanding of where extractable (plant-available) P resided in the soil profile and how P fertiliser applications impacted the extractable-P profile of the soil.

4.2 Discussion: Output 4(a) – Conduct participatory plot scale experiments in areas of WA and NSW where serradella is already grown to assess serradella production in response of P fertiliser application and quantify the critical P level required by the legume.

4.2.1 Relative yields of the serradellas and subterranean clover

At these sites, which represent areas where serradella is already widely used, the cultivars of French and yellow serradella generally yielded as well (WA), or better (northern NSW) than subterranean clover across the range of dry to very-dry growing seasons under which they were tested. In northern NSW, yellow serradella has now been promoted over many years as a viable alternative to subterranean clover which underperforms in the light-textured, highly acidic soils of this area (Freebairn 1996). It is of interest that the French serradella cv. Margurita often yielded marginally better that yellow serradella cv. King which is the preferred serradella for this area. Older, soft-seeded French serradellas were examined previously in this district but were not considered viable. Margurita is among the first of the new hardseeded French serradellas (Nutt 2004a; 2004b) and these yield results may indicate that further testing of the newer hardseeded varieties of French serradella should be considered with the objective of examining their yield and persistence relative to yellow serradella varieties under average to good seasonal conditions. If proven to be a viable alternative, the option of French serradella would assist growers in this region to diversify their pasture base with another P-efficient legume species.

It was notable that when soil moisture conditions deteriorated after sowing (e.g. Grass Patch and Spring Ridge 2017, Purlewaugh 2018) that both French and yellow serradellas were able to establish and remain viable under moisture deficits that caused subterranean clover to fail. In each case, this ability to "hang-on" resulted in useful amounts of herbage when rain eventually fell, compared to no herbage availability in the subterranean clover plots. Similar observations were made in farm group participatory experiments and serradella persistence experiments in tableland areas of southern NSW (**see Section 6**).

4.2.2 Response to applications of P

The original objective of these experiments was to determine the critical STP concentrations for serradellas and subterranean clovers growing in soils where serradellas are widely used. This was important because:

(i) yellow serradella had been shown on more than one occasion to have a significantly lower P-fertiliser requirement than subterranean clover in sandy WA soils (e.g. Paynter 1990, Bolland and Paynter 1992). However, this had not led to changes in fertiliser practice because the lower critical STP level for the serradella was never determined.

(ii) it was considered necessary to verify the recent specification of serradella critical STP requirement (Sandral *et al.* 2019) because it had been determined for clay- and sandy-loam soils in areas where serradella is not used.

(iii) current advice in northern NSW suggested a critical STP for serradella that was at least twice the recent determination of critical STP for serradella (Freebairn 2013).

The drought conditions that persisted over all three years of these experiments are unsuitable for, and do not permit analysis of "benchmark" critical P requirements of any plant species. Benchmark critical P requirements are defined under moist spring conditions suitable for near maximum pasture growth (e.g. Sandral *et al.* 2019). Nevertheless, the contrasting yield responses of the legumes to P addition observed at the WA and northern NSW sites have provided novel insights into the P management of serradella pastures that can lead to improved efficiency in P fertiliser use.

4.2.2.1 Pasture growth in response to P application at WA sites

The serradella and subterranean clover pastures at all four WA sites were unresponsive to P application, in most instances. Possible exceptions were subterranean clover pastures at Katanning and Neridup in 2018 where clover yield appeared to increase with increased P application up to STP concentrations that were well in excess of the published benchmark critical STP value for subterranean clover. This is analogous to the linear response to P observed in dry soils in northern NSW. As discussed above, dry soil restricts the availability of P in soil. Nevertheless, both serradella varieties in these experiments continued to be unresponsive to P application. It is highly likely that this observation is a further reflection of (i) the greater ability of serradella to extract P from low P soil and (ii) soil P supply (irrespective of P fertiliser application) that exceeded the P requirement of serradella for maximum growth.

The soil P profiles consistently demonstrated that lack of response to P application at the WA sites was associated with legacy P at depth. This was a common feature of these deep sandy and very-low PBI soils (PBI <12) because P moved readily to depth when it was applied as fertiliser to the soil surface. Some soils (e.g. at Katanning) clearly contained a large reserve of P at depth even before P fertiliser was applied. The reserves of plant-available P within soil profiles were not detected by traditional surface-soil (0-10 cm) testing.

4.2.2.2 Pasture growth in response to P application in northern NSW

Drought severely constrained the yield of pasture at all northern NSW locations. The extent of the constraint ranged from no germination or pasture failure in some years to very modest moisture-limited production in others. When there was sufficient soil moisture for all three of the legumes to establish and grow (e.g. 2019), the responses to P application were linear and a critical P supply for maximum growth was not achieved for any of the species.

Phosphate is supplied to roots predominantly by diffusion in soil solution. Diffusion of phosphate can be described by Fick's Law:

$$J = -D * A * dC/dX$$

where: J is the diffusive flux (µmol/s), A is the area of the root-soil interface through which P is taken up (cm^2), D is the isothermic diffusion coefficient which is typically determined by soil chemical and physical properties (cm^2/s), C is the phosphate concentration in the soil solution of the bulk soil ($\mu mol/cm^3$), X is the length of the diffusion path and dC/dX is the concentration gradient that is created between the root-soil interface and the bulk soil when the concentration of phosphate at the root-soil interface is depleted as P is taken up by the root.

The rate of phosphate diffusion in soil is very low compared to other nutrients (e.g. nitrate or potassium) and is rate limiting for P acquisition from soils up to the point where soil P fertility is high enough for maximum growth rates to be achieved (Barber, 1995; Tinker and Nye, 2000). Consequently, successful agronomic and plant-based strategies to increase P acquisition by plants are guided by the key principles embodied in the diffusion equation.

These principles are that the rate of P diffusion to a root is directly proportional to the P concentration gradient between the bulk soil and the absorptive surface of the root system, which is determined by:

(i) X - the distance over which P diffusion occurs. In dry soil the amount of soil solution is reduced and the water-filled path for P diffusion becomes longer and can be broken completely as the soil

dries further. This initially causes a "green drought" because plant growth slows down because P diffusion is slowed, and P becomes harder to source. Ultimately, however, drought takes over and moisture becomes the most limiting resource.
(ii) ***C*** - the concentration of phosphate in the soil solution. In a P-deficient soil this is increased by applying P fertiliser. Applications of P fertiliser increase pasture growth by increasing ***C***, and, consequently, the concentration gradient (*d**C**/d**X***). Fertiliser applications that increase ***C*** will also counteract the negative impact of a drying soil (longer ***X***) on P diffusion to roots.

The impacts of dry soil conditions on pasture growth and P nutrition were very clearly shown by the yield responses of all three legumes to the increase in STP achieved by P fertiliser application at the northern NSW sites, particularly in 2019. A linear increase in moisture-limited yield in response to P fertiliser addition (i.e. increased Colwell P concentrations of the topsoil) reflects very closely the restrictions to P supply to pasture caused by a drying soil (e.g. Mariotte *et al.* 2020) as is described by Ficks' Law.

A third principle arising from the application of Fick's Law is that plant root systems with a large absorptive surface area for P acquisition can access more of the P diffusing to the roots when growing in low P soil. Indeed, the high P-acquisition efficiency of the serradellas when compared with subterranean clover is due to their high specific root length (large root length per unit root mass; Yang *et al.* 2017) and very long root hairs (2 to 3-fold longer than the root hairs of clover; Yang *et al.* 2017) which allow them to deploy a substantially larger root-soil interface for P uptake (Haling *et al.* 2016a, Haling *et al.* 2016b, Sandral *et al.* 2018).

The 'yield - soil test P' relationships at the northern NSW sites in 2019 (Fig. 4.1 - 2) allow a relative assessment of the P efficiency of French serradella, yellow serradella and subterranean clover in the field. In most instances, the yield and STP concentrations were linearly related and did not reach an asymptote indicating that all three legume species were growing with deficient P supply irrespective of P application level. This was deduced to be caused by dry soil conditions. The gradients of the relationships are a measure of P efficiency (yield per unit Colwell P). The data suggest that the P-efficiencies of the serradella species were similar to or greater than that of subterranean clover.

Differences in P efficiency in P-deficient soil is often a predictive indicator of the critical P ranking of pasture legumes (e.g. Sandral *et al.* 2018). On this basis, it is expected that these data indicate that the critical P requirement of subterranean clover will prove to be higher than that of the serradellas in these locations where serradella are widely used. This provides indirect evidence that serradellas can be fertilised to a lower critical STP benchmark than recommended for subterranean clover pastures (i.e. partially achieving the initial objective of these experiments to confirm the critical STP requirements of the legumes in these agroecosystems).

4.2.3 The implications of having legacy P at depth in soil profiles with low Phosphorus Buffering Indices

Legacy reserves of plant-available P (Colwell P) were observed in all WA soil profiles and especially after P had been applied as fertiliser to the soil surface. This has significant implications for the management and efficiency of P-fertiliser use in these soils:

(i) ***traditional topsoil (0-10 cm) testing does not account adequately for P in these soil profiles***.

The principle that underpins the use of topsoil testing is that P is usually relatively concentrated in the topsoil layer under a pasture. This occurs because soils under pasture are subject to only minimal disturbance, P in excreta and leaf-litter is deposited on the soil surface and most soils have sufficient P buffering capacity to prevent movement of P from the surface layers to depth. The "natural" surface P band is further enhanced by broadcasting soluble P fertiliser onto the soil surface in granular form. Phosphate moves rapidly from the granule into the soil (laterally and vertically) by diffusion forming a P enriched hemisphere below the granule (Benbi and Gilkes, 1987, Hedley and McLaughlin, 2005). The extent and speed of P movement into the soil is influenced by the P buffering capacity of the soil (Benbi and Gilkes, 1987). Empirical evidence indicates that ~70-80% of the increase in plant-available P after fertiliser application occurs in the topmost 10 cm of most soil profiles. The remaining impact is usually measured in the 10-20 cm layer of the topsoil, even after a century of fertiliser use (Simpson *et al.* 2015, Schefe *et al.* 2015). In this way, surface soil testing (0-10 cm) directly measures ~70-80% of the P reserve that is modified by fertiliser use.

This principle cannot be met in soils with very low P buffering capacities, such as those at the WA sites, because P moves readily from the topsoil layer to depth. An alternative way of assessing the availability of P in the root zone of low PBI soil profiles is required.

In this project, soil sampling in the farm group participatory experiments to 60 cm depth was commenced as soon as the issue of legacy P in soil profiles was identified. Results from these deep soil sampling activities are outlined in **Section 4.3**.

(ii) ***legacy P is a reserve that can be tapped to improve the efficiency of P fertiliser use***.

The Colwell P profiles of soil under pasture at all WA sites demonstrated that the amounts of P present at depths down to, and sometimes below 60 cm were substantial and often exceeded, by many-fold, the P available in the surface soil. Soils that were identified originally (using standard soil test procedures) as P deficient were found to be unresponsive to P fertiliser applications presumably because there was more than enough P at depth for serradella growth.

The clear implication from these results is that the existence of legacy P needs to be recognised, accounted for, and utilised in pasture production in these areas. Because P applied to the soil surface will continue to move into these soil profiles, this will lead to immediate and on-going improvements in the efficiency of P fertiliser use.

It is well-established that serradella develops much deeper roots (e.g. 1.5 m depth) than subterranean clovers (e.g. 0.45-0.8 m depth, Ozanne *et al.* 1965) in deep sandy soils. We conclude that they are ideal legumes species for utilising legacy P at depth in deep sands soils and are a better option in this regard than relatively shallow-rooted subterranean clovers. It has been proposed previously that the deep rooting characteristic of serradella assists it to also resist potassium deficiency because it can acquire potassium leached to depth in sandy soil profiles (Ozanne *et al.* 1965)

(iii) ***High P mobility and legacy P at depth in low PBI soils present potential environmental risks.***

The very high P mobility and movement of P to depth that was measured at the WA sites in dry seasons also leads to the unavoidable conclusion that these soil profiles must also pose a high risk for leakage of P to the wider environment. Issues such as this are already documented for natural resources with high environmental value (e.g. Summers and Weaver 2006). Recognising that legacy P exists in soil profiles, assessing the amounts that are available and actively budgeting for deep soil P use as part of a fertiliser program should lead to more efficient use of P fertilisers with the added advantage of reducing the potential risk of P loss from farms to waterways and other environmentally sensitive environments.

4.3 Results: Output 4(b) – Conduct participatory paddock scale experiments with farmer groups in areas of WA and NSW where serradella is already grown to improve knowledge of the P-fertiliser and general management of serradella-based pasture systems.

4.3.1 Western Australian sites

Over the course of these experiments, seasonal conditions were characterised by late breaks with dry starts, and drier than average growing seasons. The conditions were not conducive to testing whether soils fertilised to the expected critical P requirement for serradella were returning maximum pasture yields. Nevertheless, several observations from data collected across the sites pointed to soil P fertility issues that were not widely appreciated and could lead to improved P fertiliser management:

(i) The first soil test results indicated very low PBI in the surface soils at all sites; the soil profiles were typically sandy in the surface and to depth. This immediately alerted the team to the potential for P leaching within the soil profile.
(ii) All sites had been selected as P deficient, on the basis of surface soil tests (0-10 cm depth) as per standard practice, however soil P levels (0-10 cm) were not increased after P fertiliser application, and
(iii) Pasture growth was typically not responsive to P fertiliser application.

The farm groups were encouraged to conduct additional deep soil sampling at depth intervals of 10 cm, or as a single 0-60 cm deep soil core, to determine the P status of the soil profile. Unfortunately, the soil P profiles under treatments that had been fertilised at farm-relevant P rates did not always provide a conclusive understanding of the issues in these soils (e.g. Fig. 4.3 - 1). However, adjacent deep soil sampling in the "P response" experiments (Fig. 4.1 - 3), where larger (experimental) amounts of P fertiliser were being applied, showed conclusively that: (a) P applied at the surface moved to depth very rapidly, and (b) that some soils had reserves of "legacy" P at depth within the root zone of the pasture.

The deep soil P profiles from the "P response" experiments indicated that soil coring to 60 cm depth would often account for much of the P that moved into the soil profile after P fertiliser had been applied. Deep cores (0-60 cm) taken in 2018 and 2019 at Katanning and Broomehill were analysed for Colwell extractable P. It was anticipated that pasture yield responses to Colwell P in these soil samples may provide clues to how these soils should be sampled to get a better indication of their "true" P fertility. However, severe droughts prevented sensible analysis of pasture responses.

The Colwell P contents of the deep cores were instead compared with the amounts of Colwell P sampled using the standard (0-10 cm depth) soil sampling method. The soils at these sites are uniform deep sands, so it was assumed that the bulk density of the topsoil and at depth was similar. The ranges in the Colwell P concentrations of each soil layer were not large regardless of P treatment, so the assessment was made using average Colwell P results across all P treatments and years (i.e. 2018 and 2019 - the years in which 0-10 cm and 0-60 cm depth samples were taken). The results were surprisingly similar for both sites, with about 7-fold more Colwell P (i.e. plant-available P) accounted for by measuring the soil profile to a depth of 60 cm instead of using a standard soil P testing depth (0-10 cm) (Table 4.3 – 1).

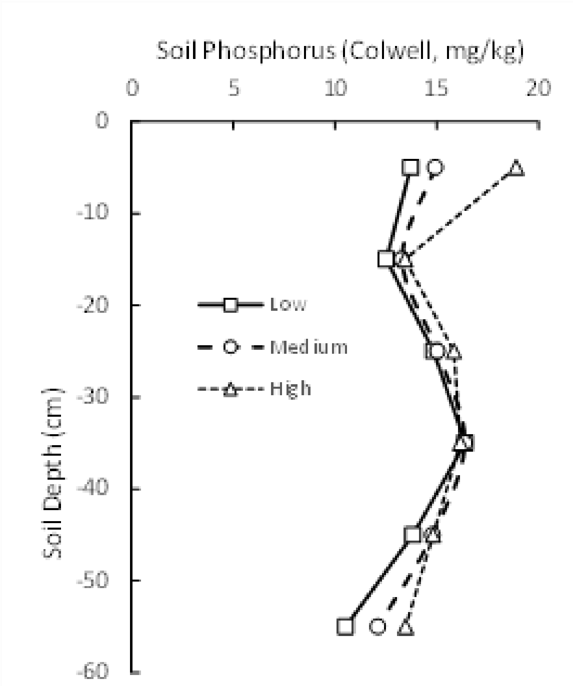


Figure 4.3 - 1. Colwell P profiles (mg/kg soil) under the low, medium and high P treatments at Neridup in December 2017 (ASHEEP participatory site). There were no significant differences for P treatment or depth nor any significant interaction between the two ($P < 0.05$). However, when larger amounts of P were applied (see Fig. 4.1 – 3), it was clear the P moved readily to depth in this soil.

Table 4.3 - 1. Relative size of the legacy reserve of available P found to depth in soils at Broomehill and Katanning (Southern Dirt participatory sites). The calculations assume the bulk density of both soil layers was 1.6.

	Broomehill	**Katanning**
Average amount of Colwell in the topsoil (0-10 cm depth) (averaged over 2018 and 2019, all P levels)	76 kg Colwell P/ha	220 kg Colwell P/ha
Average amount of Colwell in the profile (0-60 cm depth) (averaged over 2018 and 2019, all P levels)	523	1,524 kg Colwell P/ha
Relative amount of extractable P held in the soil profile (0-60 cm) compared to the topsoil (0-10 cm)	**6.9-fold more available P** accounted for when taking a deep soil sample	**6.9-fold more available P** accounted for when taking a deep soil sample

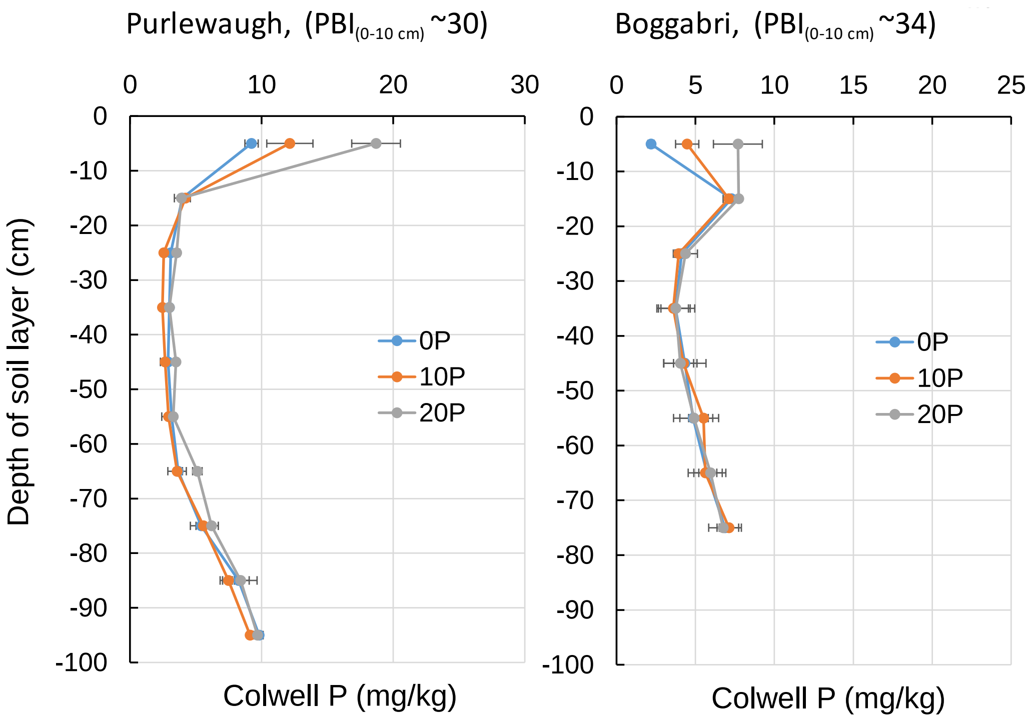


Figure 4.3 - 2. Colwell P profiles to depth in the three soil P treatments (spring 2017) at the Purlewaugh (PBI = 30) and Boggabri (PBI = 34) farm-group participatory experiment sites. Despite the low PBI values for the topsoils (0-10 cm) at these sites, there was no evidence of P movement to depth below 20 cm in these soils. It is unclear why there was a "bubble" of Colwell P in the 15-20 cm layer at Boggabri. This may conceivably be associated with previous cultivation of fertilised soil for cropping (however, this was known to have not occurred in the last 30 years with the current owner) or may be a result of the low topsoil PBI. However, there was no evidence of movement of P as a result of applying fertiliser in the 20P treatment.

4.3.2 Northern NSW sites

As was the case in WA, rainfall in all three years of the experiments was well below average, with poor autumn, winter and spring conditions. Rainfall over all three years was ~60% of average annual rainfall. Pasture yields in spring reflected these conditions and were very low (i.e. never greater than 1.1 t DM/ha).

The soils in the northern NSW regions where serradellas are used are also light, sandy soils. The topsoil PBI at the Purlewaugh and Boggabri participatory sites were low enough (i.e. PBI <35) to trigger a check of the soil P profiles. In contrast to the "leaky" soil P profiles found in WA, the Colwell P profiles of the northern NSW soils did not show any indications that P was moving to depth after it had been applied as fertiliser. There was an unusual Colwell P spike at 15-20 cm depth in the Boggabri profile which was the subject of much speculation. It may have been a result of previous cultivations for cropping, or movement of P from the low PBI topsoil layer that was caught by a higher PBI layer at this depth. However, there was no evidence of movement of P below 20 cm depth as a result of applying fertiliser in the 20P treatment. It was concluded that standard surface soil testing protocols were relevant for these soils despite their light texture. The farm groups achieved good soil P management outcomes over the experimental period by using soil test data to guide P inputs to achieve the target soil P fertility levels designated in these experiments.

The prevailing drought conditions and extremely low spring pasture yields were not conducive to testing whether soils fertilised to the expected critical P requirement for serradella pasture were returning maximum yields on these soil types. However, it was demonstrated that seedling establishment and pasture yields responded to P fertiliser applications at the intermediate treatment levels and were not further improved by higher inputs of P.

The farm groups concluded that serradella can perform at near maximum capacity at soil P levels of about 20 mg/kg (Colwell P), which is lower than the levels required for subterranean clover (+30 mg/kg P). Past soil fertility recommendations in these areas have advocated higher Colwell P levels (e.g. 40 mg P/kg). However, given that these results were obtained under extremely adverse growing conditions, the conclusions must be regarded as “preliminary”. Further work in years with average to good spring growth conditions is necessary.

4.4 Discussion: Output 4(b) – Conduct participatory paddock scale experiments with farmer groups in areas of WA and NSW where serradella is already grown to improve knowledge of the P-fertiliser and general management of serradella-based pasture systems.

4.4.1 Management of P in soils prone to P leaching

The participatory research experiments conducted in WA reinforced the need to check where P is present in soil profiles when surface soil PBI results indicate that the soil has a low P retention capacity that may allow P leaching to depth. In the revised “Five Easy Steps” P management tool a threshold of PBI <35 is now recommended as a trigger for such an assessment (**Section 11; Appendix 10**). The deep sandy soils in WA were universally “leaky” with respect to P and it is anticipated, based on other soil test data collected in the experiments, that they are also problematic for retention of other nutrients (e.g. S, K).

The soil P profile of these soils indicated:
(i) Standard surface soil (0-10 cm depth) soil testing provided an inadequate and misleading assessment of the P status of the soil for pasture production.
(ii) That rapid loss of P occurred from the topsoil after application of P fertiliser with movement of P to depth in the soil, and in one case, below the measurement depth of ~1 m).
(iii) The presence of a “legacy” (accumulated) reserve of plant-available P in some soils that was presumed to have arisen from previous fertiliser applications.

There are important soil P management, P efficiency, and environmental implications arising from these observations:

- There is a need for alternative soil sampling/testing protocols for use in soil types with low P retention. Current surface soil sampling protocols are potentially very misleading. More routine assessments of soil P profiles and “deep” soil sampling to account for more of the P that is available within soil P profiles seem obvious. However, the logistic problems and costs of sampling soils in this way are large and will be major impediments.

- Critical P requirements of pastures based on surface soil testing alone will also be inadequate and misleading when P has moved to depth in a soil profile. The critical STP benchmarks for “clover-based” pastures grown on soil with very low PBI have been revised recently (Gourley *et al.* 2019). However, these benchmarks will only be reliable when a soil profile does not contain legacy P. All research examining P benchmarks for crop and pasture production on very low PBI soils must be assessed in relation to the presence or absence of legacy P reserves within the root zone of the target species; critical P benchmarks assessed in the absence of this information will be uninterpretable.

- The true “availability” of STP at depth in these soil profiles needs to be understood. This is likely to be influence by (i) the buffering capacity of the soil, (ii) P accumulation (P-fixation) potential of the soil, and (iii) may also differ depending on the rooting depth of the crop or pasture being grown.

- The very deep rooting nature of serradella (as opposed to subterranean clover) in deep sandy soils (Ozanne *et al.* 1965, Hamblin and Hamblin 1985, Sandral *et al.* 2006) is presumed to be favourable for better utilisation of “leaking” and/or “legacy” P in these soil profiles.

- The identification and use of legacy P in very low PBI soils will lead to improved efficiency in P fertiliser use and reduced potential for damaging loss of P (via leaching) to waterways. However, new protocols and guidelines on: (i) how to account for deep soil P in P fertiliser calculations and (ii) the critical threshold value of deep P reserves are required.

4.4.2 Management of soil P fertility for serradella pasture production

It cannot be assumed that soils with PBI below this threshold will automatically leak P to depth. For example, the participatory research experiments in northern NSW were also located on soils with low PBI but proved to retain P in their topsoil (<20 cm depth).

Standard STP protocols were appropriate and were used successfully to guide soil P management in Northern NSW and despite years of very low rainfall, serradella persisted well enough to provide evidence of improved P efficiency. The participatory experiments improved farmer confidence that serradella pastures have a lower critical P requirement than pastures based on subterranean clover. Previously, it has been recommended that a critical STP threshold even higher than that of subterranean clover should be pursued (Freebairn 2013), so the change in perception is very significant.

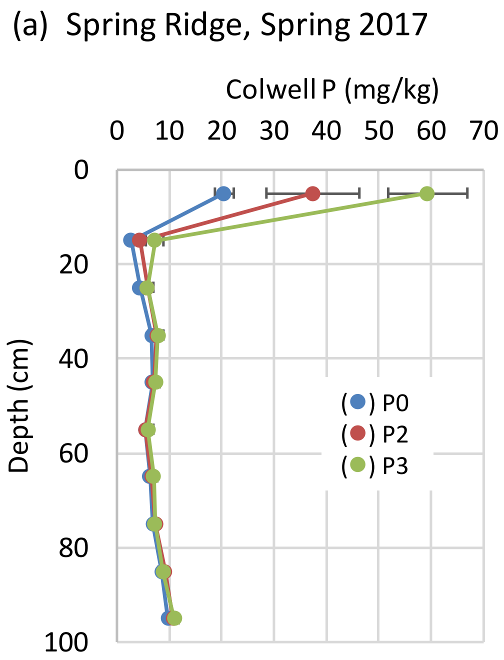
However, we contend that perceptions developed under the extreme drought conditions that prevailed during this research, are not proof. Very recent (post-drought, 2020 season) observations of the recovery of serradella on these experiments indicates that there is an on-going opportunity to test these perceptions and to achieve a real change in soil P management for serradella pastures in this area.

4.5 Results: Output 4(c) – Development of guidelines for managing soil P fertility in crop-pasture transitions involving serradella pastures.

4.5.1 Rate of P rundown when fertiliser is withheld.

4.5.1.1 Soil P profiles

Soil P profiles were assessed at P rundown sites (Fig. 4.5 - 1) and at sites on similar soil types used for farm group participatory research (Fig. 4.3 – 2). Soils at a number of these sites were light in texture and had PBI values at the margin for P movement in soil profiles (e.g. PBI <~35). In every case,


(a) Spring Ridge, Spring 2017

(b) Spring 2019

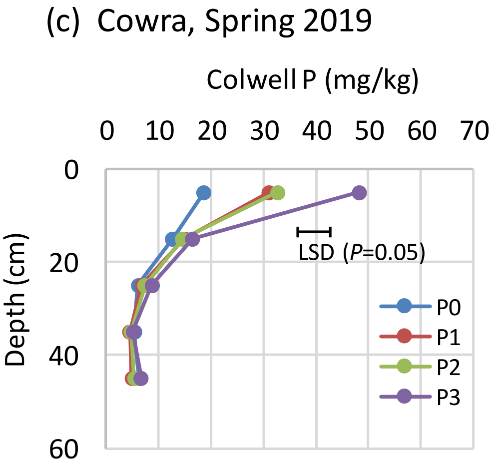

(c) Cowra, Spring 2019

Figure 4.5 - 1. Colwell extractable-P profiles at Spring Ridge (topsoil (0-10 cm) PBI = 52) in: (a) 2017, the first spring after P fertiliser application, (b) 2019, after three years of P rundown, and (c) at Cowra (topsoil PBI = ~20) after three years of P rundown. Due to the exceptionally dry conditions, deep soil cores were unable to be taken at Purlewaugh when attempted in spring 2019.

however, there was no indication of P movement below the surface 20 cm of the soil profiles, and, in every case, P applications had only altered the STP concentration of the topsoil (0-10 cm) layer. On this basis it was expected that rundown in STP concentrations would be related to the initial STP concentration of the topsoil and its P sorption capacity.

4.5.1.2 Change in soil test P concentrations over time

These experiments were all conducted during severe drought that persisted over the two years of the experiment. Consistent declines in soil P fertility in the absence of fresh fertiliser applications, only occurred in one of the three P-rundown experiments over the two-year measurement period

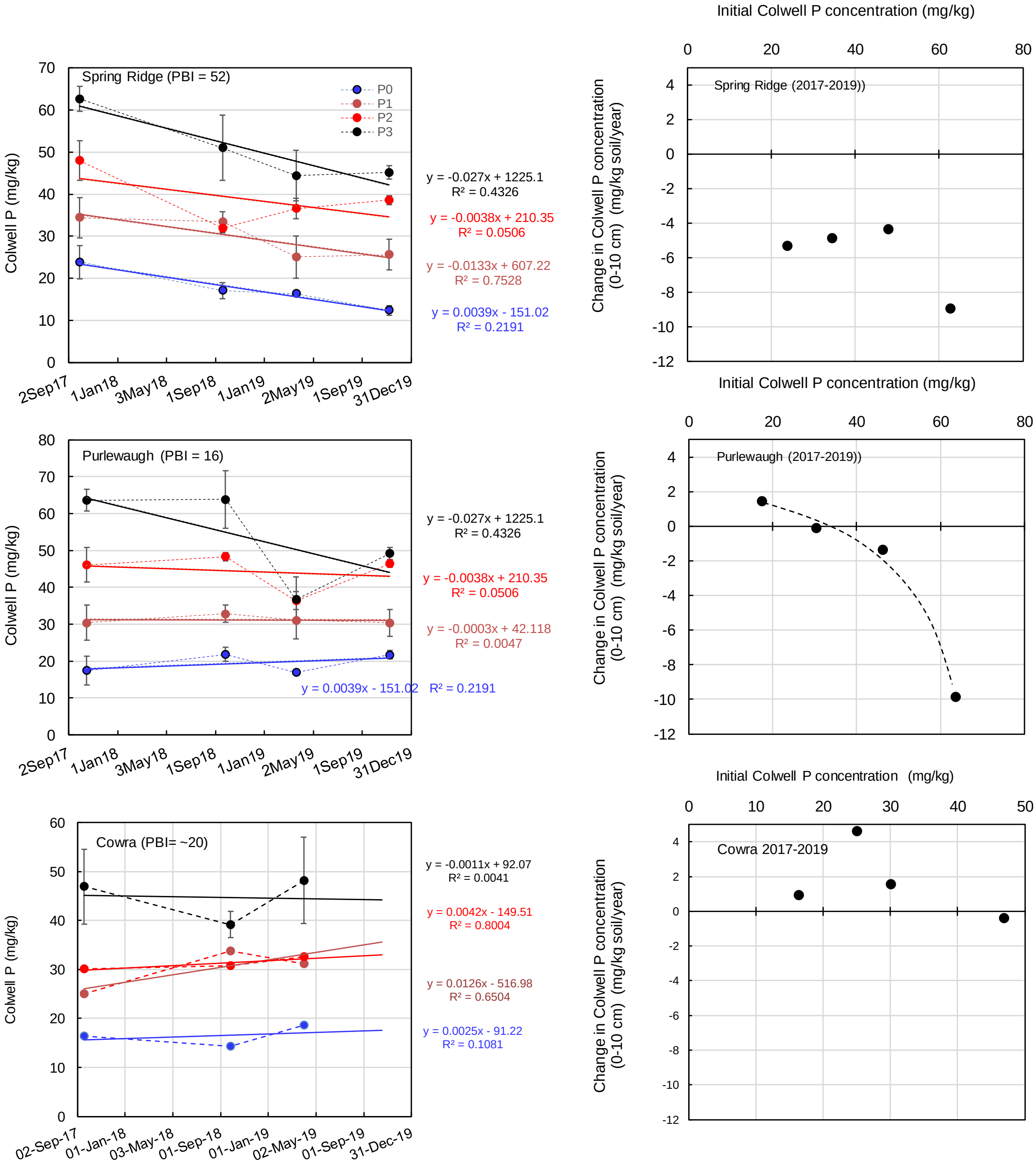


Figure 4.5 - 2. Colwell P concentrations of topsoil (0-10 cm) in the Spring Ridge, Purlewaugh and Cowra P-rundown experiments during 2017 -2019 (left panels), and rates of Colwell P declines (mg P/kg soil/year) graphed relative to the initial Colwell P concentrations of topsoil in each P treatment. A colour key to the treatments is shown in the top left panel.

(Fig. 4.5 - 2). At the other sites, STP concentrations did not change significantly during the experimental period except in the highest soil P treatment at Purlewaugh, where large declines of ~9-10 Colwell P units per year were recorded.

The hypothesis that rates of P rundown would be related to the initial STP concentration of the topsoil was not supported. In most instances, there was little evidence of a decline in Colwell P concentrations of the topsoil during the extended dry period.

4.5.2 Legacy of drill rows on spatial variability of extractable nutrients.

At the end of the pasture phase, the concentrations of extractable-P, K and S were significantly lower in the uppermost soil layers in the pasture drill row compared to the inter-row area, but depletion of nutrients in the drill row was not observed (e.g. P) or was reduced (K and S) by the end of the summer fallow (Fig. 4.5 - 3). Mineralisation of nutrients from plant residues during the summer fallow was a likely explanation for these changes.

It was concluded that drill rows can contribute to nutrient variability in pasture paddocks as a result of localised nutrient use, although a legacy effect on the concentrations of available P was not observed in this case. It is presumed, consequently, that the practice of fertiliser placement in drill rows at sowing must also exacerbate issues of spatial variability of nutrients in paddocks.

4.5.3 Spatial variability of extractable P in pasture paddock soils.

4.5.3.1 Soil test P variation across uniform areas of paddocks

Grid sampling for Colwell P (0-10 cm) universally demonstrated substantial spatial variability of STP concentrations in areas of apparently-uniform paddocks (Appendix 9). Figure 4.5 - 4 illustrates the typical high variability in STP observed in one of the paddocks assessed. These areas assessed were intentionally selected for apparent uniformity because they were to be used for an experiment. However, this also suited the objectives of the spatial variability assessments because farmers are already advised to avoid soil sampling across diverse paddock landscapes or soil types.

Irrespective of this, the distribution of STP concentrations were broad and the patterns of STP concentration were often spatially explicit (i.e. not random) but with no obvious topographical or vegetation associations. The STP concentrations, at the site shown here as an example, were normally distributed despite their non-random distribution (Fig. 4.5 - 5).

By combining typical pasture yield response data for a similar soil type (data from Simpson *et al.* 2009) with the STP profile of this paddock, it was estimated that this paddock was growing at ~80% of its yield potential at the time it was soil sampled, with a spatial range in pasture yield from 57% (lowest P areas) to 97% of maximum yield (highest P areas) (Fig. 4.5 - 6).

4.5.3.2 Spatial variogram analysis of extractable P in cropping soils

The variogram study sampled two sites in the southern cropping zone, near Wagga Wagga and Yerong Creek (NSW Riverina region). Soil samples (42 mm diam.) were taken in a random pattern at precise spacings to permit analysis by linear, spherical, Gaussian and exponential variogram models using similar statistical methods as described in Conyers *et al.* (2018). The present study compared the variance in soil P data in surface soil (0-5 and 5-10 cm depths) with the variance of the other soil parameters measured.

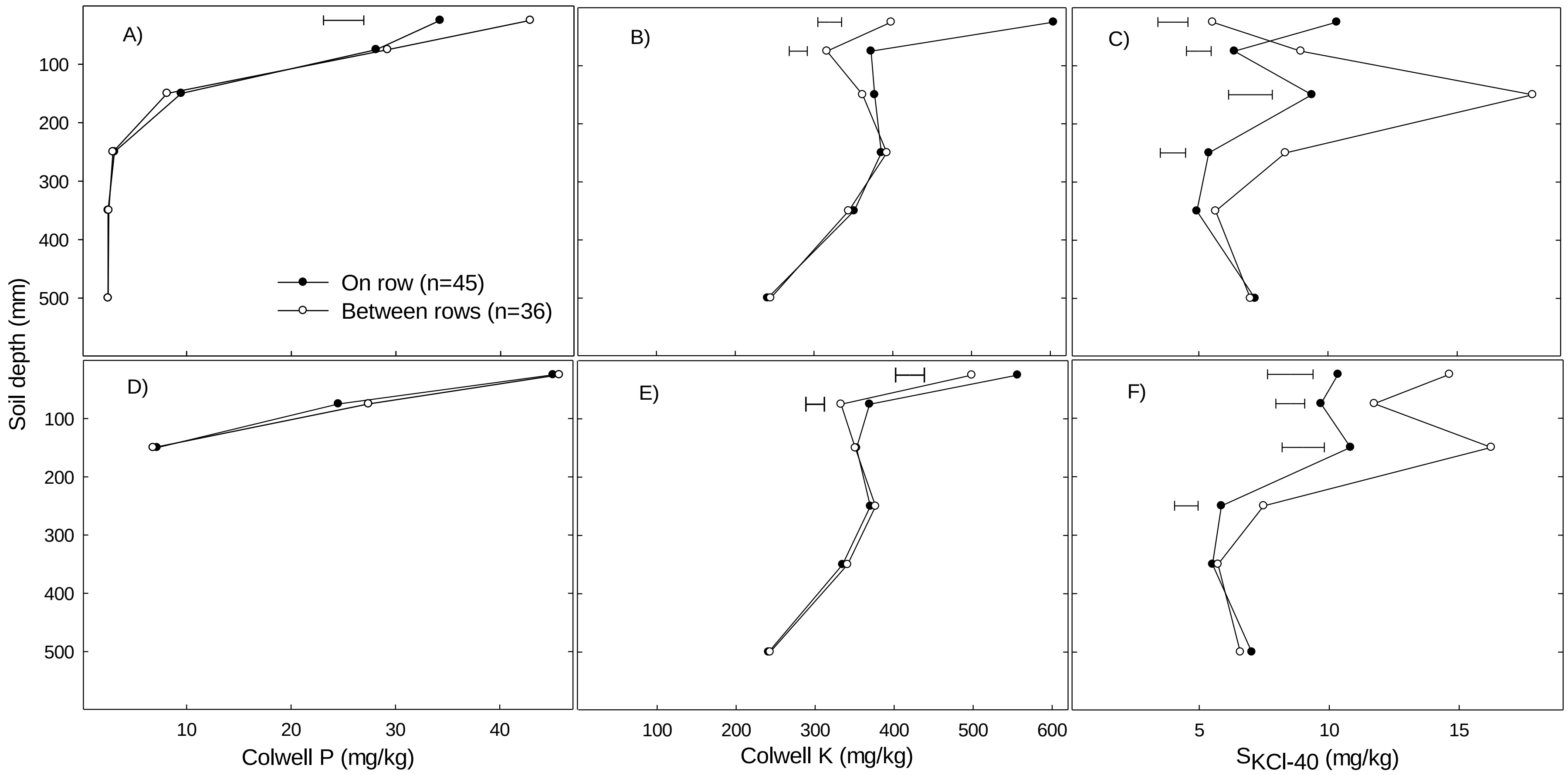
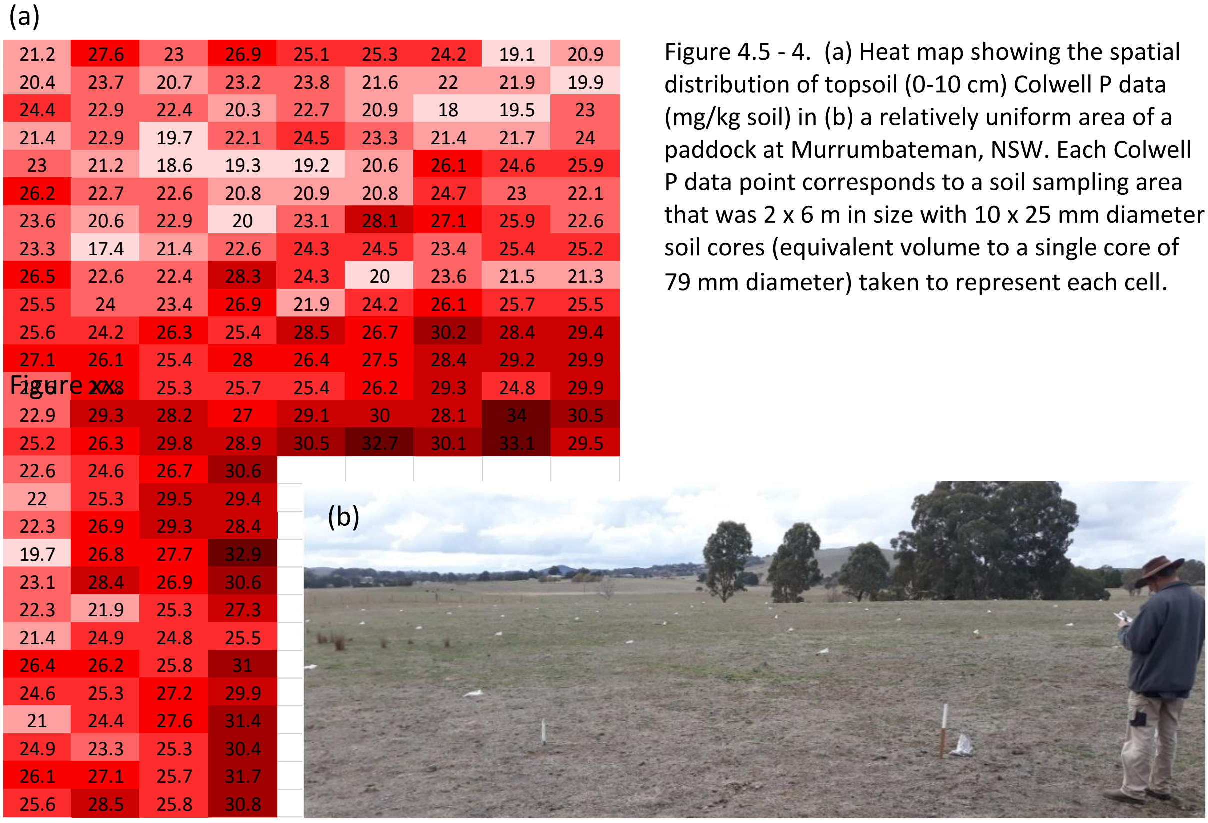


Figure 4.5 - 3. Soil available phosphorus (Colwell P; A and D), potassium (Colwell K; B and E) and sulfur (S $_{KCl-40}$; C and F) measured in the surface 0.60 m in September 2014 (A, B, C) and April 2015 (D, E, F) under pasture drill rows and in the inter-row area. Differences are not significant (ns) at *P*=0.05 unless marked with an error bar.



21.2	27.6	23	26.9	25.1	25.3	24.2	19.1	20.9
20.4	23.7	20.7	23.2	23.8	21.6	22	21.9	19.9
24.4	22.9	22.4	20.3	22.7	20.9	18	19.5	23
21.4	22.9	19.7	22.1	24.5	23.3	21.4	21.7	24
23	21.2	18.6	19.3	19.2	20.6	26.1	24.6	25.9
26.2	22.7	22.6	20.8	20.9	20.8	24.7	23	22.1
23.6	20.6	22.9	20	23.1	28.1	27.1	25.9	22.6
23.3	17.4	21.4	22.6	24.3	24.5	23.4	25.4	25.2
26.5	22.6	22.4	28.3	24.3	20	23.6	21.5	21.3
25.5	24	23.4	26.9	21.9	24.2	26.1	25.7	25.5
25.6	24.2	26.3	25.4	28.5	26.7	30.2	28.4	29.4
27.1	26.1	25.4	28	26.4	27.5	28.4	29.2	29.9
[illegible]	[illegible]	25.3	25.7	25.4	26.2	29.3	24.8	29.9
22.9	29.3	28.2	27	29.1	30	28.1	34	30.5
25.2	26.3	29.8	28.9	30.5	32.7	30.1	33.1	29.5
22.6	24.6	26.7	30.6					
22	25.3	29.5	29.4					
22.3	26.9	29.3	28.4					
19.7	26.8	27.7	32.9					
23.1	28.4	26.9	30.6					
22.3	21.9	25.3	27.3					
21.4	24.9	24.8	25.5					
26.4	26.2	25.8	31					
24.6	25.3	27.2	29.9					
21	24.4	27.6	31.4					
24.9	23.3	25.3	30.4					
26.1	27.1	25.7	31.7					
25.6	28.5	25.8	30.8					

Figure 4.5 - 4. (a) Heat map showing the spatial distribution of topsoil (0-10 cm) Colwell P data (mg/kg soil) in (b) a relatively uniform area of a paddock at Murrumbateman, NSW. Each Colwell P data point corresponds to a soil sampling area that was 2 x 6 m in size with 10 x 25 mm diameter soil cores (equivalent volume to a single core of 79 mm diameter) taken to represent each cell.

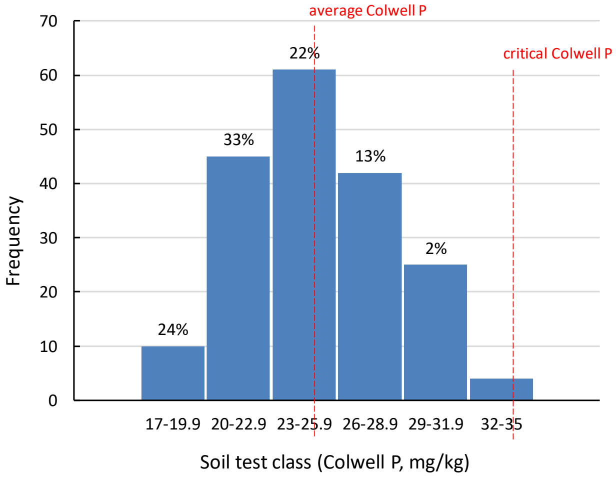


Figure 4.5 - 5. Colwell P data appeared to have a non-random spatial distribution but were normally distributed for statistical purposes. The range in STP data were estimated using information in Figure 4.5 - 6 to correspond with a range in pasture yield from 57% to 97% of maximum potential yield.

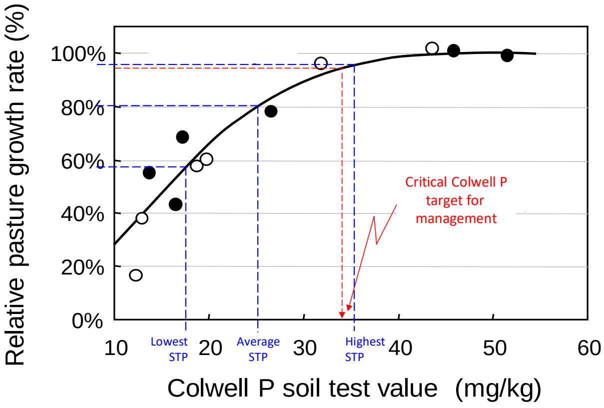


Figure 4.5 - 6. Spring growth response of subterranean clover-rich pasture to soil P fertility after superphosphate application at Bookham, NSW in 2002 (open circles) and 2003 (close circles) (from Simpson *et al.* 2009). This pasture was growing on a similar soil in a similar district to the paddock at Murrumbateman and is used to estimate likely pasture production at the Murrumbateman site.

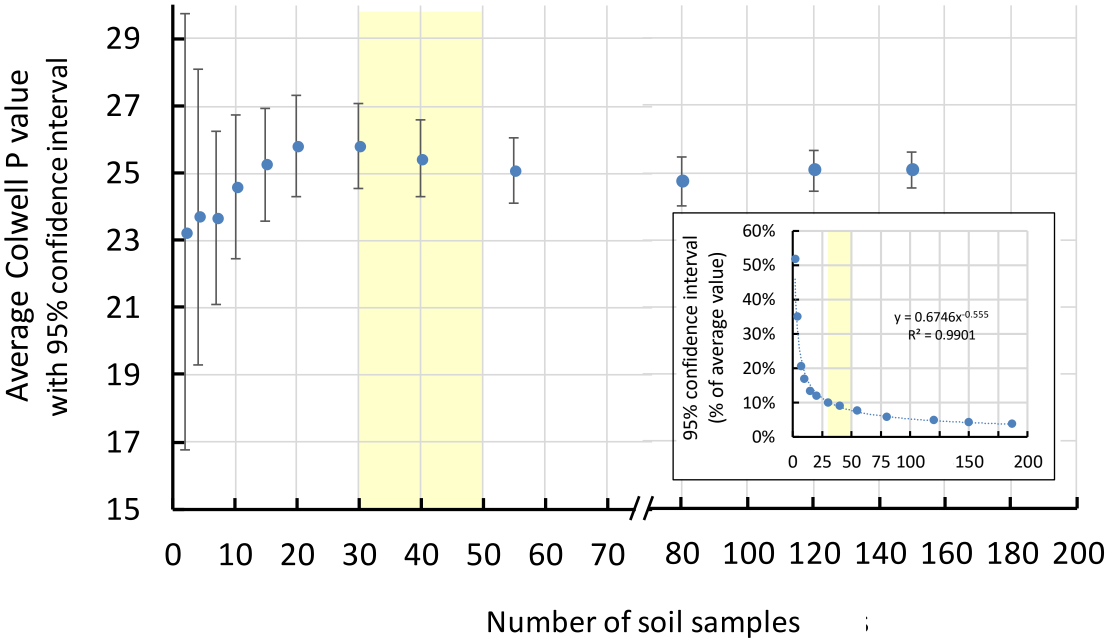


Figure 4.5 - 7. Average Colwell extractable P concentrations of topsoil (0-10 cm) samples collected from an apparently-uniform paddock at Murrumbateman NSW. This figure was generated by randomising the STP dataset and then selecting increased sample sizes for determination of average STP values and their error term. The total data set of 187 values was collected by grid sampling the site (Fig. 4.5 - 4). Bars indicate the ±2x standard error (i.e. the ~95% Confidence Interval for each estimate). Note the change of scale in the x-axis. Inset shows impact of sampling frequency on the confidence interval expressed as a percentage of the average Colwell P value. Presently it is recommended to soil sample farm paddocks at frequencies of 30 to 50 cores per paddock (core diameter = 19 mm) (Simpson *et al.* 2009, Gourley and Weaver 2019).

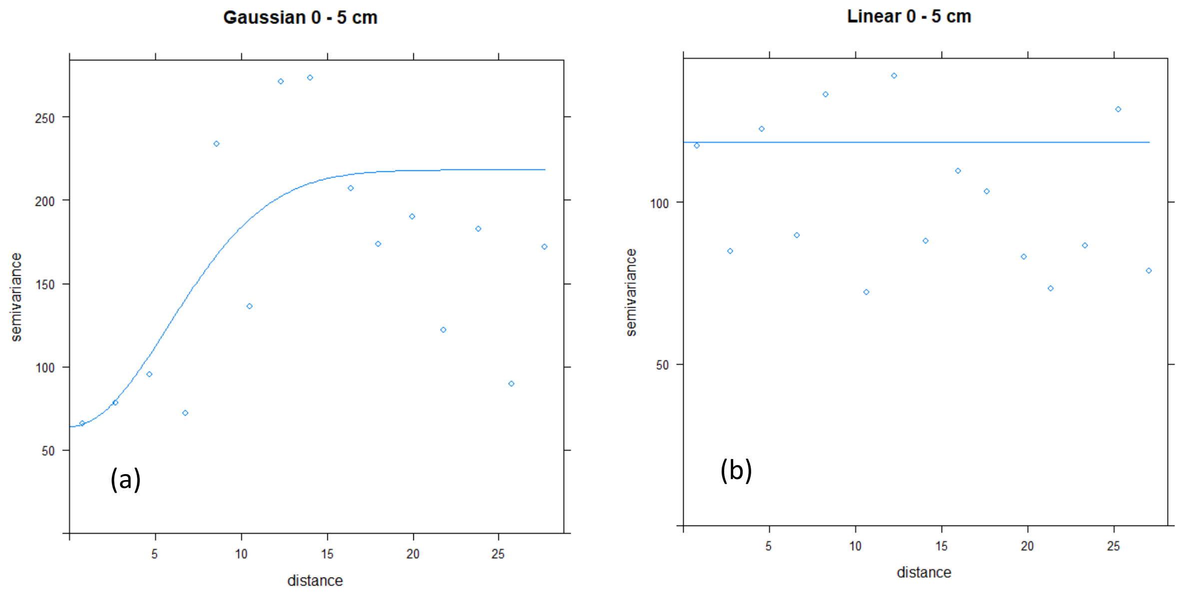


Figure 4.5 - 7. Two variogram models the best fits to Colwell P data at the 0-5 cm depth at a) Wagga Wagga, and b) Yerong Creek. Nevertheless, these fits were not significant (P >0.05) and indicated that the scale of Colwell P variability at those sites was greater than the scale of sampling.

Only a small proportion of the variation in the Colwell P data was explained by fitting variogram models to soil samples taken from these two cropping soils from (e.g. Fig. 4.5 - 7). This relatively poor fit indicated that the scale of variability of P at those sites was beyond the scale of sampling; in many ways similar to the issue of high spatial variability in STP concentrations that was also observed in pasture paddocks.

An analysis of the sample size at both sites revealed that Colwell P was significantly more variable than the other parameters tested; pH, total N and total C. At the Wagga Wagga site it was calculated that 19-26 (average 23) soil cores were required to provide an estimate of Colwell P with 10% precision in the surface 10 cm of the profile. At the Yerong Creek site, 10% precision was achieved with 12-151 (average 57) soil cores. The range in values at the Wagga site was substantially less than the range at Yerong Creek and this may be due to any number of factors. However, it is noted that no fertiliser was banded in drill rows in the year of sampling at the Wagga Wagga site (see next section) which is a likely source of variance in surface soil P, as opposed to Yerong Creek where a commercial cereal crop was being grown where fertilised was banded. On the same soil samples, a similar number of soil cores was calculated to be necessary to estimate values at 5% precision; soil pH (estimated average of 3 and 6 cores for Wagga Wagga and Yerong Creek, respectively), total C (average 33 and 36 cores, respectively) and total N (average 31 cores, at both sites). To achieve 5% precision in Colwell P an average of 90 cores would be required at the Wagga Wagga site compared to 225 cores at the Yerong Creek site. Several thousand soil cores would be required to estimate Colwell P at 1% precision at both sites.

4.6 Discussion: Output 4(c) – Development of guidelines for managing soil P fertility in crop-pasture transitions involving serradella pastures.

4.6.1 Rates of rundown in soil test P

These experiments were designed to provide information about how rapidly STP concentrations fall when fertiliser is withheld for the purpose of lowering soil P fertility. This is likely to be useful when transitioning from a crop to a serradella pasture with a lower critical P requirement than that of the crop. It was hypothesised that the rate of P rundown under average seasonal conditions would be a consequence of P removal in plant or animal products, plus P accumulating in the soil due to the slow P-sorption reactions that convert phosphate to less plant-available forms (i.e. ”P-fixation” reactions), plus any losses in runoff, erosion or leaching. With good agronomic management, losses of P from soils with moderate to high P buffering capacities should be minimal.

The uncertainty in this equation is the rate at which P is accumulated in the soil. The experiments were managed by mowing to reduce P removal in products to zero, and soil P profiles were examined, after P had been applied, to assess whether P movement to depth was occurring. In this way it was anticipated that P rundown would primarily reflect the slow P sorption (fixation) reactions of the soil. The rate of phosphate sorption reactions is known from laboratory incubation experiments, to be directly proportional to the phosphate concentration of soil solution (Barrow 1974, Barrow 1999) and, therefore, it was anticipated that it would be related to the STP concentration at which the soil was being managed (see also Simpson *et al.* 2014).

The hypothesis that declines in STP concentration would be related to the initial STP concentration was not supported by the results of these experiments. However, the experiments were conducted under conditions of severe drought. Adjacent experiments at the northern NSW sites recorded no pasture growth in some years and when there was sufficient moisture for some pasture growth, there was good evidence that the dry conditions were severely constraining the plant-availability of soil P (Fig. 4.1 - 2; **Section 4.2.2.2**).

It was concluded that the same environmental conditions would also constrain P sorption reactions in soils and that the results from these experiments demonstrate that plant-available P will often be conserved in dry soils because pasture growth and P uptake are occurring at low rates, and because low moisture conditions will not support the usual rates of chemical reactions in soils.

These results added weight to the revised advice in the “Five Easy Steps” P tool, where farmers are encouraged to use STP monitoring to guide fertiliser practice and to be alert for evidence of conservation of P after drought years. This is potentially very important for farm budgets because during and immediately after droughts, farm cash flow is poor. If soil P fertility is adequate and STP conservation has occurred during drought, it may be feasible to temporarily reduce fertiliser P inputs without significantly reducing pasture growth during the immediate period of drought recovery (e.g. see **Sections 4.6.2 and 10**).

4.6.2 Dealing with spatial variability in paddocks

Spatial variability in the STP concentrations of farm paddocks is well known. Some of this variability is associated with gross differences in topography and variations in soil type across farm paddocks (e.g. Trotter *et al.* 2014, Gourley and Weaver 2019) and can be dealt with by fencing and soil sampling that recognises differences in topography, land class and soil type. However, we observed surprisingly large variability even in areas of paddocks selected to avoid obvious topographical and soil type issues. Variability in the STP such as this can only be managed by using an appropriate soil sampling frequency to collect a volume of soil that reflects the soil in the paddock with acceptable accuracy.

The spatially-distributed STP data were, therefore, used to check the soil sampling frequency recommendations for estimating the average STP concentration of farm paddocks. The standard recommendation for soil sampling in pasture paddocks is to take between 30 and 50 soil samples using a 19 mm diameter soil corer when testing for extractable P (e.g. Simpson *et al.* 2009). Relatively large soil volumes per STP estimate were collected in the present pasture and crop paddock experiments to improve accuracy of the STP estimates. However, larger soil sample volumes mean that the analysis of sampling frequency will not be directly applicable to 19 mm diameter soil cores.

If the differences in sample size used in the Murrumbateman pasture experiment are ignored, for example, and the data are assumed to reflect the distribution in STP detected using a standard soil corer, 30-50 soil cores would result in estimates of the STP concentration with a 95% confidence interval of ±1.1-1.3 Colwell P units. The true average Colwell P concentration of the experiment test area was 25.1 mg/kg soil, so the maximum error in the STP value would have been ~±4-5%. Likewise, ~ 23-57 soil cores (0-10 cm depth) were required to provide an estimate of Colwell P with 10% precision in croped paddocks at Wagga Wagga and Yerong Creek, respectively.

These estimates are generally better than the error that is estimated to be associated with the pragmatic sampling recommendation made by Gourley and Weaver (2019): i.e. 30-40 soil samples per paddock giving ±15% error when using a 19 mm diameter soil corer.

All of the assessments of STP variation in “uniform” pasture paddocks indicated that topsoil extractable P concentrations were, nevertheless, highly spatially-variable with a large range in potential productivity across the paddock due to variation in STP concentration. Data from the cropped paddocks indicated that Colwell P is in fact more variable than some other soil test parameters (e.g. pH, total N and total C).

Many of the causes of nutrient variability in paddocks cannot be controlled (e.g. nutrient depletion under plants, the influence of drill rows, uneven deposition of animal excreta, etc.). However, the factors that can be controlled are: (i) separation of non-uniform zones for STP management and (ii) using a soil sampling frequency that can deliver a reasonably accurate STP outcome. Inevitably, there will always be a trade-off when sampling soils from farm paddocks between the need for precision (very high sampling frequencies) and practicality (reasonable sampling loads).

The results from the present investigations provided insights into how soil sampling procedures and the recommendations regarding sampling frequency should be formulated for the revised *'Five Easy Steps*', P decision tool (**see Section 10**).

The advice to farmers now includes:

- recommendation to collect soil samples from relatively 'uniform' areas by sampling separately from paddock areas with obvious differences in topography, land class and soil type,
- recommendation to sample soils by taking at least 30 and preferably up to 50 soil cores (19 mm diam.) per paddock,
- additional descriptions of the factors that contribute to STP variability to encourage high levels of sampling accuracy and the importance of accurate subsampling of collected soil before despatch to the testing laboratory,
- repeated recommendations to monitor soil fertility annually and make decisions based on STP trends rather than individual test results.

4.7 Results: Output 4(d) – Provide farmers with materials on the characteristics of low-P pasture systems based on the serradellas.

4.7.1 Managing soil P fertility for efficient use of P in pasture systems on soils with moderate to high P buffering capacity (the majority of soils in southern Australia)

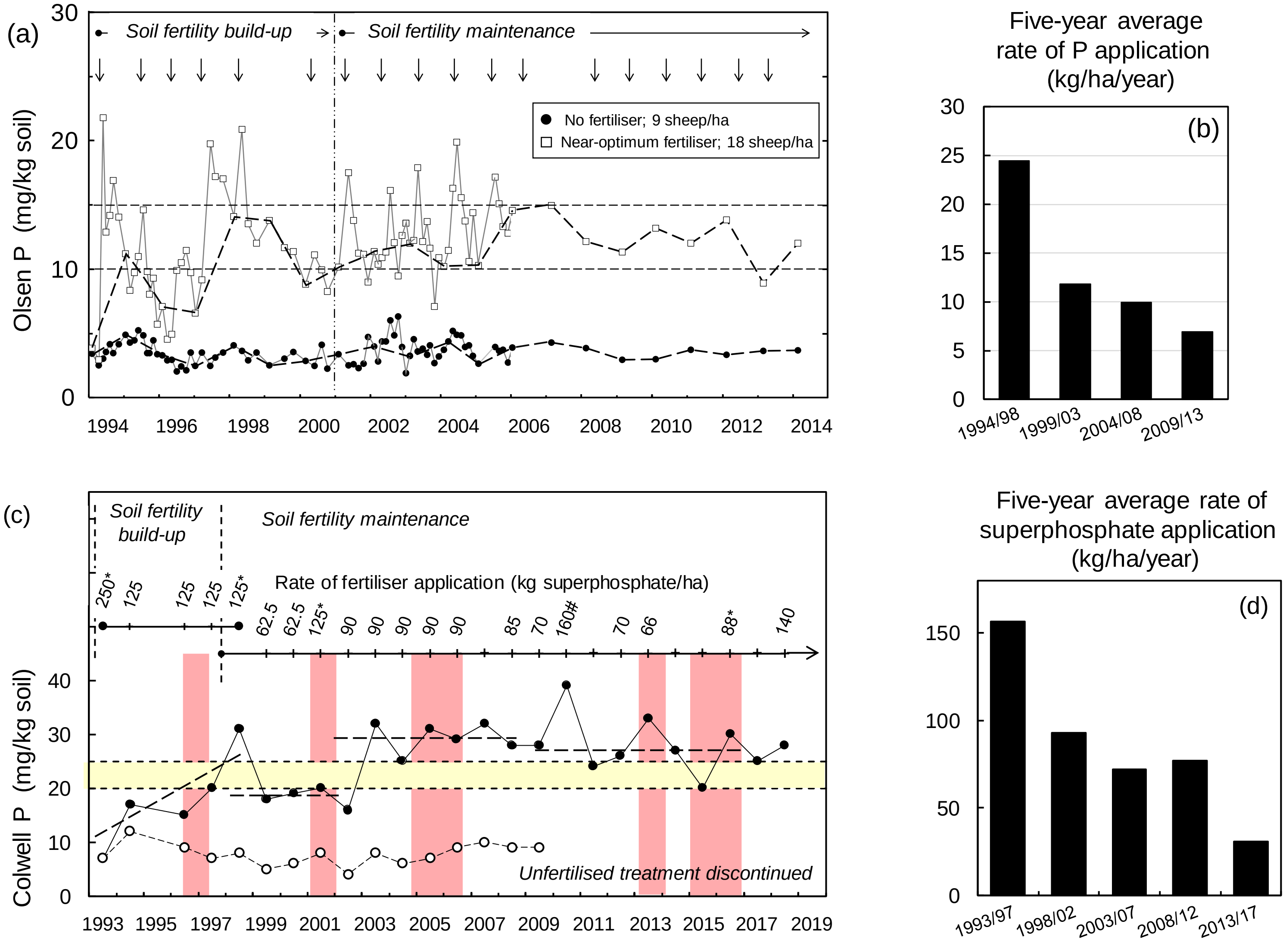
Application of P fertiliser is the easiest and most economical means to achieve high land-use efficiency and realise the productive potential of most farms (Carter and Day, 1970, Cayley *et al.*, 1999, Lean *et al.*, 1997) because low soil fertility reduces pasture yield and carrying capacity and limits the efficiency of rainfall use (Mills *et al.*, 2006). However, P fertiliser is a significant production cost for a grazing business and may account for 20-25% of annual variable costs; it is often the largest cost after labour and debt-servicing (McEachern and Brown, 2010). This has typically made Australian farmers very sensitive to fertiliser price and to any doubts they may have about the benefits of fertiliser use.

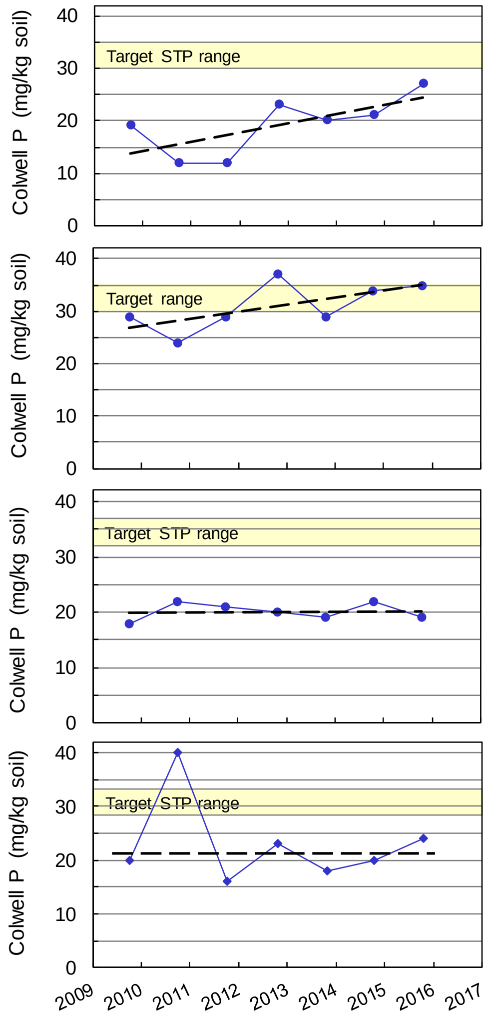
The most effective way to ensure efficient use of P fertiliser is to target its application using the critical STP benchmarks for high production as a guideline (Reuter *et al.* 1995). Managing soil P fertility to target levels is also essential for capturing lower P fertiliser costs when using a serradella-based pasture. Lower P inputs are only realised when serradella pastures are fertilised to their lower critical STP requirements. The available evidence indicates that fertilising soils to maintain a lower STP concentration results in reduced rates of P accumulation in the soil. This, in turn, means there is a lower requirement for P fertiliser to maintain production (Simpson *et al.* 2014, Simpson *et al.* 2015).

Figure 4.7 - 1. (a) The Olsen P concentration of topsoil (0–10 cm) in selected grazing system treatments from a long-term grazing experiment at Hall, ACT (adapted from Simpson *et al.,* 2015). Three phases of the experiment are shown: 1994–2000, a soil fertility building phase, and 2001–2006, a soil fertility maintenance phase during which pasture was grazed continuously and STP was monitored at approximately 6-week intervals; and 2007–2014, a phase in which soil P fertility continued to be maintained, but with changed grazing management and only annual monitoring of the STP concentration. The fields that received no P fertiliser (closed circles) were stocked with 9 sheep/ha. Phosphorus was applied to the fertilised fields (open squares) with the intention of entering the spring period of pasture growth with a soil test P (STP) level within an Olsen P target band of 10-15 mg P/kg soil. These fields were stocked with 18 sheep/ha. Dashed horizontal lines delineate the target range for STP management. Arrows indicate when P fertiliser was applied (typically in autumn close to the break of each season). Each symbol represents the average STP concentration of three replicate fields. Soil test P monitoring points are joined by a solid grey line to illustrate seasonal variability in soil test results. The dashed black line joins soil tests that were taken in Jan/Feb and illustrates the variation in STP results that would be typical of annual monitoring at the same time each year. These STP values were used to estimate the amount of P to apply each year. Phosphorus fertiliser was typically applied in autumn close to the break of each season. (b) Five-year average rates of P application for the duration of the experiment. (c) Fertiliser application history and results of annual Colwell P (mostly during spring) in a Grazing Systems Demonstration at, Bookham, NSW. Shaded vertical panels indicate years in which spring droughts occurred. The fertilised field carried 12-15 sheep/ha once the target STP concentration was achieved (zone delineated by dashed horizontal lines). Soil test results from an adjacent unfertilised paddock grazed continuously by 6 sheep/ha are also shown. The dashed lines show “trends” in the data used at intervals to gauge progress in STP management. Source: Graham, (2006, 2017) and R.P. Graham *unpublished data*. (d) Five-year average rates of superphosphate (9% P) application for the duration of the demonstration experiment.

From the revised edition of: *“Five Easy Steps to ensure you are making money form phosphorus fertiliser*”.
(see **Section 10; Appendix 10**).

The soil fertility management demonstration at Bookham NSW was supported by farmer members of the Bookham Agricultural Bureau.


(a)
Soil fertility build-up
Soil fertility maintenance
No fertiliser; 9 sheep/ha
Near-optimum fertiliser; 18 sheep/ha
Olsen P (mg/kg soil)
0
10
20
30
1994
1996
1998
2000
2002
2004
2006
2008
2010
2012
2014
Five-year average rate of P application (kg/ha/year)
(b)
0
5
10
15
20
25
30
1994/98
1999/03
2004/08
2009/13
(c)
Soil fertility build-up
Soil fertility maintenance
Rate of fertiliser application (kg superphosphate/ha)
250*
125
125
125
125*
62.5
62.5
125*
90
90
90
90
90
85
70
160#
70
66
88*
140
Colwell P (mg/kg soil)
0
10
20
30
40
Unfertilised treatment discontinued
1993
1995
1997
1999
2001
2003
2005
2007
2009
2011
2013
2015
2017
2019
Five-year average rate of superphosphate application (kg/ha/year)
(d)
0
50
100
150
1993/97
1998/02
2003/07
2008/12
2013/17



Paddock name: “China wall”
PBI: 63
Predicted critical Colwell P: 30 mg/kg
Ave. fertiliser rate: 100 kg superphosphate/ha/year
Decision for 2017: Progress towards the STP target is good; no change to fertiliser.

Paddock name: “Jimmy’s Road”
PBI: 54
Predicted critical Colwell P: 29 mg/kg
Ave. fertiliser rate: 100 kg superphosphate/ha/year
Decision for 2017: Soil P now reliably within STP target range; reduce P fertiliser to maintenance rate; continue monitoring STP trend.

Paddock name: “Ring-a-bells”
PBI: 84
Predicted critical Colwell P: 32 mg/kg
Ave. fertiliser rate: 128 kg superphosphate/ha/year
Decision for 2017: Achieving only maintenance of suboptimal STP at current fertiliser rate; increase fertiliser to build STP towards target.

Paddock name: “Sunnyside”
PBI: 34
Predicted critical Colwell P: 27 mg/kg
Ave. fertiliser rate: 100 kg superphosphate/ha/year
Decision for 2017: Farmer chooses to ignore very high STP result. Current fertiliser rate is maintaining STP below target; increase fertiliser.

Figure 4.7 - 2. Examples of STP monitoring by a farm business near Bombala, NSW, that illustrate how STP data are being used to “fine-tune” fertiliser management plans and to check that fertiliser application rates are achieving soil P management goals.
Although, testing paddocks every 3 or so years has been promoted in the past, research, demonstration and data from farmer practice (such as these) demonstrate that infrequent soil tests are not reliable enough for fertiliser decision making because of the intrinsic seasonal and interannual variability in P availability.

These data were provided by farmer members of Monaro Farming Systems.

Figure 4.7 - 1 provides two examples of managing soil STP levels to targets that are guided by the critical P requirement for production of clover-based pasture (Gourley *et al.* 2019). Figure 4.7-1a shows a series of 6-weekly soil tests that illustrate the typical seasonal variability in STP concentrations that is experienced on farms as a consequence of the timing of fertiliser applications and seasonal soil moisture and temperature conditions. Inability to deal with this variability in STP concentrations can be a major disincentive for effective fertiliser management.

The project team have collated research data and worked with the participating farm groups to develop examples and extension materials that can assist a wider cohort of farms to adopt simple practices that overcome these issues. These practices include:

- testing pasture soils at the same time each year (typically spring) (e.g. Figure 4.7 – 1c),
- setting a pragmatic target range for soil P management that is guided by the critical P requirement of the soil-pasture system, and
- commencing an annual soil-fertility monitoring program.

As much as possible, fertiliser decisions are then based on trends in the STP monitoring data (e.g. Fig. 4.7 - 2), rather than one-off soil test results which may provide an unrealistic fertility assessment due to sampling and/or seasonal errors.

An unexpected outcome of the work to record and validate this approach to soil P management has been evidence that it also appears to deliver a slow, but steady reduction in the fertiliser cost of production over time (Figs. 4.7 -1b and 4.7 - 1d).

The reduction in P costs was surmised to be due to at least three factors:

(i) once the critical level P fertility is achieved, a lower fertiliser rate is employed to maintain the target soil fertility level than rate that was required to build soil P fertility; this delivers an obvious and immediate cost saving,

(ii) application of P to soil with moderate to high P-buffering and sorption capacities has a "P-sparing effect" (Barrow, 2015, Barrow and Debnath, 2018). It is argued that the P-sparing arises because the negative charge on reactive surfaces in the soil is increased when sorbed P diffusively penetrates the soil particle; this reduces the P buffering capacity of the soil and the rate of further sorption reactions. Put simply, each application of P is expected to slowly improve the effectiveness of subsequent P applications (Bolland and Baker, 1998, Barrow *et al.*, 1998, Barrow and Debnath, 2018).

(iii) improved confidence in P fertiliser decisions because of STP monitoring enables decisions to skip fertiliser applications after dry seasons when there is evidence that soil P-fertility had been conserved (most probably because dry seasons result in less P utilisation (pasture growth), lower microbial activity and less P sorption) (see Fig. 4.7 - 1c and **Section 4.5.1**)

4.7.2 P use in serradella-based pasture systems

4.7.2.1 The critical STP requirements of serradellas

Yellow serradella was known to have a significantly lower P-fertiliser requirement than subterranean clover (e.g. Paynter 1990, Bolland and Paynter 1992) but critical STP concentrations for serradella were not determined. The collation and processing of data from previous field experiments was completed to enable peer-reviewed publication of the first estimates of the critical STP benchmarks for serradellas (*O. compressus* and *O. sativus*; Table 4.7 - 1, Sandral *et al.* 2019) in comparison with a range of other alternative pasture legumes.

The work also identified three forage legumes (crimson clover, *T. incarnatum*; purple clover, *T. purpureum*; arrowleaf clover, *T. vesiculosum*) with lower critical P requirements than subterranean clover and provided important confirmation of the critical STP requirement of subterranean clover.

However, the serradellas were the only pasture legumes capable of being used as potential P-efficient alternatives to subterranean clover.

The field experiments demonstrated that the P-efficient legumes could be grown with an Olsen STP concentration (0-10 cm) of 10 mg P/kg soil to achieve near-maximum spring yields in soils where it is recommended that *T. subterraneum* be grown with an Olsen STP concentration of 15 mg/kg (Sandral *et al.* 2019, Gourley *et al.* 2019). The Colwell P equivalent of these benchmarks for serradellas and subterranean clover growing in soil with PBI in the range 40-80, were 20-21 mg/kg and 33 mg/kg, respectively.

4.7.2.2 More effective use of P fertiliser in areas where serradellas are used currently

Early in the project it was realised that the participatory research, in areas of WA and northern NSW where serradella pastures are already used, had been established on contrasting soil types. Both regions have light, sandy soils and serradellas are commonly grown on these soil types. In WA, at all participatory sites and their co-located research experiments had topsoil PBI's that were below 12 (extremely low P buffering capacities). In addition, some of the participatory and aligned research sites in northern NSW had topsoils with PBI of ~30 (very low P buffering). These results alerted the research team to the potential for P movement in poorly P-buffered soil profiles. Soil testing protocols were revised to enable the assessment of soil extractable-P profiles to depth.

Soils with very-low P buffering - WA sites: rapid movement to depth of P applied as fertiliser was found in the extremely-low P buffered soils. These sites have been identified previously as P-deficient on the basis of traditional surface-soil tests. Many of the sites were reassessed as P-adequate because of legacy P at depth in the soil profiles. These results underpinned conclusions that:

(i) traditional surface-soil tests do not provide an adequate assessment of the P status of poorly P-buffered soil profiles; a change to standard practices is essential.
(ii) these soil profiles can contain substantial amounts of legacy P at depth that is accessible to pasture legumes depending on their rooting depths; planning to use legacy P reserves as part of a soil P management program should substantially improve the efficiency of P-fertiliser use.
(iii) serradella is characteristically very deep rooting in sandy soil profiles (e.g. ~1.5 m as opposed to 0.6-0.8 m for subterranean clover) and is surmised to be ideally suited to utilisation of legacy P at depth and for reducing the risk of loss of P to the wider environment where it can be a significant pollutant.

(**see Section 4.4.1**)

Soils with low P buffering – northern NSW sites: extractable-P profiles to depth at these sites indicated that fertiliser P was predominantly confined to the top 20 cm of the soil profile and that the conditions of reliable use of standard surface-soil tests (Gourley *et al.* 2019) were not violated. Despite severe drought, the aligned research experiments confirmed that serradellas were more P efficient that subterranean clovers. Considered together, these results have led to acknowledgement that substantially lower critical P benchmarks for serradella pastures (than previously advocated) were likely to apply in this region.

(**see Sections 4.1.2 and 4.4.2**)

Table 4.7 - 1. Mean critical soil test phosphorus (P) values for those species that had established and grown well in experiments at Yass, Burrinjuck. Each site-by-year result was regarded as independent. Standard deviations (sd) are provided as a measure of the repeatability of the critical P determinations. From: Sandral *et al.* (2019).

Species	Cultivar(s) tested	Sample size[1]	Critical Colwell P[2] (sd)	Critical Olsen P[3] (sd)[4]
			for 95% of maximum yield (mg/kg)	
Medicago sativa	SARDI 10	6	>45-50	>15.5-21.4
Trifolium ambiguum	Kuratas	1	36	12.5
T. subterraneum	5xLeura/2xIzmir/2xNarrikup	9	33 (4)	13.2 (1.8)
M. truncatula	Sultan-SU	2	33 (6)	14.5 (4.3)
T. glanduliferum	Prima	2	30 (3)	13.1 (0)
T. spumosum	Bartolo	3	28 (3)	11.6 (1.6)
Biserrula pelecinus	2xCasbah/1xMauro	3	28 (7)	11.3 (2.0)
T. hirtum	Hykon	4	28 (6)	11.0 (2.0)
Dactylis glomerata	Porto	2	26 (1)	9.3 (0.3)
T. michelianum	Bolta	1	25	9.0
T. incarnatum	Dixie	4	25 (5)	9.8 (1.8)
Phalaris aquatica	Advanced AT	2	23 (2)	8.3 (0.8)
Lotus corniculatus	breeders line LCO7AUYF	2	21 (0)	8.2 (0.5)
Ornithopus compressus	6xSantorini/1xAvila	7	21 (5)	8.2 (1.9)
O. sativus	Margurita	5	20 (6)	7.7 (2.4)
T. vesiculosum	Zulu II	3	21 (7)	8.3 (2.8)
T. purpureum	Electra	3	20 (4)	7.5 (1.3)

[1] Sample size refers to the number of 'cultivar-site-year' instances that yielded a critical soil test P result for each of the species.

[2] The critical Colwell P concentrations were derived from experiments grown in soils with a Phosphorus Buffering Index range: 40-80.

[3] Critical Olsen P values were determined from critical Colwell P values using relationships between Olsen P and Colwell P determined for each site in each year of the experiment.

[4] Standard deviation (sd) is presented as an indicator of the repeatability of each critical soil test P value because it is independent of sample size.

4.8 Discussion: Output 4(d) – Provide farmers with materials on the characteristics of low-P pasture systems based on the serradellas.

4.8.1 Materials provided to farmers

A variety of communication materials were prepared for farmer audiences covering the key areas described above, i.e. soil P fertility management for pastures, P-efficient legumes, and serradella performance in the experiments. A record of these communications, including communications to farm groups involved in the project and field days, seminars, webinars for wider audiences, is provided in **Section 14; Appendix 13**.

Some key communications have included:

(a) NSW Grasslands Society Newsletter (circulation 300)

Vol.34, No2:	Phosphorus soil test benchmarks for productive legume-based pastures. *by Richard Simpson and Cameron Gourley*
Vol.34, No3:	Practical application of soil test P benchmarks for phosphorus. *by Richard Simpson and Phil Graham*
Vol.34, No4:	Progress in research to develop a more P-efficient pasture system. *by Richard Simpson, Richard Hayes and Rebecca Haling*

(b) The Land Newspaper articles (major rural weekly with articles also distributed to a wider audience via Twitter; circulation 82,470 weekly readers)

DOWN TO EARTH #1821 (2017),	GOD'S OWN COUNTRY. DEFINITION CHANGES *by Bob Freebairn*
DOWN TO EARTH #1831 (2017),	SERRADELLA SHINES IN TOUGH SEASON *by Bob Freebairn*
DOWN TO EARTH #1855 (2018),	INSIGHTS INTO MORE PHOSPHATE EFFICIENT PASTURE LEGUMES *by Bob Freebairn*
DOWN TO EARTH #1884 (2018),	REFINE PHOSPHORUS USE *by Bob Freebairn*
DOWN TO EARTH #1921 (2019),	RESEARCH DEFINES FERTILITY TARGETS FOR NUTRIENT-EFFICIENT PASTURES *by Bob Freebairn*

(c) "Five Easy Steps to ensure you are making money from phosphorus fertiliser" (2020)
An updated and revised version of the decision-support booklet and tool first released in 2009 and available for download by farmers at: https://www.mla.com.au/extension-training-and-tools/tools-calculators/phosphorus-tool/

The revised phosphorus management booklet and tool updates advice on critical STP requirements for clover- and serradella-based pastures, soil testing method and management of soil test variability, and how to use fertiliser rate calculations. Experience and farm group data from the current project is included to illustrate application of the advice. A new section summarises the latest thinking on P fertilisation of native grass pastures (**see Section 10**)

(d) Further factsheets and articles for farm audiences are being prepared currently.

5 Conclusions/recommendations – (Activity B4) Lowering P costs of serradella systems

5.1 Output 4(a) – Conduct participatory plot scale experiments in areas of WA and NSW where serradella is already grown to assess serradella production in response of P fertiliser application and quantify the critical P level required by the legume.

Output 4(b) – Conduct participatory paddock scale experiments with farmer groups in areas of WA and NSW where serradella is already grown to improve knowledge of the P-fertiliser and general management of serradella-based pasture systems

5.1.1 Serradella observed to be 2-fold more P-efficient than subterranean clover

Recurring droughts made the assessment of critical STP requirements an impossible task because pasture failed to establish in some seasons and, when pasture did establish successfully, subsequent growth was water-limited. In low soil moisture conditions, the supply of P to roots by diffusion in soil solution is constrained. This was illustrated clearly by the linear responses of serradellas and subterranean clover to soil P fertility in northern NSW during 2019. These linear responses extended to STP concentrations well above the known critical P requirement of subterranean clover (Gourley *et al.* 2019) and expected requirements of yellow and French serradella (Sandral *et al.* 2019).

It was shown very clearly at both northern NSW sites that, under P-limiting conditions, the herbage yield increase per unit increase in Colwell P of the serradellas was 2-fold that of subterranean clover. The present research was aiming to build on early reports that yellow serradella can yield as well as subterranean clover in light, sandy soils with about half the application of P fertiliser (e.g. Bolland and Paynter 1992) by determining critical STP levels. Critical STP concentrations are needed to guide fertiliser practice. Whilst, this has not been possible, the differences in P efficiency between serradellas and subterranean clover in these experiments does provide further field evidence of the high P efficiency of serradella relative to subterranean clover.

This evidence will reinforce the changing attitude of farm group leaders to the very high Colwell P concentrations that were recommended previously for serradella pasture production in northern NSW (e.g. Freebairn 2013). By inference, the research supports the view that the critical STP requirements of serradella will be less that that required for subterranean clover which, in turn, can be predicted for these soil types using PBI (Gourley *et al.* 2019). (**see Section 4.3 and Appendix 8**).

5.1.2 Planning to use legacy P in deep sandy soils will reduce the P-fertiliser cost of production

Extremely low PBI values for soils at all of the WA sites alerted the project team to the potential for rapid movement of P to depth in these soil profiles. Soil sampling protocols were revised immediately, and a very large effort was made to sample soil profiles to depth. The results confirmed that P applied as fertiliser moved readily to depth in these soil profiles and did so even under relatively dry seasonal conditions. It is likely that P may leach below the root zone in these soils during wet growing seasons. This poses a potential environmental risk if it is not managed. Some soil profiles had existing reserves of legacy P at depth and all soils developed deep P reserves when P was applied as fertiliser.

The extremely low P buffering by these soils will ensure that large proportion of the P at depth will remain plant available (and potentially mobile). Indeed, P at depth was considered the most likely reason why few responses to P application were detected.

Co-location of the farm group (paddock relevant) and small plot (research scale) experiments proved invaluable. The extractable P profiles from the research-scale experiments helped to explain results observed when only typical farm rates of fertiliser application were being used; they also enabled decisions to begin sampling soil using a 0-60 cm soil sample in the farm group participatory experiments. The Colwell P profiles indicated that this was likely to capture a reasonable proportion of the deep P without incurring an arduous soil sampling regime. Soil samples taken this way at the Broomehill and Katanning sites accounted for ~7-fold more plant-available P than traditional surface soil (0-10 cm depth) testing.

It was concluded that development of strategies to routinely assess and utilise deep P in these sandy soil profiles would reduce the need for P fertiliser and would lower the risk of P loss to the wider environment.

The deep root system of serradella in these soils appears to be ideally suited to capturing P that has moved to depth and beyond the shallower root zone of subterranean clover.

5.1.3 Traditional surface soil testing is not suitable for assessing the P status of soil with extremely low P buffering capacity

All sites in WA were identified as P-deficient and selected for use in the experiments using standard (0-10 cm) soil testing procedures. Most soils in southern Australia have moderate to high P buffering capacities (PBI >35) and fertiliser P modifies the top ~20 cm of the soil profile. Typically, 70-80% of applied as fertiliser can be found in the top 0-10 cm of soil, with the rest in the 10-20 cm soil layer even after long periods of regular fertiliser use (Simpson *et al.* 2015, Schefe *et al.* 2015). In these soils, standard soil tests directly sample the majority of the zone that is influenced by P fertiliser. This is not the case in soils with exceptionally low P buffering where P moves readily to depth.

Alternative ways to assess the amount of extractable P in the root zone of the pasture are needed.

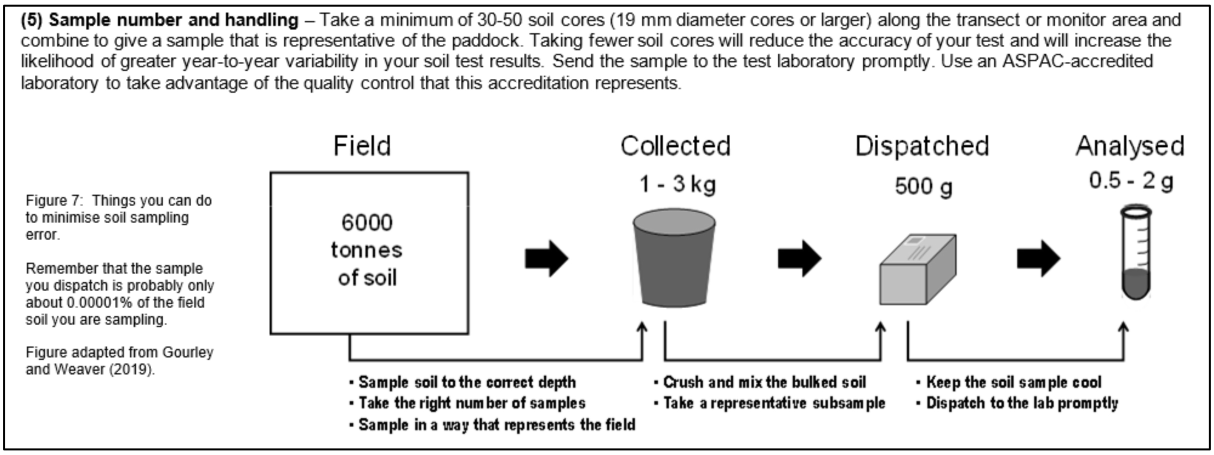
Advice that forewarns farmers about the consequences of very low P buffering for soil testing and soil P management needs to be included in soil testing protocols. Experiences at these sites has directly impacted advice to farmers in the revised "*Five Easy Steps*" P management tool (**see Section 10**). It is now advised: "*When the PBI of your soil is <35 there is an increased risk that P may be lost in runoff and/or leaching. When this is the case, do not use this booklet alone to support your fertiliser decisions. Seek further local advice; check whether P from previous fertiliser applications has moved to depth in your soil profile; consider the merits of using a less soluble form of P fertiliser*."

5.2 Output 4(c) – Development of guidelines for managing soil P fertility in crop-pasture transitions involving serradella pastures.

Data intended to assist development of a guideline for predicting the rate at which soil P fertility can be reduced by withholding P fertiliser was not able to be collected during the project because severe drought conditions persisted during the experiment period. Under these conditions consistent decline in STP concentrations were only observed at one site most probably because "P fixation" reactions in soil were slowed by dry conditions. The observations did, however, reinforce pragmatic observations that the STP concentration of fertilised soil does not decline during droughts as would otherwise be expected. Farmers who are monitoring STP concentrations in farm paddocks can make use of this knowledge and may opt to reduce P fertiliser use immediately after droughts when STP monitoring confirms that soil P fertility has been conserved.

All experiments examining the variability of STP concentrations in farm paddocks confirmed the very high variability in STP levels that occurs over short distances (metres) in paddock areas that are otherwise judged to be uniform. The variability in paddocks that had been cropped was no greater than in undisturbed pasture paddocks. The results highlight the importance of following STP sampling guidelines. Soil sampling frequency is the only variable that farmers can manage (after taking obvious topographical, soil type, etc. variations into account) to obtain an STP measurement that reflects the fertility status of their paddock. In fact, higher sampling frequencies than currently recommended would be ideal.

High sampling frequencies make manual soil sampling of large paddocks a major and laborious task. We know that many practitioners cut corners when soil sampling because of this. It was concluded that sampling frequency advice, and the reasons for it, should be promoted more clearly to farmers (e.g. Fig. 5.2 – 1.)



(5) Sample number and handling – Take a minimum of 30-50 soil cores (19 mm diameter cores or larger) along the transect or monitor area and combine to give a sample that is representative of the paddock. Taking fewer soil cores will reduce the accuracy of your test and will increase the likelihood of greater year-to-year variability in your soil test results. Send the sample to the test laboratory promptly. Use an ASPAC-accredited laboratory to take advantage of the quality control that this accreditation represents.

Figure 7: Things you can do to minimise soil sampling error.

Remember that the sample you dispatch is probably only about 0.00001% of the field soil you are sampling.

Figure adapted from Gourley and Weaver (2019).

- Sample soil to the correct depth
- Take the right number of samples
- Sample in a way that represents the field

- Crush and mix the bulked soil
- Take a representative subsample

- Keep the soil sample cool
- Dispatch to the lab promptly

Figure 5.2 – 1. An example of the sort of advice now included in the “Five Easy Steps” technical booklet that aims to reinforce to farmers the importance of following soil sampling guidelines that will help to ensure reasonable soil test accuracy.

5.3 Recommendations: Activity B4 - Lowering P costs of serradella systems.

5.3.1 Frequency of soil testing

- After taking all obvious sources of paddock variability into account when soil sampling, sampling frequency is the only variable that farmers can manage the less obvious, highly variable distribution of STP in farm paddocks. Sampling frequencies need to be sufficient to obtain an STP measurement that accurately reflects the fertility status of the paddock, but high sampling frequencies make manual soil sampling of large paddocks a major and laborious task. It is important that soil sampling frequency advice, and the reasons for it, be promoted more often and more clearly to farmers to avoid practitioners cutting corners when soil sampling.

5.3.2 Recognising and using legacy P in soil profiles

- It was something of a surprise to realise that surface soil testing is promoted to, and routinely accepted as good practice when farming soils that have very low P retention characteristics. Deep sandy soils with very low PBI values (<35) have the potential for P to leach from the surface layer and were demonstrated to be doing so under serradella pastures. Soils such as these may have legacy P accumulated at depth as a result of previous fertiliser applications and may also be leaking P to the wider environment where it can be a significant pollutant.

- The first step to improving P efficiency on these soil types is to assist farmers to recognise that there may be P accumulated at depth in their soils and to encourage them to factor this into their fertiliser input calculations.

- Serradellas develop very deep root systems in these sandy soils and are presumed to be more ideally suited for capturing P at depth than more shallow-rooted pasture species (e.g. subterranean clover).

- Unfortunately, there is little, if any, work that demonstrates the value of deep P to farmers at present. The lack of responses to P fertiliser applications in the present project was taken as an indication of the value of legacy P reserves, but it has always been necessary to qualify these results because of the prevalence of severe drought during the project. The soils on which the experiments were based had been identified as P-deficient after soil testing by the farm groups using standard sampling protocols. However, it was subsequently estimated using soil P profile information that they often had enough P at depth to be regarded as P sufficient.

- Definitive work examining pasture responses to P fertiliser applied to soils with known and measured legacy P within the root zone would provide the impetus for rapid uptake. Farmers in the Southern Dirt farm group were quick to realise the importance of P deep in their soil profiles and began to measure P profiles in all of their pasture and cropping research projects. However, a protocol for routine soil sampling and for accounting for deep P when estimating P fertiliser inputs is yet to be developed.

5.3.3 Development of alternatives to surface soil testing for deep sandy soils

- Currently, other research (not this project) is seeking to confirm critical surface soil STP concentrations for pasture production that have been revised recently (Gourley *et al.* 2019) for low PBI soils. This sort of research needs to also account for the possibility of legacy P at depth in soil profiles. Critical surface soil STP concentrations remain important to know and will be relevant when sites have not had previous applications of P fertiliser. However, the application of surface soil testing in low PBI soils in the absence of understanding the soil P profile, will provide a misleading guide for efficient use of P fertiliser.

- Alternative soil sampling/testing protocols need to be developed for use in soil types with low P retention. Promotion of more routine assessments of soil P profiles and “deep” soil sampling to account for more of the P that is available within a soil P profile is supported by the current project results. A soil test depth of 0-60 cm may be appropriate, but this should be critically examined. The logistic problems and costs of deep soil sampling soils are large and will be major impediments.

- The true “availability” of legacy STP at depth in these soil profiles needs to be understood and requires investment in systematic research that considers the likely impacts of: (i) soil P buffering capacity, (ii) P accumulation (P-fixation) rate, and (iii) the rooting depth of the crop or pasture being grown.

6 Results and Discussion – (Activity B5) Extending the adaptation range of serradella systems.

6.1 Results and Discussion: Output 5(a) - Conduct participatory research experiments with farmer groups in areas serradella is not used to examine productivity, persistence and potential use.

6.1.1 Experiment network in south-eastern Australia

A network of participatory experiments in permanent pasture areas of southern NSW and Victoria was set up to investigate the persistence and production of serradella in areas where subterranean clover is the dominant sown pasture legume. The experiments were to be conducted on soils with Colwell P values at about two-thirds the critical value for subterranean clover. The overview of sites is presented in Table 6.1 - 1. In each of the regions, four experiments were to be sown to capture variation in landscape/soil factors. Whilst only 12 experiments were planned, by the conclusion of the project 21 experiments had been sown. Each experiment added further opportunities to explore the potential for serradella in areas where they are not being grown.

Considered together, these experiments provided some clear messages about the comparative yield responses of serradella and subterranean clover, persistence of serradella, difficulties in serradella establishment and problems with weed management.

6.1.2 Establishment and persistence of serradella

Serradella failed to establish adequately in a number of experiments. For sites sown on the Monaro in NSW, establishment was inadequate in 6 out of the 10 sites. Drought was often a factor at these locations with insufficient weed control also implicated.

Persistence and/or establishment was also poor in experiments sown in central Victoria. Serradella was only present in appreciable levels in two out of six sites by the end of the experiment period. Serradella emerged in each of the experiments sown but through the combined influences of drought and high competitive pressure from weeds it failed to persist. Of the other two sites sown in central Victoria, in one seedling numbers were very low (Sidn Nth) whilst in the other, serradella established well but regenerated at low densities in the second year (Sidn Sth). Recent observations of these plots indicate good regeneration of serradella in year 3 which suggests a year 2 regeneration issue, probably associated with slow hardseed breakdown during the summer after establishment.

In the experiments sown on the southern tablelands of NSW, serradella persisted well in four of five experiments. Poor establishment at the fifth site in 2018 was largely as a result of drought; subterranean clover did not establish better than serradella but the plots were eventually dominated by subterranean clover because of regeneration from the soil seed bank. Serradella did not persist adequately into the third year at two of these sites. The serradella plots were invaded by subterranean clover and naturalised clovers (chiefly *Trifolium glomeratum*) but were also severely drought affected. It may be notable that at the site where serradella persisted very well (BAB-Hill) late rainfall events in the dry springs of 2017, 2018 and 2019 allowed the serradella species to respond at a time when most of the subterranean clover had died.

Table 6.1-1. Key results and challenges from the participatory trials investigating serradella performance in areas where it is not currently grown.

Site and sowing date	Key results	Learnings/Comments
Monaro Farming Systems (D. Alcock)		
Glenfinnan East aspect (A) 2017	Sown to sub clover and yellow serradellas Avila and Santorini. No clear conclusions – due to inadequate establishment/persistence and high competitive pressure from weeds.	Difficulty in establishment an issue – much better weed control needed for successful serradella establishment. A hint that Avila may have been able to produce more dry matter than sub clover at low soil P but measurements drought affected, so not convincing.
Glenfinnan Sthn aspect (A)2017		
Glenfinnan East aspect (B) 2017	Sown to sub clover, yellow serradella Avila and French serradella Margurita but failed to establish due to high competitive pressure (sorrel, winter grass, naturalised clovers) and severe drought.	
Glenfinnan Sthn aspect (B)2017		
Glenfinnan Eastern aspect 2018	Re-sown in May 2018. No key results, poor serradella establishment and regeneration in 2019. Drought most likely cause of failure – not enough seed set – no evidence of the weed control difficulties of 2017.	Some sub clover survival – but unclear if it was the sown cultivar
Glenfinnan Sthern aspect 2018		
Redcliffe Eastern aspect (A) 2017	Sown to sub clover and yellow serradellas Avila and Santorini. Excellent persistence and regeneration of Avila which produced more dry matter than sub clover at low soil P in 2017 and 2019	Successful sites, despite drought conditions that demonstrated potential of serradella (particularly Avila) at levels of soil P lower than optimum for sub clover
Redcliffe Western aspect (A) 2017	Sown as above. Acceptable establishment and persistence of serradella. Serradella produced as much biomass as clover at low soil P in 2017 only	
Redcliffe Eastern aspect (B) 2018	Sown to sub clover, yellow serradella Avila and French serradella Margurita. Good establishment but very poor regeneration in 2019. Dry matter production in 2018 confirmed superior (East) or equal (West) performance to sub clover by Avila at low soil P.	Evidence that Avila equal-to-superior compared to sub clover in terms of phosphorus use efficiency but only in year of sowing; notable issue of low regeneration by serradellas in year 2 after sowing.
Redcliff Western aspect (B) 2018		
Grassland Society Central Ranges Victoria (L. Warn)		
Bayn1 2016	Serradella established at low density but did not persist in farmer sown trial; sub clover did.	Evidence of poorer establishment by serradella in lower lying areas of the experiment; transient waterlogging suspected.
Bayn2 2018	Serradella and sub clover established at low density but only sub clover persisted into 2019. Weed competition in establishment year a significant issue.	Very dry spring in 2018 may have reduced seed set by all species; persistence of clover may be due to background population.
Past1 2017	All legume species emerged and established in 2017, regeneration of all serradella cultivars poor in 2018 and worse in 2019.	Weeds an issue during establishment. Year 2 regeneration issue for Avila. Serradella persistence poor.
Past2 2017	Adequate emergence and establishment, yellow serradella poor regeneration in 2018, both serradella very poor regeneration 2019.	Year 2 regeneration issue for Avila . Serradella fails to persist.
Sidn Sth 2018	Avila most productive legume in establishment year, Margurita the least. Serradella less competitive with grass than sub clover.	Serradellas persisting, Avila more than Margurita, but subclover better still. In 2019, dry matter production proportional to seedling density.
Sid Nth 2018	Serradella persisted at this site but at very low density. Avila most productive legume in establishment year, Margurita the least.	
Tableland Farming Systems & Bookham Agricultural Bureau (J. Virgona)		
TFS-Western aspect 2017	Serradella established and persisted to some extent but eventually outcompeted by other legume species. Dry matter responses compared to sub clover. Saffron thistle in plots could not be controlled with conventional herbicide (MCPA) because serradella is sensitive.	Drought a major problem at these sites – plants dead before seed production in 2019. Sub clover and naturalised clovers appeared to persist better than serradellas.
TFS-Eastern aspect		
BAB-Hill 2017	Excellent persistence of both serradella species to 2019. Avila higher density than other legume species in 2019. Dry matter results only confirmed superiority of Margurita in establishment year – 2017.	Most successful site of the 5 sown for these groups.
BAB-Flat 2017	Established well and provided dry matter results consistent with previous work but could not continue due to owner	Better communication between site manager and owner warranted!!
BAB-Flat2 2018	Poor establishment due to drought in establishment year and high background population of sub clover (serradella less than 20% of legume dry matter production). Serradella species persisted through first year but at lower levels than sub clover.	Poor establishment and performance mainly due to drought but invasion of background legume species suggests critical need for good paddock preparation wen establishing serradella.

In summary, problems establishing serradellas were relatively common and were attributed to:

(i) **poor prior weed control and/or drought**. This may indicate a need for greater attention to weed control at sowing because subterranean clover appeared to have been able to cope under these conditions. However, it is highly likely that the apparent success in establishing subterranean clover was due to its regeneration from an existing seed bank, rather than the clover necessarily being more successfully established from the sown clover seed.

(ii) **competition from subterranean clover germinating from the soil seed bank**. This is likely to be a widespread issue in areas where subterranean clover has been grown for many years.

(iii) **low year 2 regeneration of serradellas**, most probably due to slow seed softening during the first summer after establishment.

(iv) in one instance only, **poor establishment in low lying areas of the paddock** – anecdotally attributed to intolerance of transient waterlogging.

6.1.3 Serradella and weed management

There are some issues relevant to the management of weeds in serradella production systems that were highlighted as a result of these experiments. At some sites (in southern NSW and on the Monaro), hand rogueing of weeds, particularly saffron thistle, took place to reduce competition from this species. In subterranean clover-based pastures on farms, saffron thistle is managed by applications of MCPA. Serradella is susceptible to MCPA so this herbicide is not an option to control saffron thistle or other species that are controlled by this herbicide. In Western Australia and the NSW Riverina serradella is often grown as a pasture monoculture ley. In the pasture-based systems of eastern Australia, serradella would be sown with a perennial grass. This also reduces the range of herbicides that can be applied if serradella is present. The experiments in Western Australia used imazamox (Raptor) which controls a range of grass and broadleaf species. This type of herbicide could not be used in permanent grass-based pasture. Hence, the development of management packages for serradella will need to include herbicide options for common weeds that require control but do as little harm as possible to serradella.

6.1.4 Dry matter production: comparing serradella with subterranean clover

The participatory experiments were not intended or designed to verify the claimed high P efficiency of serradellas relative to subterranean clover. However, the measurements taken in these experiments can be used for that purpose. It is important to remember that in the P-response experiments conducted by Sandral *et al.* (2019), all legume species were grown as monocultures and re-sown each year so that differences between species in response to soil P levels could be fully expressed and were not confounded by issues of plant density. In the participatory experiments, legumes were not grown as monocultures and in many of the site-by-year combinations serradella was either not present or at much lower density than subterranean clover. However, there were occasions when plant density was similar for each of the sown legume species; this usually occurred in the year of establishment and, in a few cases, in subsequent years. Of the 16 instances where at least two of the species were present at comparable plant densities, on 13 occasions the yield of a serradella species was greater than that of the subterranean clover (Table 6.1-2). There were four instances when the yield of the serradella was equivalent to subterranean clover; this coincided with high STP levels (i.e. higher than the critical STP requirement of subterranean clover). The pattern was encouraging – spring herbage production by the serradella(s) grown in soils with P levels near

Table 6.1 - 2. Relative production of dry matter between legume cultivars. "Year" denotes when the result was obtained (not year of sowing).

Site	Year	Soil test P (mg/kg)	Rankings in terms of legume dry matter production during spring (all differences were significant unless specified)
SIDN Nth	2018	14-16*	Avila > subterranean clover, Margurita density too low. Soil P levels higher than ideal.
SIDN Sth	2018	17-20*	Avila > subterranean clover, Margurita density too low. Soil P levels higher than ideal.
TFS-West	2017	23	Margurita > Avila > subterranean clover.
TFS-East	2017	24	Margurita > than subterranean clover. Avila intermediate and not significantly different from other two cultivars
BAB-Hill	2017 2019	23 9	Margurita > (Avila = Subterranean clover). No differences between cultivars despite Avila having twice the seedling density of others
BAB-Flat	2017	13	Margurita > (subterranean clover= Avila)
Redcliffe East (A)	2017 2019	14-15	Avila > subterranean clover, Santorini intermediate and no different from other cultivars Avila > other cultivars but had much higher seedling numbers
Redcliffe West (A)	2017	12-21	Avila > subterranean clover, Santorini intermediate and no different from other cultivars
Redcliffe East (B)	2018	24-30	Avila > subterranean clover. Margurita intermediate and not different from other cultivars
Redcliffe West (B)	2018	36-51	Subterranean clover = Avila = Margurita. Soil P levels too high
Glenfinnan South (A)	2017	49-52	(Avila = subterranean clover) > Santorini. Very high soil P. Avila also greatest seedling density.
Glenfinnan East (A)	2017	20-25	Avila > (subterranean clover = Santorini). Santorini density much lower than others
Glenfinnan South (B)	2018	58-81	(Avila = Margurita) > subterranean clover. Seedling densities equal but low. Very high soil P.
Glenfinnan East (B)	2018	40-41	(Avila = Margurita) > subterranean clover. Equal seedling density.

*Olsen P – all other results are Colwell P.

the target STP for serradella was often greater than that of subterranean clover provided that the alternative legumes were present at a comparable plant density. Maintaining high densities of serradellas (i.e. serradella persistence) is the major issue constraining their adoption in permanent pastures.

The serradella yield results from the participatory experiments were not entirely consistent with those of Sandral et al (2019) who found that yellow (cvs Avila or Santorini) did not yield as well as French serradella (cv. Margurita). In the participatory experiments cv. Avila was the most productive serradella cultivar in many of the experiments. However, cv. Margurita was the most productive cultivar at low levels of soil P at southern tableland sites (TFS and BAB sites, Table 6.1 - 2) which were located in a similar environment to some of the Sandral *et al.* (2019) experiments. It is concluded that there may be much yet to learned about the performance of serradella species/cultivars at a regional level.

6.2 Results and Discussion: Output 5(b) - Conduct field experiments with serradella to quantify the range in serradella flowering time, seed production, hardseededness/ breakdown, persistence, seedling recruitment and survival traits that underpin cultivar development.

6.2.1 Flowering time

6.2.1.1 Flowering date and flowering date stability

Median flowering dates (the date on which 50% of plants of each cultivar had produced their first flower) of the cultivars of each species were usually highly correlated when compared among the sites in 2017 and 2018 and between sowing dates in 2018 ($R^2 = 0.79$-0.99; Figures 6.2 - 1 and 6.2 - 2) indicating that, in relative terms, flowering date rankings of the cultivars were expressed consistently at all locations. However, unlike the situation usually anticipated for subterranean clovers, the maturity-type rankings of the serradellas were often not similar among the test locations (see **Section 6.2.1.2**).

The time from sowing to median flowering often differed among the sites and was usually longer at the three eastern sites than in Perth (e.g. commercial cultivars of French serradella flowered an average of 42 days later at Canberra than at Perth). The only exceptions were mid-late maturing subterranean clovers for which the time to flower tended to be similar at all locations. Time from sowing to flowering in Perth was most similar to that in Tamworth, and least similar to that in Canberra.

It is essential for persistence of annual species in permanent pastures, that pasture varieties display relatively stable flowering dates irrespective of sowing (germination) date. This is necessary in self-regenerating swards because the date on which the opening seasonal rainfall occurs in southern Australia is highly variable, yet flowering must reliably occur after periods with high frost risk. Frost kills flowers and developing pods and limits seed production (Donald 1970). Farmer's control this by choosing a cultivar of suitable “maturity type” for the growing season length at their location. Implicit in this choice is the expectation that cultivars will have stable flowering dates and will, therefore, flower reliably after the risk of frost has declined.

Flowering date stability was tested by sowing subterranean clover and serradella varieties at times that represented a moderately-early and a late break to the growing season. The median flowering dates of all subterranean clover cultivars was highly stable (varying by only 1-2 days (average for all five cultivars) when sown at the earlier date (~6 weeks earlier) in Perth, Tamworth and Cowra, and moderately so at Canberra, where the average median flowering date of the five cultivars occurred ~7 days earlier when sown at the earlier date (Fig. 6.2 - 3).

In contrast, median flowering dates of many serradellas were “unstable” to “very unstable” at all of the sites in the national experiment. For example, in Perth early sowing brought forward the median flowering dates of the yellow serradella cultivars by 10-25 days and that of the French serradellas by 22-31 days, depending on the cultivar (Fig. 6.2 – 4; Table 7.2 - 1). In Canberra, similar shifts in median flowering date were observed for the yellow serradellas, but the median flowering dates of French serradella cultivars occurred 13-42 days earlier depending on the cultivar (Fig. 6.2 - 4).

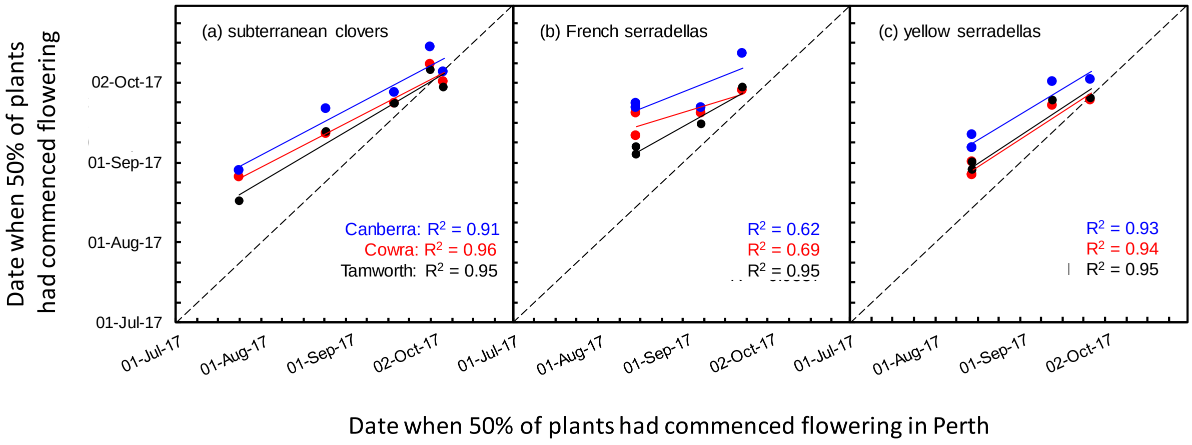


Figure 6.2 - 1. Relationship between median flowering date in Perth (when sown 9 May 2017) and that recorded in Canberra, Cowra and Tamworth in 2017 after sowing dates of 27 April, 5 May, and 15 May, respectively.

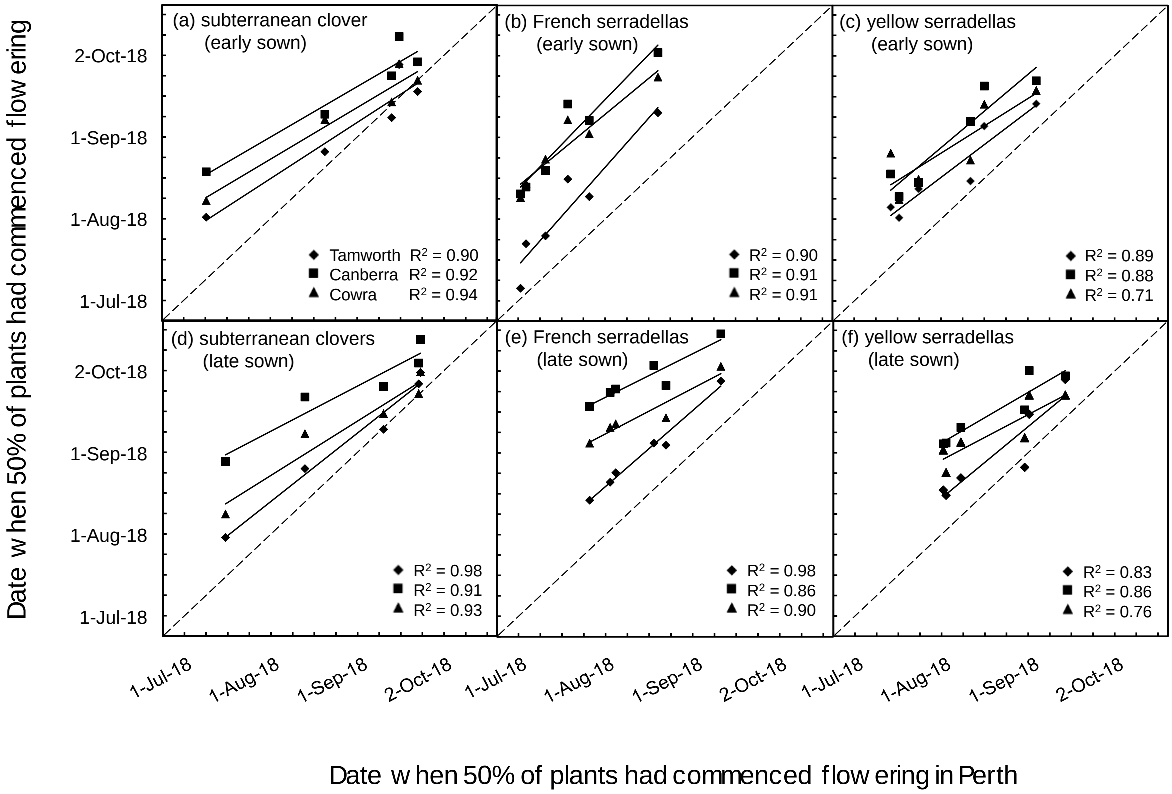


Figure 6.2 - 2. Relationship between median flowering date in Perth and that recorded in Canberra, Cowra and Tamworth in 2018 after an early sowing in the week of 19 March (4 April at Cowra), and a later sowing date in the week of 1 May (7 May at Cowra).

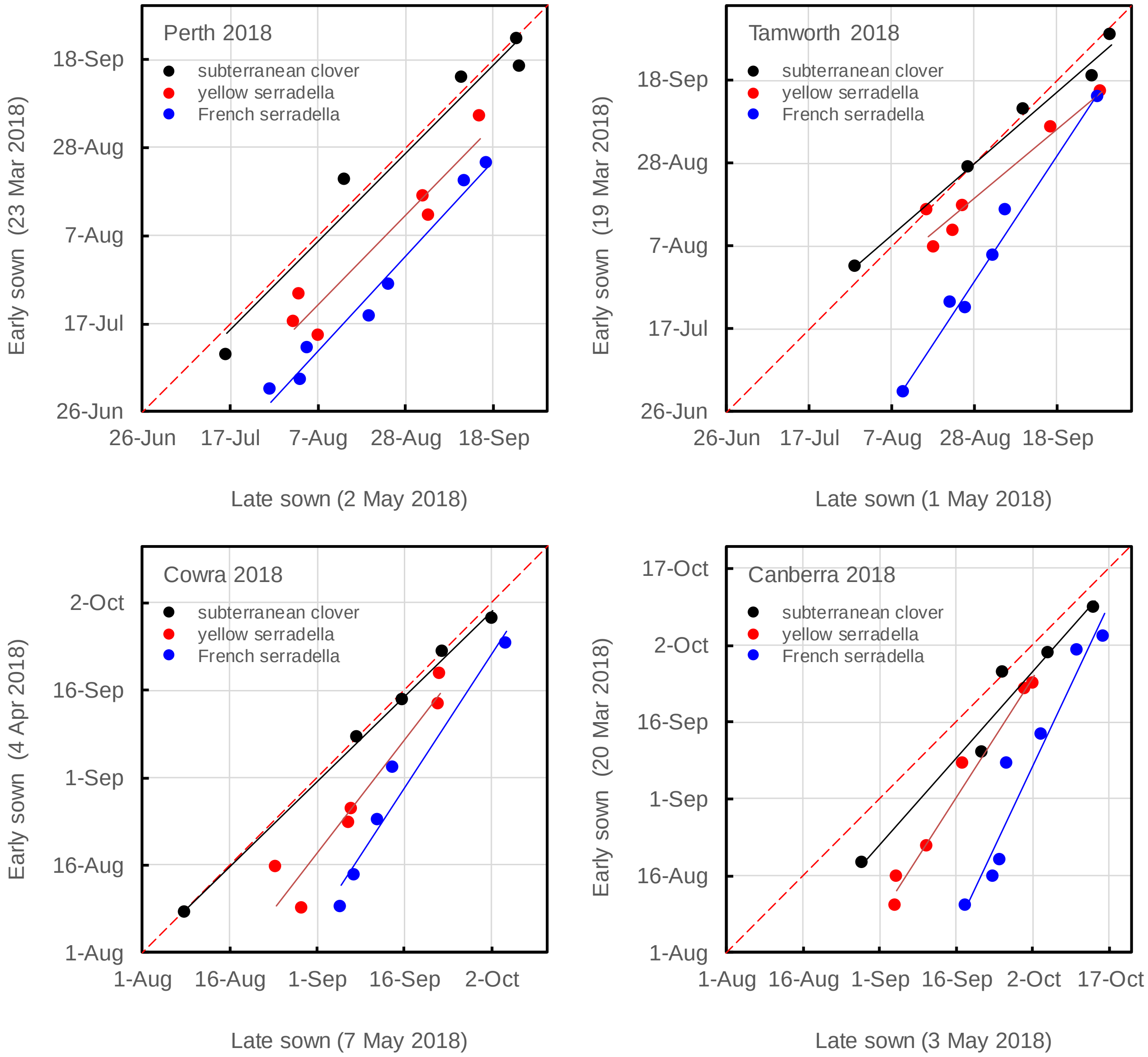
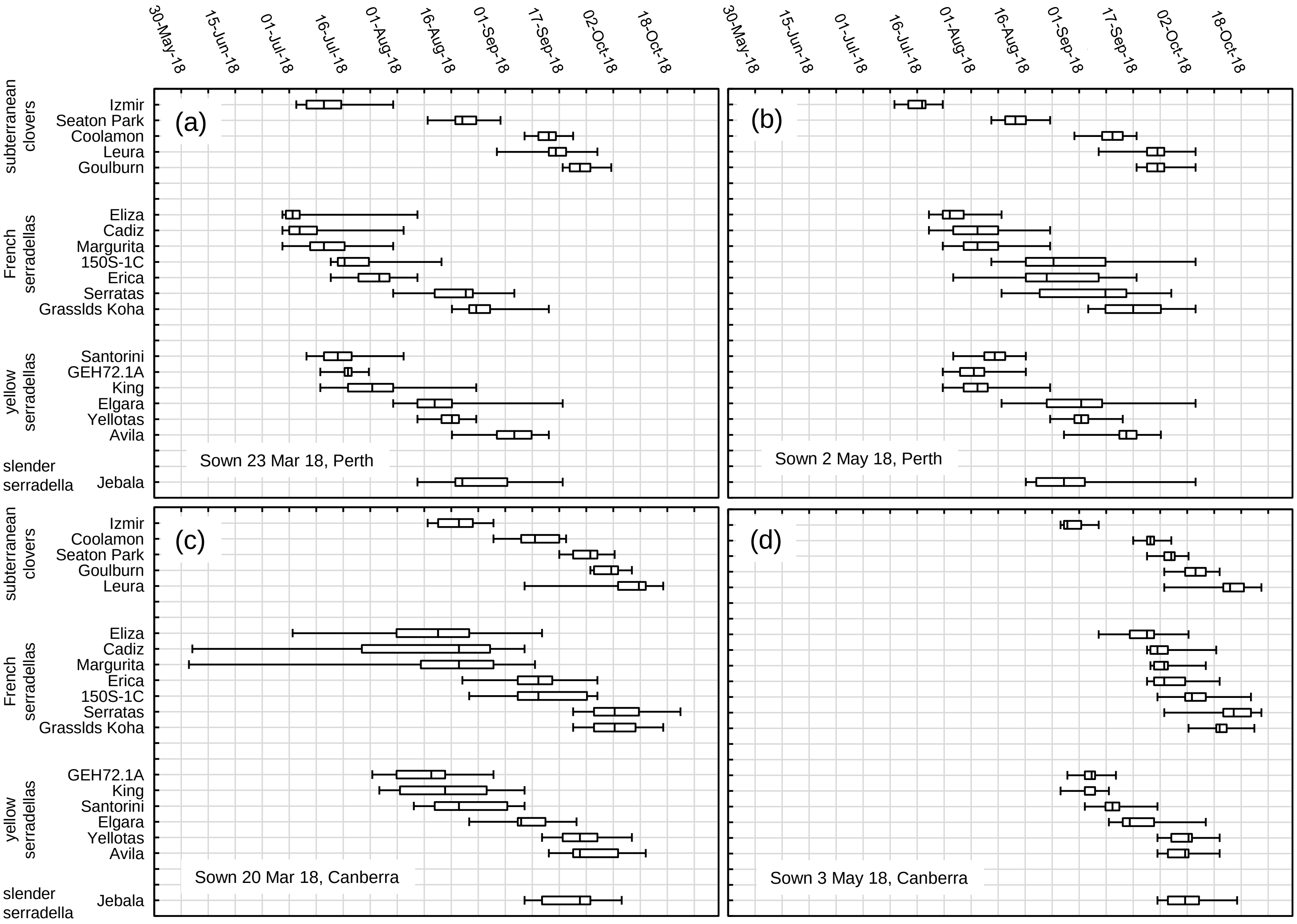


Figure 6.2 - 3. Flowering date stability of subterranean clover control varieties (black circles) compared with yellow serradella (red circles) and French serradella varieties (blue circles) when grown in Perth, Tamworth, Cowra and Canberra in 2018. Flowering date stability is indicated by flowering dates that do not change appreciably when sowing date is varied (e.g. see results for subterranean clover varieties at Perth, Tamworth and Cowra0. In this experiment, flowering date of each variety after “late” sowing (first week of May) was compared with its flowering date after “early” sowing (between 19 March and 4 April). The dashed red line indicates where varieties with “perfect’ flowering date stability (i.e. no change in flowering date) would be plotted; deviations below this line indicate instability in flowering dates (i.e. earlier flowering when a variety is sown early).

Figure 6.2 - 4 (*following page*). Box plots of the dates on which flowering commenced within populations (max. n=60) of the serradella and subterranean clover cultivars sown early and late in 2018 at (a, b) Perth and (c, d) Canberra. The boxes show the interquartile range (i.e. when 25% to 75% of plants began flowering); middle bar is the median flowering date and the whiskers show the dates of first and last plants to commence flowering. NB: Grasslands Koha was included at these sites in addition to the core cultivars used at all sites in the national experiment.


30-May-18
15-Jun-18
01-Jul-18
16-Jul-18
01-Aug-18
16-Aug-18
01-Sep-18
17-Sep-18
02-Oct-18
18-Oct-18
(a)
(b)
(c)
(d)
subterranean clovers
French serradellas
yellow serradellas
slender serradella
Izmir
Seaton Park
Coolamon
Leura
Goulburn
Eliza
Cadiz
Margurita
150S-1C
Erica
Serratas
Grasslds Koha
Santorini
GEH72.1A
King
Elgara
Yellotas
Avila
Jebala
Sown 23 Mar 18, Perth
Sown 2 May 18, Perth
Izmir
Coolamon
Seaton Park
Goulburn
Leura
Eliza
Cadiz
Margurita
Erica
150S-1C
Serratas
Grasslds Koha
GEH72.1A
King
Santorini
Elgara
Yellotas
Avila
Jebala
Sown 20 Mar 18, Canberra
Sown 3 May 18, Canberra

Flowering dates of all French serradellas were “very unstable” at all locations; cvs Eliza, Cadiz and Margurita was particularly variable following early sowing as shown by the boxplots that compare data from Perth and Canberra (Fig. 6.2 - 4). Their median flowering dates moved forward substantially after the varieties were sown early. The duration of flowering by these varieties was also highly attenuated.

The late season cultivars of French serradella (cvs Serratas and Grassland Koha) and yellow serradella (cvs Yellotas and Avila) expressed the most stable flowering dates of the serradella cultivars within each serradella species when grown in eastern Australia. However, they were no more stable than any other cultivar when grown in Perth.

6.2.1.2 Serradella maturity type

It is to be expected that there will be some variation in flowering dates of any one variety due to differences in seasonal temperature patterns, and differences in the combinations of temperature and photoperiod among the experiment sites. However, the unstable nature of flowering dates among the serradella varieties was a significant and unexpected confounding factor in their maturity type classification. We avoided this complication by using data from the late sowing treatments to assign a maturity type to the serradella cultivars. Flowering dates observed in 2017 were plotted against those observed in 2018 to obtain a “typical” maturity rank and classified each serradella variety relative to the maturity types assigned previously by Lattimore and McCormick (2012) to the subterranean clover “control” varieties (Figs 6.2 – 5a and 6.2 – 5b).

The experiments demonstrated that a wide range in maturity types (similar in range to early- through late-maturing subterranean clovers) are available among the yellow and French serradella cultivars (Figs 6.2 – 5a and 6.2 – 5b). However, there were some significant gaps in maturity type coverage of the current serradella varieties (e.g. no very early serradella varieties). It was necessary to reclassify the maturity-type of a number of the serradella varieties depending on the site at which they were grown. Notable examples of this were cvs Margurita and Cadiz which were early-maturing varieties in Perth, early-mid season in Tamworth, and of mid-season maturity in Cowra and Canberra. The maturity types of other varieties (e.g. cv. Yellotas) also varied at the margin indicating that local experience will always be important when evaluating cultivar performance.

The data can be used to correct and extend the information available to farmers regarding the maturity type of serradellas in current use (see Fig. 5.2 – 6 and Table 7.2 - 1). However, this information must now be qualified by an understanding of the unstable nature of flowering dates in many of the serradellas.

Median flowering dates of the serradellas were highly correlated among the test locations, but their maturity-type classifications often differed depending on the location. It is common practice to predict subterranean clover maturity type based on flowering dates in Perth. However, maturity-type classification of the serradellas in Perth were not sufficiently indicative of maturity type across southern Australia for this to be considered an adequate practice for the current cohort of serradella cultivars.

Key messages include:

(i) many serradella cultivars differed substantially in their maturity type when grown in different locations indicating that local experience with the cultivars remains important when formulating recommendations to farmers,

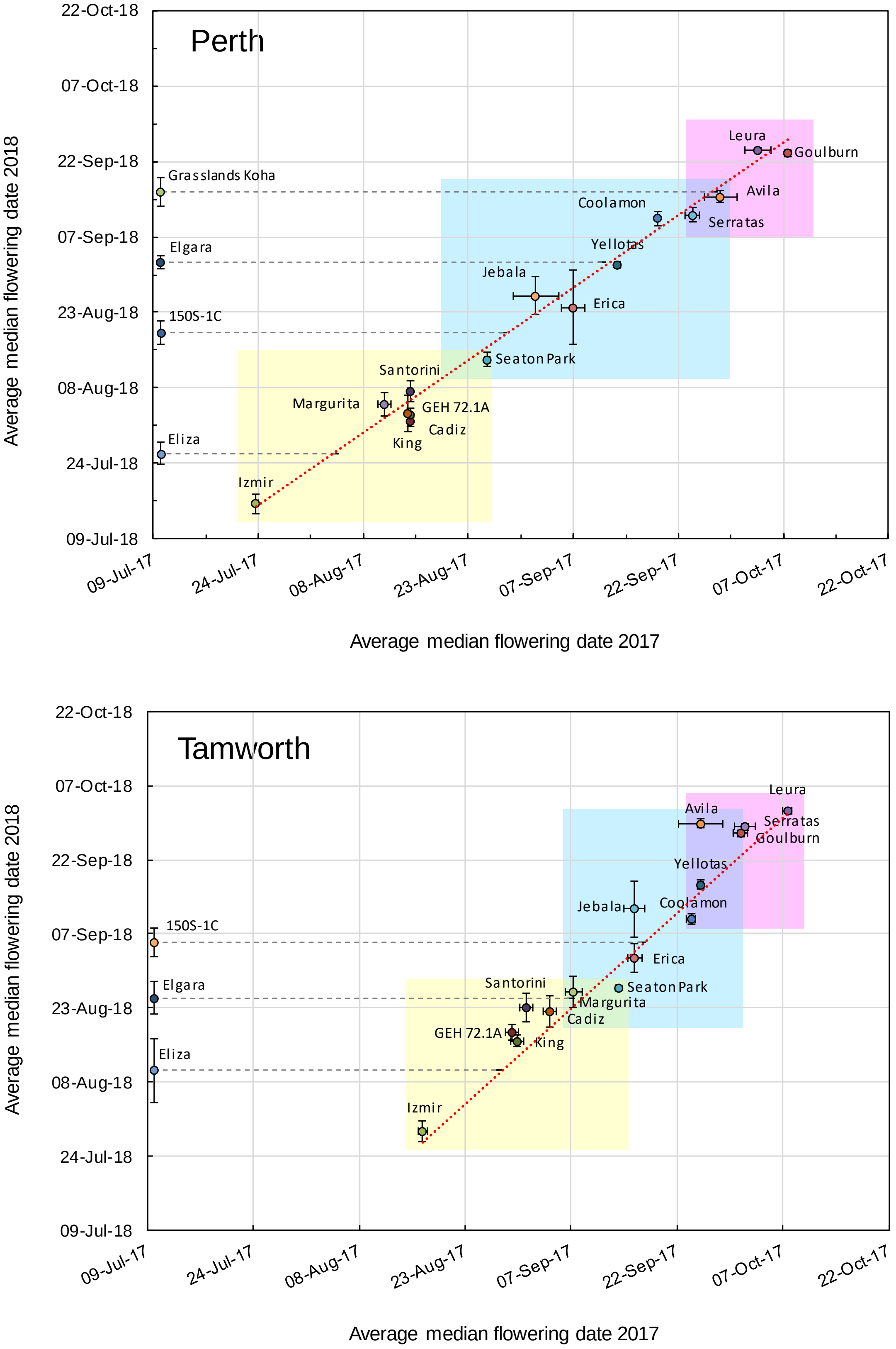


Figure 6.2 - 5a Flowering dates in 2017 and 2018 after “late” plantings were graphed relative to each other for Perth and Tamworth to facilitate determination of maturity classes based on those assigned to subterranean clovers by Lattimore and McCormick (2012). The red dashed line was plotted using only the data from the subterranean clover varieties. Key: early (yellow), mid (blue) and late-season (magenta) maturity.

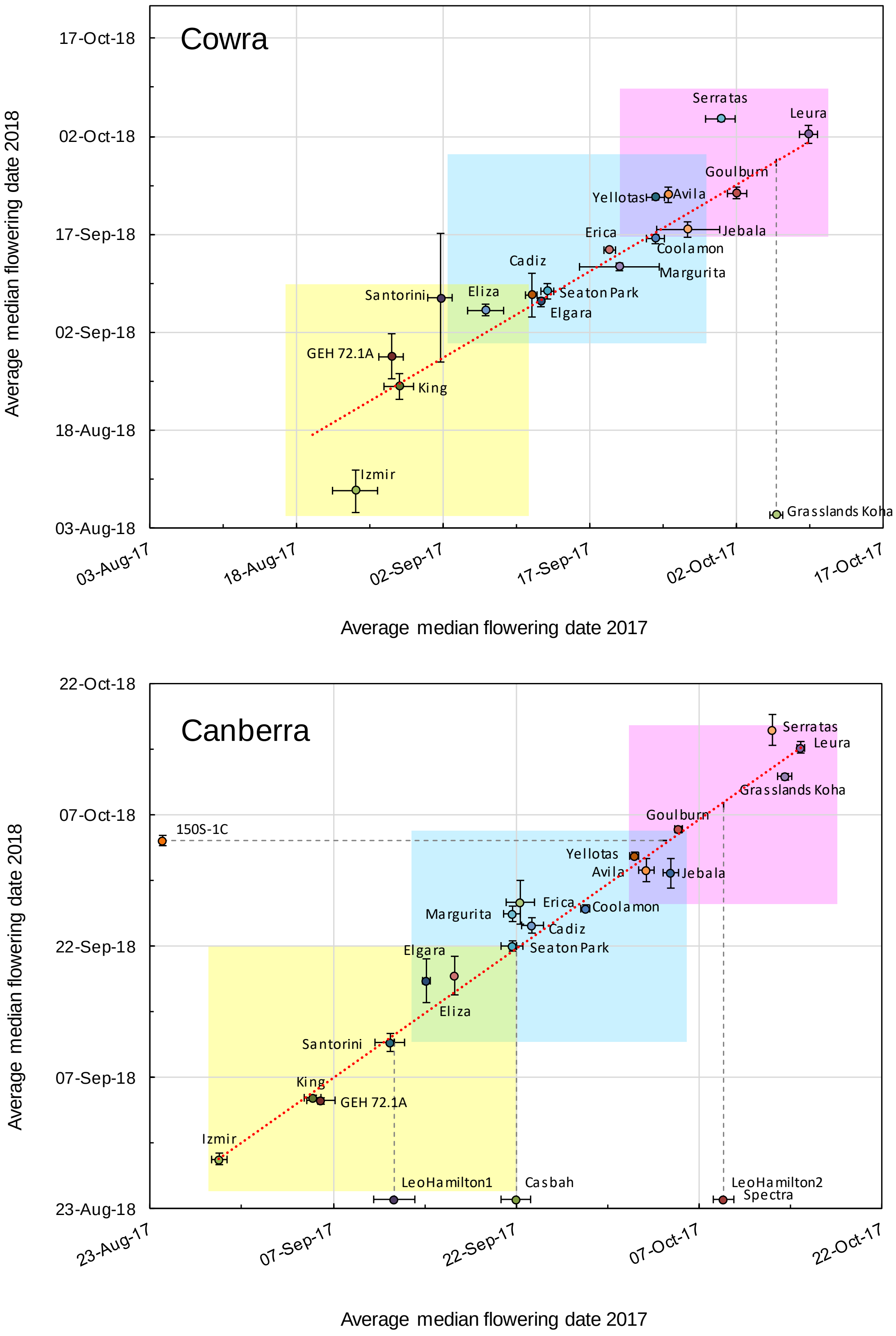


Figure 6.2 - 5b Flowering dates in 2017 and 2018 after “late” plantings were graphed relative to each other for Cowra and Canberra to facilitate determination of maturity classes based on those assigned to subterranean clovers by Lattimore and McCormick (2012). The red dashed line was plotted using only the data from the subterranean clover varieties (NB: Izmir excluded at Cowra, due to inconsistent flowering dates between years at this location). Key: early (yellow), mid (blue) and late-season (magenta) maturity.

Pasture varieties used in New South Wales 2012–13,
M Lattimore, L McCormack (eds), NSW DPI.

* Yellow serradella varieties

Variety/brand	Comment	Main seed source	Revised maturity type advice based on National experiment
Mid – late season, high hard-seed levels			
Avila		Public variety	✓
Early – mid season, high hard-seed levels			
Charano Ⓟ		Seedmark	
Elgara	mod to high tol Al	Public variety	✗ Early-mid (Tamworth, Cowra, Canberra), Mid-season (Perth)
Madeira	sensitive to Al	Public variety	
Santorini Ⓟ		Ballard Seeds	✗ Early-season
Yelbini Ⓟ		Ballard Seeds	
Early season, medium hard-seed levels			GEH72.1A - also early season
King	suits acid sands with high Al	Ballard Seeds, GN Lummis	✓
Yellotas	50:50 hard seed	Auswest Seeds, Tasglobal Seeds	✗ Mid-season (Perth), Mid-late (Tamworth, Cowra, Canberra)

* French (pink) serradella varieties

Variety/brand	Comment	Main seed source	Revised maturity type advice based on National experiment
Soft-seeded, erect growth habit			
Cadiz Ⓟ	mid maturity, extended flowering	Ballard Seeds, Seedmark	✗ Early (Perth), Early-mid (Tamworth), Mid-season (Cowra, Canberra)
Eliza Ⓟ	early–mid maturity, RLEM tolerant, Moderately tolerant of aphids	Ballard Seeds	✗ Early-season (Perth, Tamworth), Early-mid (Cowra, Canberra)
Hard-seeded, prostrate growth habit			
Erica Ⓟ	mid maturity, grazing tolerant, moderately tolerant of RLEM	Ballard Seeds, Seedmark	✓
Hard-seeded, erect growth habit			
Margurita Ⓟ	mid maturity, hard-seeded	Ballard Seeds	✗ Early (Perth), Early-mid (Tamworth), Mid-season (Cowra, Canberra)
Serratas	soft-seeded	Tasglobal Seeds, Auswest Seeds	✗ Mid-late (Perth, Tamworth), Very late (Cowra, Canberra)

Hackney B, Rodham C, Piltz J (2013) Using French serradella to increase crop and livestock production (Meat & Livestock Australia)

Table 1. Comparison of French serradella varieties ******

	Cadiz	Eliza	Margurita	Erica	Grasslands Koha	Serratas
Maturity	Mid	Early	Mid	Mid	Late	Late
Days to flowering #	125	100	121	121	–	175
Hard seed level	Low (5%)	Low (5%)	Moderate (55%)	Moderate (55%)	Low (0%)	Low (0%)
Revised maturity type advice based on National experiment	✗ see above	✗ see above	✗ see above	✓	✓	✗ see above

\# days to flowering will be misleading unless target district is specified; this is impractical for broad recommendations

GENERAL NOTE OF CAUTION: *when germinating on 'early' opening rainfall, many early and mid-season serradella cultivars may not exhibit stable flowering dates as is expected of equivalent subterranean clover cultivars. The flowering dates of cvs GEH72.1A, King, Eliza, Cadiz and Margurita, in particular, may be advanced significantly under these circumstances. This may cause them to have unreliable seed production. Persistence in permanent pastures may be affected adversely.*

Figure 6.2 - 6. The most recent farmer serradella information sheets can now be substantially updated with improved maturity-type classifications, and information concerning flowering date stability, hardseededness and seed softening. For example, the hardseededness descriptions of cvs King and Yellotas (above) are also incorrect (**see Section 6.2.3**).

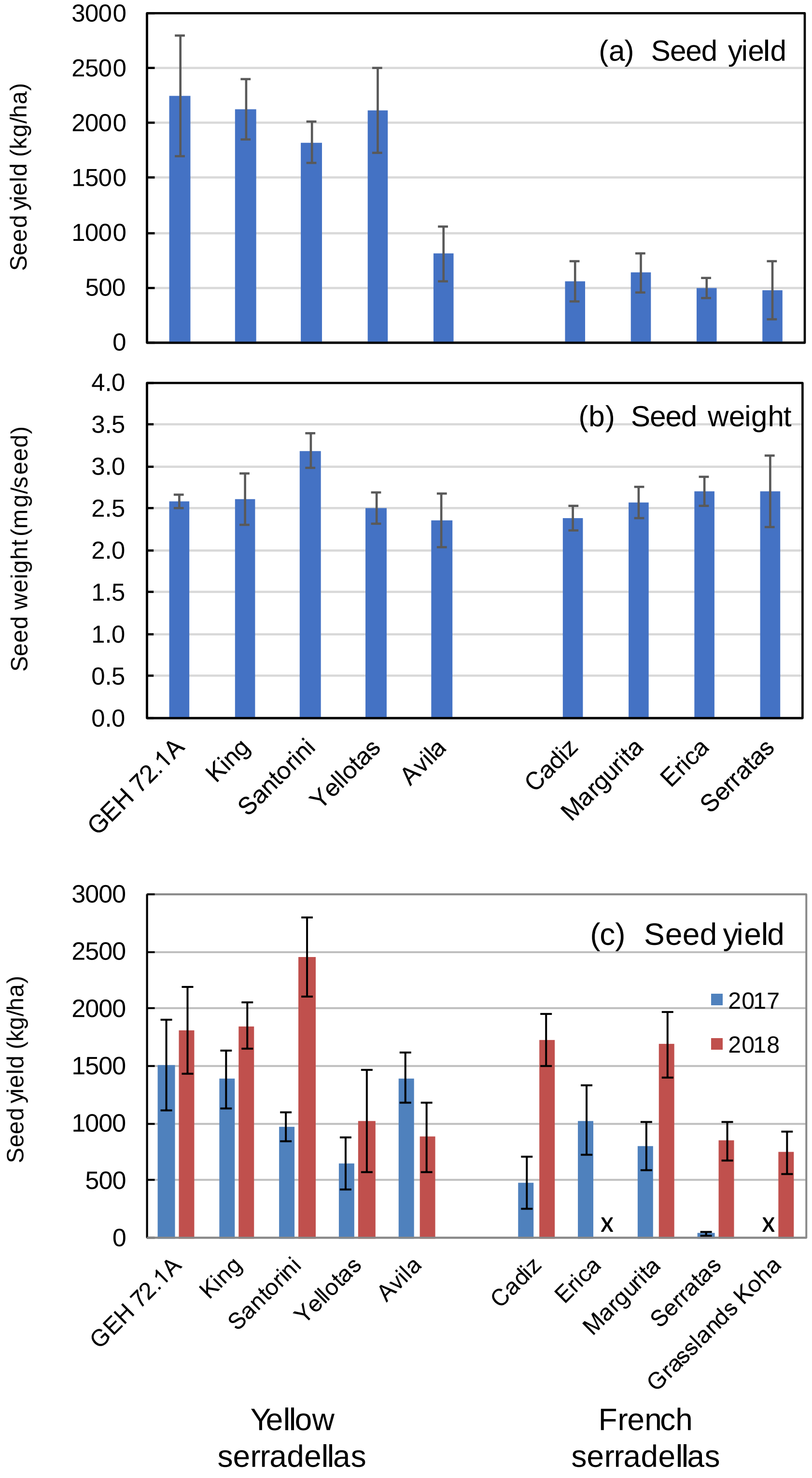


Figure 6.2 - 7. Seed yields and seed weights of a representative group of yellow and French serradellas grown in microswards at (a, b) Canberra, 2017 and (c) Cowra, 2017 and 2018. Due to shortages of viable seed cvs Erica and Grasslands Koha could not be grown at Cowra in both years. Bars indicate 2x SE.

(ii) the unstable flowering dates exhibited by many serradella varieties (all French serradellas and, in particular, early and mid-season serradella cultivars) indicate a need to provide additional cautionary information to farmers, to the effect that: *when germinating on 'early' opening rainfall, many early and mid-season serradella cultivars may not exhibit stable flowering dates as is expected of equivalent subterranean clover cultivars. The flowering dates of many serradellas may be advanced significantly under these circumstances. This may cause them to flower during frost periods and to have unreliable seed production. Persistence in permanent pastures may be affected adversely.*

(iii) specification of maturity types using days-to-flowering, as is occasionally the practice, is misleading and should not be promoted.

6.2.2 Serradella seed production

Seed yields in microswards: Serradella seed size serradella was ~2.5 mg for most cultivars of both yellow and French serradellas (Fig. 6.2- 7). The exception was French serradella cv. Erica, which had a slightly heavier seed (3.1 mg). However, seed production by the yellow and French serradella cultivars was highly variable between sites, growing seasons and genotypes, with no obvious pattern to the variability. In 2017, most cultivars of yellow serradellas produced between 1.5- to 4-fold more seed per hectare (i.e. 1.5 - 2 t/ha) than the French serradella varieties (0.5 – 1.8 t/ha), but in 2018 (at Cowra) seed yield among the cultivars was more variable and there was no obvious difference in the average seed production by the yellow serradella and French serradella groups. A few serradella varieties exhibited large unexplained variation in seed production between years and/or sites.

It has been noted in farmer advisory material that dryland yields can be highly variable and depends on good rainfall, the variety being grown and management. Typical yield from well-managed dryland serradella pasture is ~300 kg hulled seed/ha (NSW DPI, undated). However, an irrigation crop of cv. Avila at Cowra may yield 2.5 t of seed/ha, which is comparable to the highest yields obtained for yellow serradella varieties in the present experiments.

With only one exception, the minimum amount of seed production by the monocultures in the present experiments was ~500 kg/ha among the cultivars and years. Assuming an average seed size of 2.5-3.0 mg, this translates to a minimum seed production of about 16,700-20,000 seeds/m^2.

Seed reserves and seedling establishment in a soil fertility experiment: Despite achieving the planned Colwell P targets for soil P management in this experiment, the prevailing dry seasonal conditions resulted in low (deficient) availability of P for all of the legume varieties as shown by the fact that herbage yields were relatively low and responded to all levels of Colwell P concentration (Fig. 6.2 - 8). This is analogous to the P deficient condition induced by droughts in northern NSW (**see explanation in Section 4.2.2.2**).

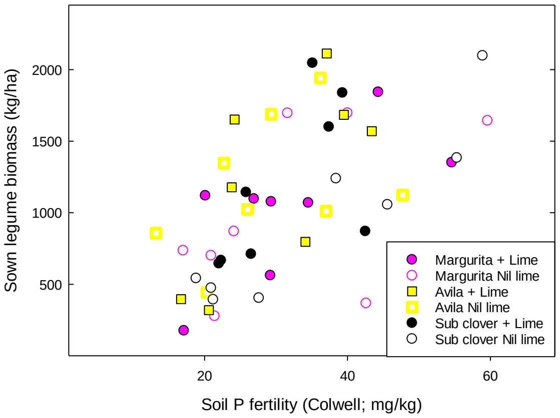


Figure 6.2 - 8. Relationship ($R^2 = 0.37$) between topsoil Colwell P concentration (mg/kg) and winter legume biomass (kg/ha) in year 3 at Gunning.

Under these conditions, there was a highly significant ($P<0.001$) difference in the seed reserves accumulated over two years by the three species (Fig. 6.2 - 9). Yellow serradella (cv. Avila) accumulated a seed reserve that was 1.4-fold larger than that of French serradella (cv.

Margurita) and at least 5-fold greater than that of subterranean clover (cv. Leura). Averaged across all species and lime treatments there was ~30% more seeds/m^2 in the high P compared to the low P treatment although differences were only significant at the P=0.08 level. There was no effect of lime nor any significant interaction between legume, lime or P treatments on total seed reserves (P>0.05).

The density of sown legume seedlings in the experiment increased progressively over time (Fig. 6.2 - 10). In the establishment year, seedling densities were low (range: from 46 subterranean clover plants/m^2 to 132 French serradella plants/m^2) reflecting the difficult seasonal conditions after sowing. Yellow serradella (cv. Avila) was particularly notable because its seedling density increased from amongst the lowest (years 1 and 2) to being the highest in years 3 and 4. There was no main effect of soil P fertility on legume density in any year, but lime depressed seedling densities by 11-17% in years 3 and 4 (P<0.05). In year 4, a significant cultivar x P interaction (P<0.05) was observed. High P improved the density of yellow serradella seedlings (+15%), reduced the density of the clover (-21%) and had no impact on the French serradella. Irrespective of this, the regenerating densities of all legumes were relatively high (1800-4000 plants/m^2) by year 4 and adequate for the coming growing season (year 2020).

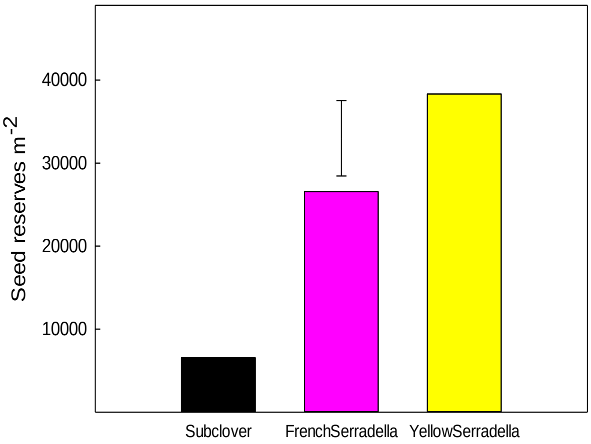


Figure 6.2 - 9. Total seed reserves (seeds/m^2; averaged over P and lime treatments) developed after two years under subterranean clover (cv. Leura), French serradella (cv. Margurita) and yellow serradella (cv. Avila) in the Gunning P x lime experiment. Only 3.5% of the total seed count could be attributed to background levels of subterranean clover (as measured in the serradella treatments) Bar indicates LSD (P=0.05).

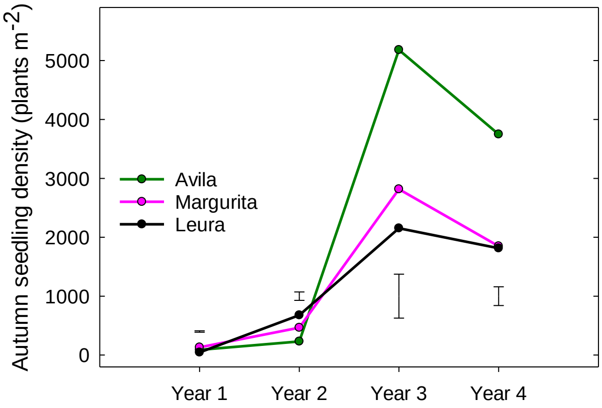


Figure 6.2 - 10. Autumn seedling density of yellow serradella (cv. Avila), French serradella (cv. Margurita) and subterranean clover (cv. Leura) (averaged over P and lime treatments) over four years at the Gunning site. Bars indicate LSD (P=0.05) for comparison within years.

Seed production in serradella persistence experiments:

Serradella almost universally produced more seed in the establishment year than all subterranean clover cultivars at the three sites established in 2017 (Fig. 6.2 - 11). The magnitude of difference between the serradella and subterranean clover cultivars was greater at Bombala and Goulburn compared to the Bigga site, with no difference (P > 0.05) between total seed yield of the French and yellow serradella cultivars. Subterranean clover cv. Goulburn consistently had the highest seed yield of any cultivar of that species across all sites. This was reflected in its year 2 regeneration data.

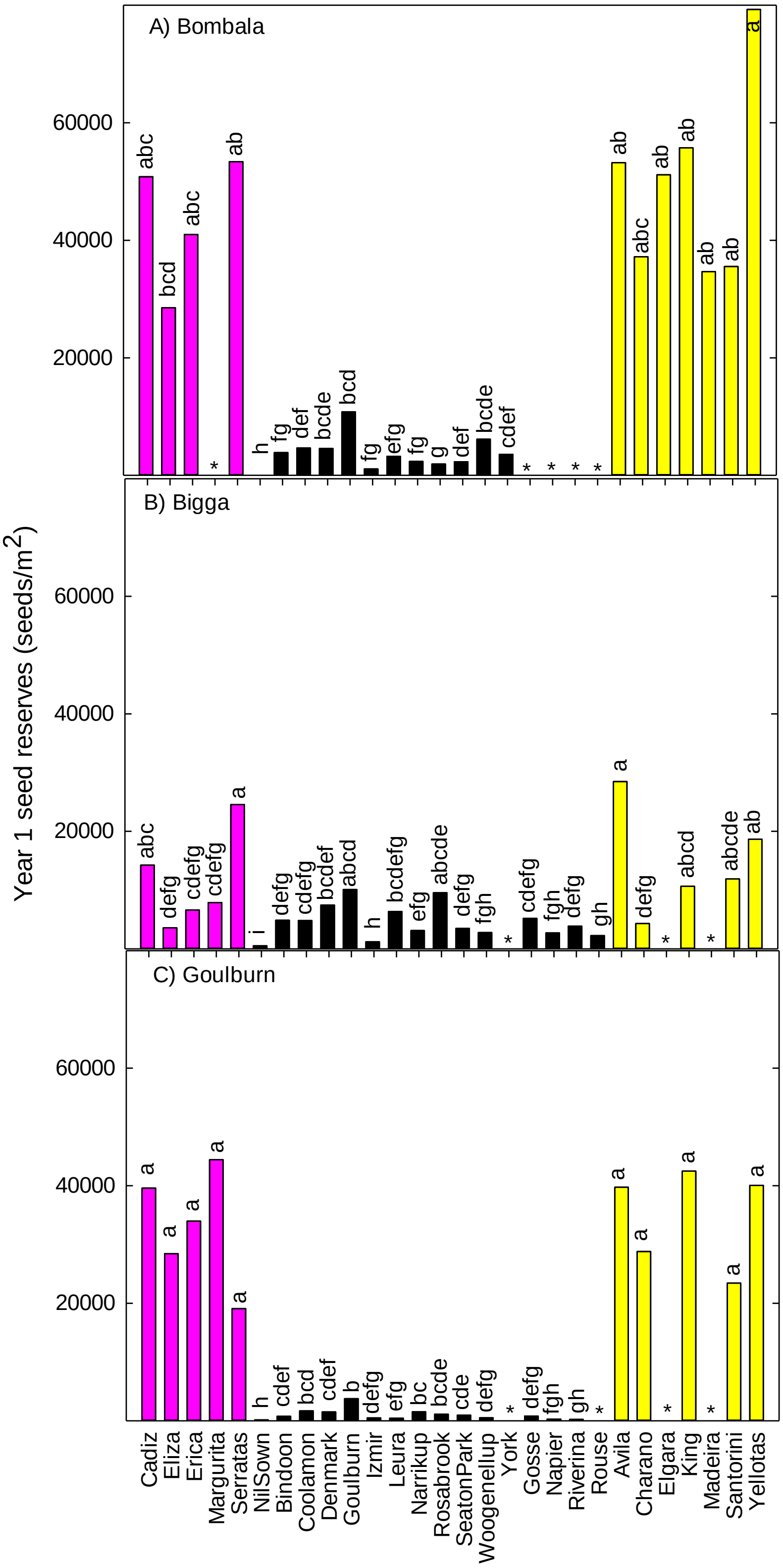


Figure 6.2 - 11. Seed reserves (seed number/m^2) of French serradella (pink), subterranean clover (black) and yellow serradella (yellow) cultivars at the end of year 1 at the 2017-sown experiments at (A) Bombala, (B) Bigga and (C) Goulburn. Data points with the same letter in each panel indicate differences were not significant ($P<0.05$; data transformed for analysis). The 'NilSown' treatment indicates levels of background subterranean clover at each site. * Indicates that the cultivar was not tested at the particular site.

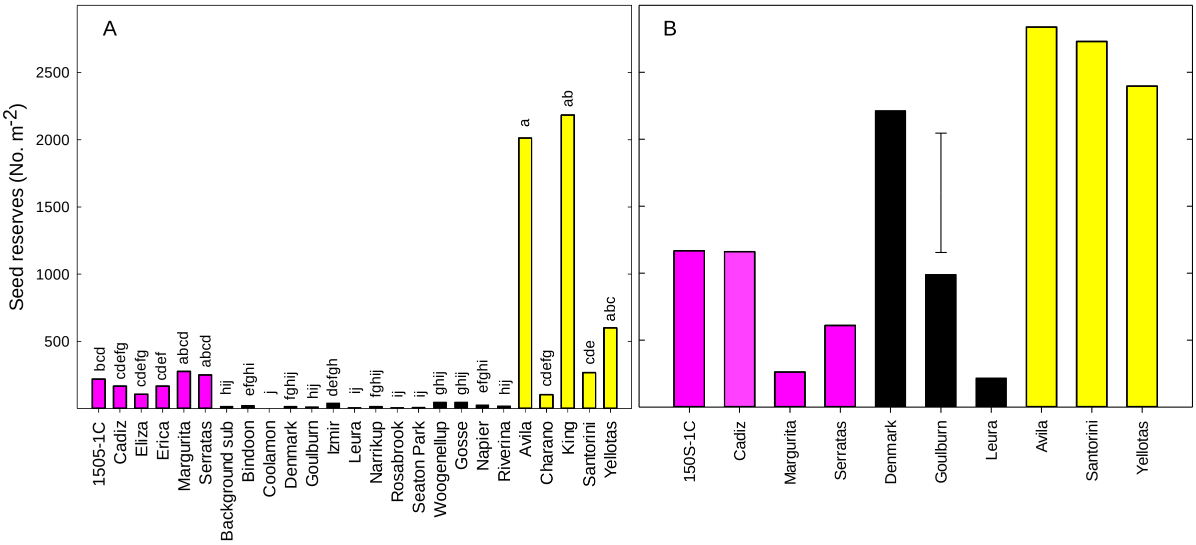


Figure 6.2 - 12. Seed reserves (seed number/m^2) of French serradella (pink), subterranean clover (black) and yellow serradella (yellow) cultivars at the end of year 1 (February 2019) at (A) Yass and (B) Gunning. Data points with the same letter in panel A indicate differences were not significant (P<0.05; data transformed for analysis). "Background subterranean clover" shows the number of seeds/m^2 in nil control plots and indicates that the seed bank developed in the subterranean clover treatments was no better than the existing level of seeds present in the soil at the Yass site. Error bar (panel B) indicates LSD (P=0.05) for the experiment at Gunning.

Subterranean clover seed reserves were always lowest in the nil-sown control plots adding confidence that the year 1 seed yield data reflected performance of the sown variety and not the background subterranean clover seed bank at any site.

In the 2018-sown experiments, seed banks developed by most legumes at Yass were low (Fig. 6.2 - 12A). Legumes sown at this site had failed to establish in very dry seasonal conditions during 2017. The site was re-sown in 2018, also a challenging dry season. All subterranean clovers failed to develop a significant bank of seeds under these conditions. In contrast, seed production by some yellow serradella cultivars Avila, King and Yellotas, was significantly greater than that of the clovers. All French serradella cultivars also produced more seed than the clovers, but at low levels equivalent to the poorest yellow serradella cultivars.

The persistence experiment at Gunning was established using only the "most-promising" serradella cultivars that had been identified by the time it was sown. Yellow serradella cultivars produced significantly more seed/m^2 (P<0.05) than most French serradella and subterranean clover cultivars, except for subterranean clover cv. Denmark (Fig. 6.2 - 12B). The lower seed production by cv. Margurita reflected its low seedling density at establishment (33 plants/m^2). However, this was similar to the density of yellow serradella cv. Santorini (47 plants/m^2) which, by comparison, developed a high seed bank. The relatively low seed banks developed by the late maturing cultivars, Serratas and Leura were presumed to have been a consequence of the very dry spring conditions in 2018.

Collectively, the seed yield experiments indicated that French and yellow serradellas can produce high seed yields and, in a series of very difficult dry seasons, were often capable of producing substantially more seed than subterranean clover cultivars.

6.2.3 Hardseededness and seed softening

6.2.3.1 Patterns of seed softening

Subterranean clover controls were included in the seed softening experiments to establish the patterns of seed softening s that are characteristic of this highly successful and persistent pasture legume.

The clovers typically exhibited three phases in their seed softening pattern:
(i) a characteristic initial level of hard seeds that was present by mid-summer,
(ii) a period of rapid seed softening between February and June/July, and
(iii) a remnant proportion of the seeds that was still hard by the end of June/July and did not soften appreciably during the growing season (Figs 6.2 - 13 and 6.2 - 14).

However, aspects of this basic pattern of seed softening varied between cultivars, between the sites at which they were observed and occasionally, between years within a site.

For example:

- the initial levels of hard seeds varied between years at both sites;
- at Cowra the rate of seed softening by most clover cultivars was faster and resulted in lower levels of residual hard seeds compared with Canberra;
- the pattern of seed softening for cv. Coolamon and cv. Goulburn was substantially slower in 2019 compared with 2018 at Canberra, whilst only that of cv. Coolamon was slower in 2019 at Cowra;
- generally, softening of hard seeds had slowed by mid-winter and did not occur in spring/early summer, but seed softening continued throughout the 2019 growing season at Canberra.

The site at Cowra is generally warmer year-round than the elevated Canberra site and it was assumed that differences (especially faster rates of seed softening at Cowra) were likely to be associated with the climatic differences experienced during summer at these locations. However, in these experiments the seeds were grown in the previous season at each of the sites and consequently, it was difficult to ascribe cause and effect to the hardseededness and seed softening observations. The results would, however, reflect the seed softening patterns experienced each year on farms in these areas. The between-year variation observed for some varieties and at both sites indicates that further observations over a longer range of seasonal conditions would be useful. Nevertheless, this is the first study that has quantified the hardseed and seed softening characteristics of serradella genotypes in cooler and higher rainfall environments than those of Perth and Wagga Wagga where studies have been conducted previously.

If all subterranean clover cultivars are considered equal, there was no obvious characteristic pattern of seed softening. However, most of the control varieties would not be recommended for use in the test locations. When the seed softening patterns of cultivars that would be recommended in each location were considered (e.g. cvs Goulburn and Leura at Canberra; cvs Coolamon and Seaton Park at Cowra), a reasonable consensus emerged. Clovers adapted to each location exhibited three phases in their seed softening pattern:
(i) a characteristic level of initial hard seeds was present by mid-summer. Between 5 and 45% of seeds were soft and ready to germinate, however, most commonly ~20% were in this state of readiness by mid-summer,
(ii) ~30% of the seeds softened between February and June/July, and
(iii) the remainder (most commonly between ~20-50% of the seeds) were still hard by the end of June/July and, usually, did not soften further during the growing season. It was presumed that these hard seeds would remain viable into the following growing season.

We assumed this pattern of seed softening and readiness to germinate could be used as an ideotype against which to compare the seed softening characteristics of the serradellas. It would enable

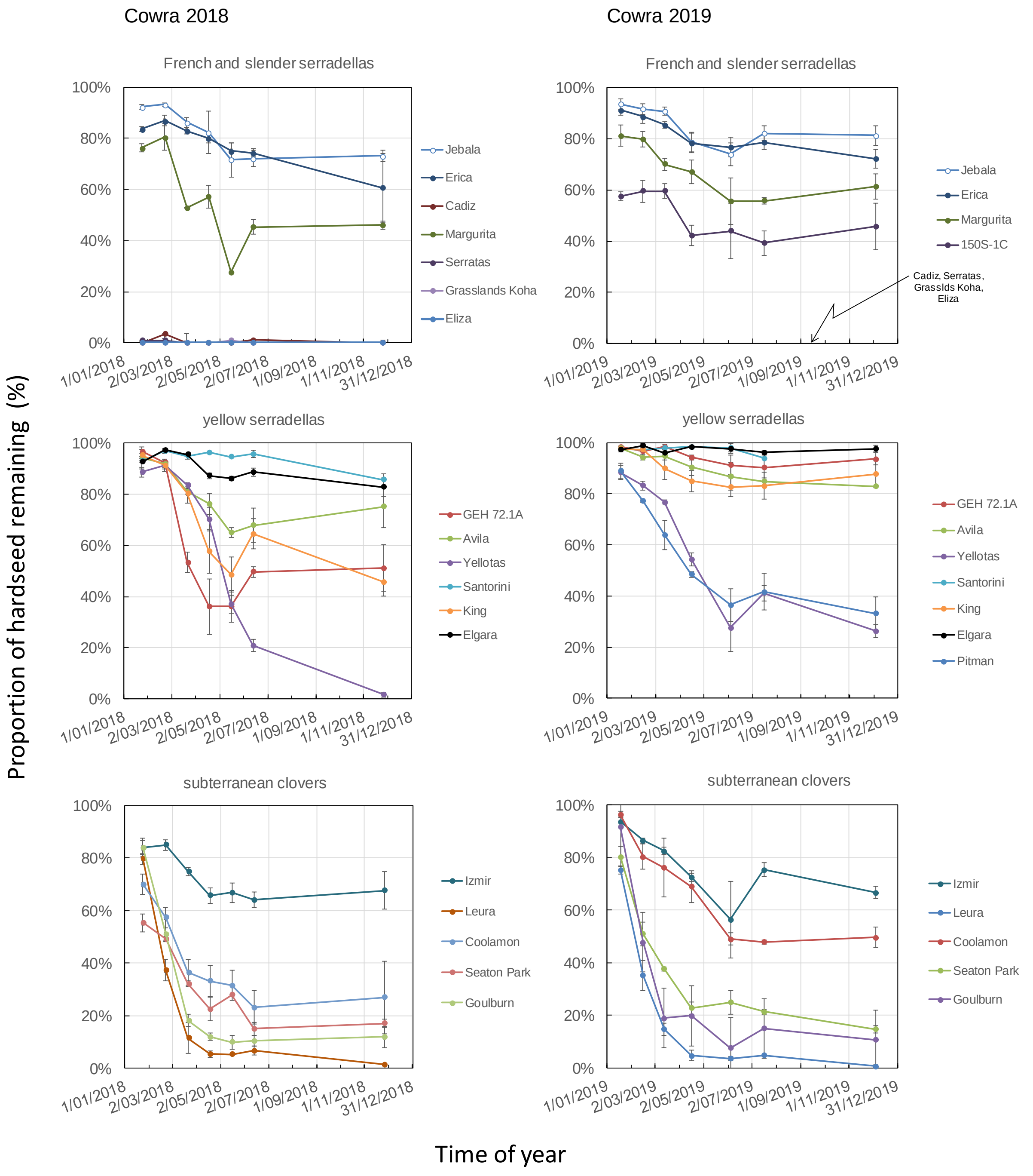


Figure 6.2 - 13. Proportions of hard seed remaining after they had been pinned in micromesh envelopes to the bare surface of the soil at Cowra in 2018 and 2019. In each case the seed had been grown at the same location in the preceding growing season and was harvested in early to mid-summer after it had matured. Each cultivar was represented by 3 replicate envelopes of ~100 seeds; bare seeds in the case of subterranean clover and seed in pod or pod fragments in the case of the serradellas. Bars indicate 2x SE.

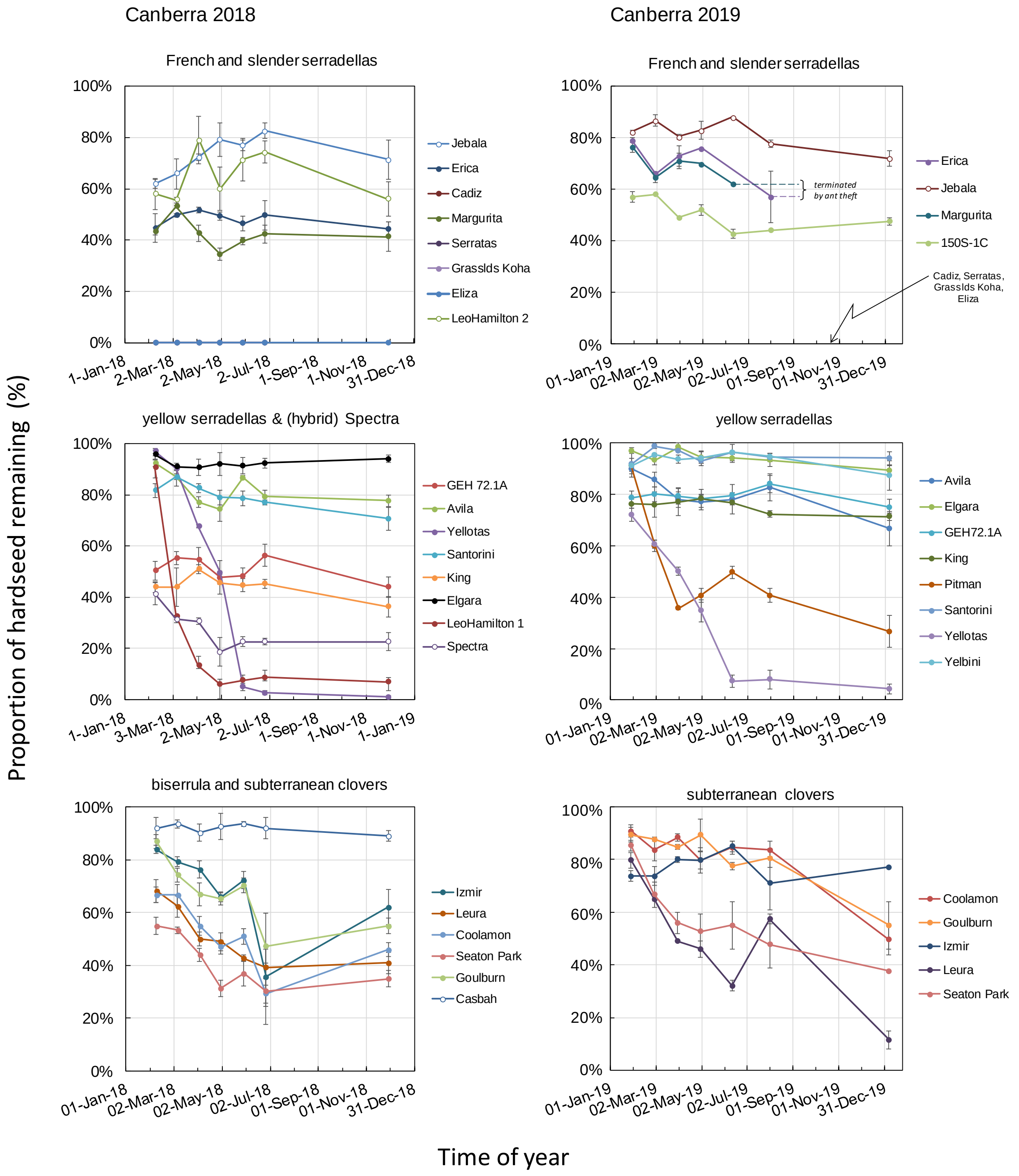


Figure 6.2 - 14. Proportions of hard seed remaining after they had been pinned in micromesh envelopes to the bare surface of the soil at Canberra in 2018 and 2019. In each case the seed had been grown at the same location in the preceding growing season and was harvested in early to mid-summer after it had matured. Each cultivar was represented by 3 replicate envelopes of ~100 seeds; bare seeds in the case of subterranean clover and seed in pod or pod fragments in the case of the serradellas. Bars indicate 2x SE.

germination of a proportion of seeds in response to an early start to a growing season, continued softening would protect against a “false” break to the season, and the residual level of hard seeds would allow development of a seed bank. Seed banks enable cultivars to persist despite significant growing season droughts and increase the seed available for germination on early rainfall.

An adaptive characteristic of subterranean clover that seemed to be an advantage in permanent pasture environments was relatively high levels of germination on early (late summer or early autumn) rainfall. This often-enabled plants to establish and grow before soil temperatures became too cold. In some years early germination risks a ‘false’ autumn break, but in many years in these cooler environment’s seedlings can survive, as was the case throughout the experiment period at the Gunning site. Early germination is an advantage for production systems looking for forage in the challenging autumn period and also helps to mitigate weed incursion into pasture swards. Subterranean clover cv. Leura was consistently observed to germinate at higher densities on early rainfall compared to yellow serradella cv. Avila and French serradella cv. Margurita throughout this experiment at the Gunning site (see Appendix 9).

Five distinct patterns of seed softening were observed among the serradella cultivars:

(1) High proportions of initially hard seeds with slow to very slow seed softening.
A majority of the hardseeded yellow and French serradella cultivars reside in this category. These cultivars often had 80-90% initial hard seeds (many varieties including cvs Avila, Santorini, etc), through to >90% hard seeds (e.g. cv. Elgara). As few as 5%, but to 20-40% of seeds soften by June/July (e.g. cv. Avila [very slow softening]). It is anticipated that seed softening may span at least two years for these varieties.

(2) Intermediate levels of initially hard seeds or initially very rapid seed softening, followed by relatively slow seed softening.
A limited number of cultivars (e.g. cvs Spectra [a yellow and French serradella hybrid] and cv. Margurita and 150S-1C) which had, as a consequence of this softening pattern, 40-60% of hard seed remaining by June/July. (NB. cv. Spectra had only 20% of hard seed remaining).

(3) High proportions of initially hard seeds with relatively fast seed softening.
A rare, but consistently expressed pattern in a small number of yellow serradella genotypes which resulted in low to very low hard seed remaining by June/July. This pattern would assist a variety to cope with false breaks, but may leave it exposed with few hard seeds to carry through a drought year (e.g. cvs Yellotas, Pitman and a field strain designated, “LeoHamilton2”), and especially in a dry establishment year before soil seed reserves have an opportunity to build up.

(4) Totally soft seeds.
A characteristic of some French serradella varieties only (e.g. cvs Cadiz, Serratas, Elgara, Grasslands Koha). However, pattern #3 may also result effectively in no hard seeds by mid-year. Soft seeded varieties are not suited for use in permanent pastures (Dear *et al.* 2002).

(5) Increasing proportions of hard seeds with little if any seed softening.
Only observed in Jebala (slender serradella) when grown in Canberra; at Cowra, Jebala exhibited softening pattern #1. Consequently, this pattern is considered unlikely to be a stable genetic trait and is probably a result of the environment in which the seeds were grown and resown.

6.2.3.2 Comparative seed softening among serradellas and subterranean clovers

Conformity with the hypothetical seed softening ideotype for subterranean clover: None of the serradellas mimicked the generalised clover seed softening ideotype particularly well. The closest varieties were cv. Spectra (a yellow and French serradella hybrid), cvs GEH72.1A and King (early-season yellow serradellas), cv. Margurita and breeder's line 150S-1C (mid-season French serradellas). All had moderate initial levels of hard seed or softened relatively quickly to achieve a residual level of 20 - 60% hard seeds by June/July.

Rates of seed softening: As mentioned previously, there was a large effect of site on rate of seed softening among the subterranean clovers and, consequently, the proportion of residual hard seeds present by mid-winter. An effect of site on rate of seed softening was less evident among the serradellas. However, there were relatively subtle impacts of site on the initial levels of hardseedeness among the serradellas. The rate of seed softening by subterranean clover (e.g. Taylor 1981) and yellow serradella seeds (Taylor and Revell 1999) is known to be increased by higher temperatures. This was assumed to be a likely reason for the marked difference in clover seed softening rates between the Cowra (relatively warm) and Canberra (relatively cool) sites. However, if temperature differences were the cause, it is unclear why there would be relatively little influence of site on the softening of the yellow and French serradella varieties.

The softening of serradella seeds has only been examined in any detail in yellow serradella (Taylor and Revell 1999, Revell *et al.* 1998) and only in Western Australia. These pioneering studies indicate a potentially complex array of environmental impacts on preconditioning of seeds to prime them for softening, on seed softening rate, and inhibition of seed softening. For example: a condition of "latent" soft seeds may be induced by exposure to moderate alternating temperature; seed softening by these "primed" seeds is reversed by cool temperatures (≤8°C); seed softening rate is increased at elevated temperatures (up to at least 70°C) and is inhibited by exposure to daylight (Revell *et al.* 1998, Revell *et al.* 1999).

Diversity in serradella seed softening patterns: Until the current research, it has generally been assumed that serradellas are either entirely soft seeded (e.g. many French serradella varieties) or are highly hardseeded, slow to soften and need two to three years for substantial seed softening and, therefore, an equivalent period to build a functional seed bank from which seeds may germinate reliably each year (e.g. many of the hardseeded yellow and French serradellas).

A consequence of determining the seed softening patterns of a wider variety of yellow and French serradella cultivars has been the realisation that the patterns of seed softening among serradellas are, in fact, potentially very diverse. Three categories of seed softening were described:
(1) High proportions (80-90%) of initially hard seeds with slow to very slow seed softening (e.g. many yellow serradella cultivars).
(2) High proportions of initially hard seeds with relatively fast seed softening (e.g. cv. Yellotas, cv. Pitman, and a field strain designated, "LeoHamilton2").
(3) Intermediate levels of initially hard seeds or initially very rapid seed softening, followed by relatively slow seed softening (a limited number of cultivars, e.g. cv. King, cv. Spectra, cv. Margurita, breeder's line 150S-1C).

These observations indicate that there may be room within existing cultivars to select varieties that have seed softening patterns that are closer to the surmised ideotypic pattern of seed softening required to for pasture establishment and persistence in permanent pastures.

Low serradella regeneration in the year after sowing a serradella pasture: The seed softening experiments provided a plausible explanation for the issue of poor serradella regeneration in the year after pasture establishment. For many serradella cultivars, seed softening is too slow and in the year after establishment there are too few soft seeds ready to germinate. It is surmised that this almost certainly contributes to the problems of serradella establishment and persistence in permanent pastures (**Section 6.1.2; Section 6.2.4**) because low seedling regeneration in year 2 opens a window for weeds and other competing species to become established.

The year 2 regeneration problem was observed in the participatory experiments (**Section 6.1.2**) and is also an acknowledged issue when serradella is used in ley farming systems. In ley farming systems, an agronomic solution to the problem is available. It is recommended that a cereal crop should be sown in the year after establishment of a serradella pasture. The crop utilises nitrogen fixed during the first year of serradella pasture, it injects cash into the farming business, and the seed that is buried during the sowing of the crop, softens and germinates to give a strong pasture stand in year three. Seed burial enhances seed softening because the seed is no longer exposed to the inhibitory effect of light on softening of serradella seeds (Taylor and Revell 1999). Unfortunately, sowing a crop in year two is an impractical solution in farming systems that rely on permanent pastures.

The seed softening results observed in the current project indicated at least three potential solutions to the year-2 serradella regeneration problem when establishing permanent serradella pastures. These options should be tested in experimental pasture establishment trials:

1. plant new pastures using a combination of softened naked seed and hard seed in the pod; the latter will germinate in year 2.
2. plant new pastures with contrasting serradella cultivars – a ‘hard, slow softening’ variety (e.g. Avila) with a ‘fast softening’ variety (e.g. Yellotas); the latter will regenerate in year 2, but may not persist.
3. select new varieties with more ideal seed softening patterns that avoid poor regeneration in year 2 by exploiting the high diversity in seed softening patterns among the serradella cultivars. This is clearly a longer-term solution. However, a wider appraisal of seed softening patterns among the serradella genome to find more favourable seed softening characteristics for permanent pastures is warranted.

6.2.4 Serradella persistence

6.2.4.1 Persistence in established legume monocultures

Three field experiments from a previous project (MLA-B.PUE.0104) at Yass, Burrinjuck and Goulburn, NSW were revisited at the commencement of the current project to assess persistence by serradella and other alterative legumes. A range of alternative pasture legume treatments had been sown or over-sown as monocultures, 2-3 years prior to assessment of their frequency and/or lack of survival. The Yass site was then monitored for a further 2 years. The experiments were maintained mostly by occasional mowing and invasion by grasses and weeds was permitted from 2015.

Up to eight alternative legumes had survived at the previously-sown sites in sufficient densities to be assessed in 2016 (Figs 6.2 – 15, 16 and 17). At all three sites, French serradella (cv. Margurita) and yellow serradella (cv. Santorini) were among the surviving legumes. These varieties were the main serradellas that could be obtained readily by farmers in the area represented by the sites. Yellow serradella (cv. Avila, an older variety recommended previously for use in NSW) was also sown but only at the Yass in 2014 (the final year of the previous experiment).

In 2016, the locally adapted cultivar of subterranean clover (cv. Leura) was observed to have maintained a high frequency (typically between 70-95%) at all sites. By comparison, the frequency of the yellow serradella cv. Santorini had declined from near-complete groundcover after sowing to between 40-60% at Goulburn and Yass, and less than 20% at Burrinjuck. The frequency of French serradella cv Margurita was similar being <20% at Goulburn, 20-30% at Burrinjuck and 40-60% at Yass. However, cv. Avila was persisting reasonably well at Yass (frequency range 60-90%). Further

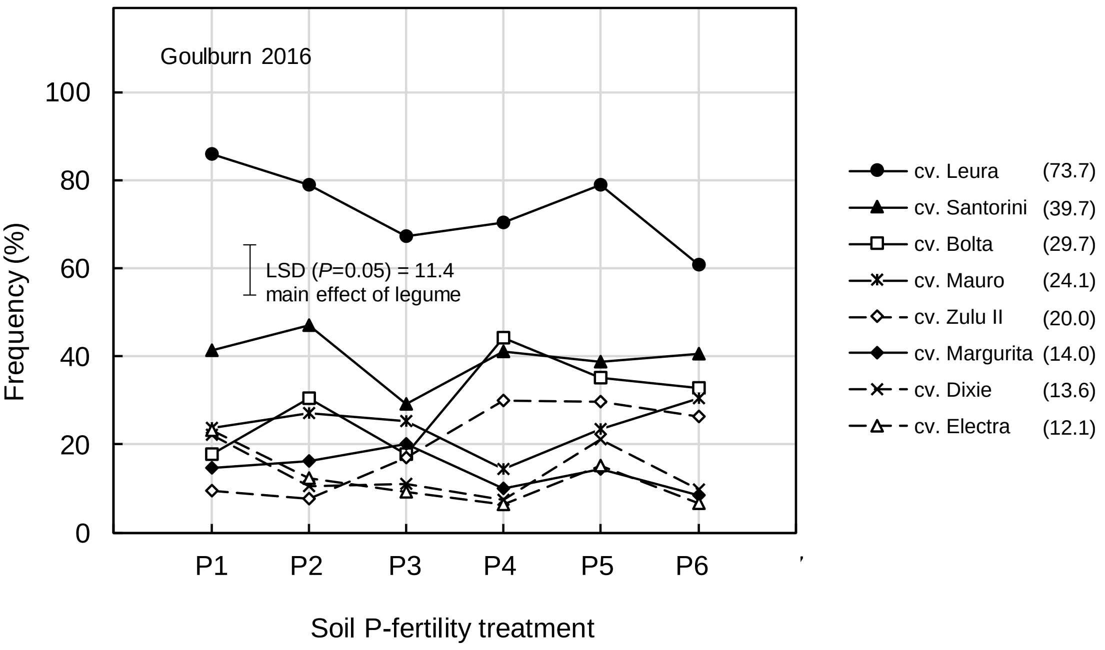


Figure 6.2 - 15. Frequency of annual legumes recorded in August 2016 in an experiment established at Burrinjuck, NSW in 2014 for the original purpose of determining the critical soil test P requirement of various alternative pasture legumes (n=3; Sandral *et al.* 2019). The experiment was subsequently closed to grazing and herbage was managed by mowing with no mowing in the lead up to, or during flowering and seed production. Grasses and weeds were permitted to invade the swards. Frequency was analysed ANOVA. Only the main effect of legume was significant ($P<0.05$). Average frequency for each legume is shown in brackets associated with each treatment label.

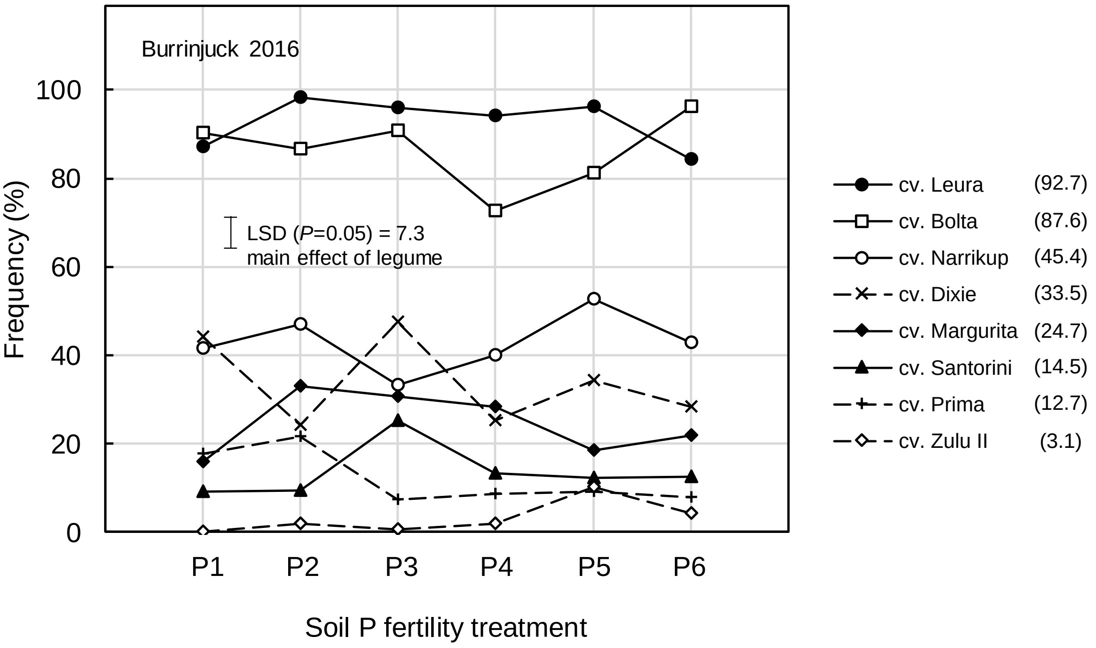


Figure 6.2 - 16. Frequency of annual legumes recorded in August 2016 in an experiment established at Goulburn, NSW in 2013 with various alternative pasture legumes (n=3; Hayes *et al.* 2015). Once established the experiment was grazed intermittently, but with no grazing during spring to aid seed production. Grasses and weeds were permitted to invade the swards. Frequency was analysed ANOVA. Only the main effect of legume was significant ($P<0.05$). Average frequency for each legume is shown in brackets associated with each treatment label.

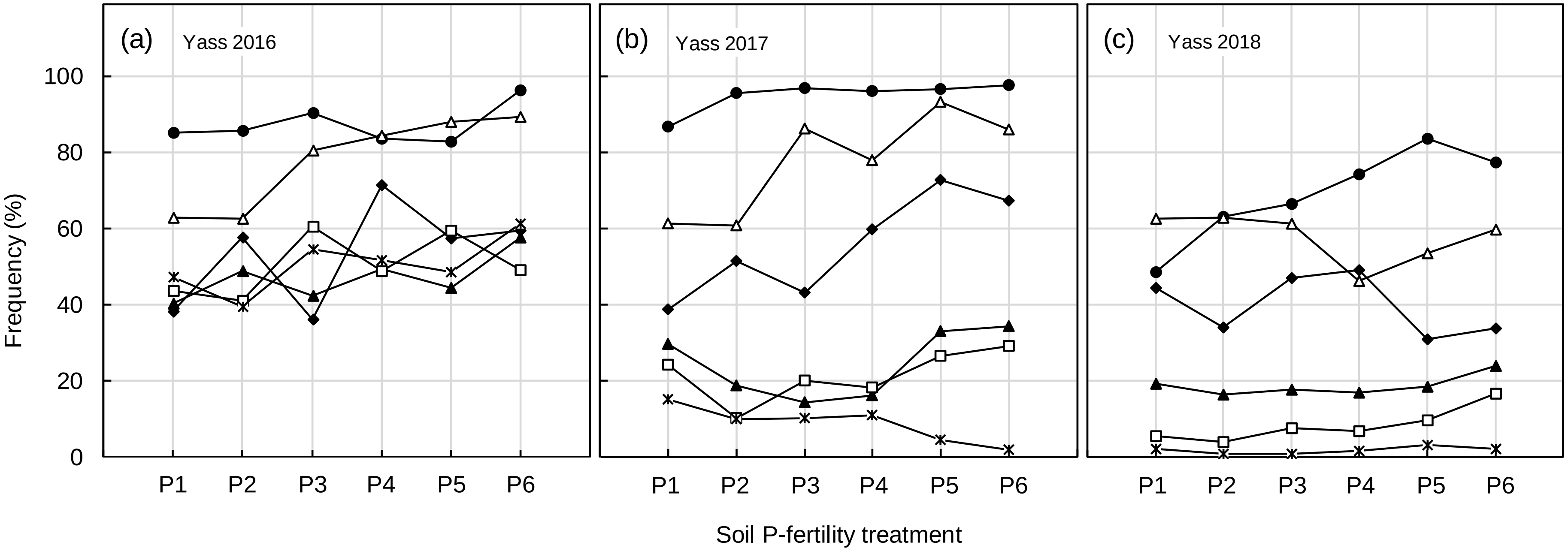


Figure 6.2 - 17. Frequency of annual legumes recorded in (a) August 2016, (b) August 2017 and (c) September 2018 in an experiment established at Yass and over-sown annually between 2012-2014 for the original purpose of determining the critical soil test P requirement of various alternative pasture legumes (n=3; Sandral et al. 2019). The experiment was closed to grazing, soil P treatment levels were maintained, and herbage was managed by mowing with no mowing in the lead up to, or during flowering and seed production. Grasses and weeds were permitted to invade the swards. For statistical assessment of these data see following page.

(d)

Analysis of variance					
Variate: Frequency					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	2716.1	1358	6.25	
Rep.*Units* stratum					
Cultivar	5	181631.2	36326.2	167.26	<.001
P level	5	4658.2	931.6	4.29	<.001
Year	2	43956.8	21978.4	101.2	<.001
Cultivar.P level	25	4594.8	183.8	0.85	0.679
Cultivar.Year	10	19758.7	1975.9	9.1	<.001
P level.Year	10	1342.5	134.3	0.62	0.798
Cultivar.P level.Year	50	9588.4	191.8	0.88	0.694
Residual	214	46477.3	217.2		
Total	323	314724			

(e)

Variable	**Average frequency** (%) values linked by shading are not signif. different						**LSD** (*P* =0.05)
Cultivar	Leura	Avila	Margurita	Santorini	Hykon	Casbah	
	83.7	71.0	49.6	29.6	26.6	20.2	5.6
P level	P1	P2	P3	P4	P5	P6	
	41.9	42.3	46.4	47.9	49.9	52.3	5.6
	▨	▨	▨				
		▨	▨	▨			
			▨	▨	▨		
				▨	▨	▨	
Year	2016	2017	2018				
	61.1	46.8	32.5				4.0
Cultivar x Year			2016	2017	2018		
		Leura	87.3	94.9	69.0		9.7
		Avila	77.9	77.3	57.9		
		Margurita	53.4	55.5	40.0		
		Santorini	47.1	23.4	18.3		
		Hykon	50.3	21.3	8.3		
		Casbah	50.4	8.6	1.7		

Figure 6.2- 17 (continued). (d) Analysis of variance for Frequency of annual legumes at Yass (2016-2018) and, (e) a matrix showing the significant main effects of Cultivar, P level and Year, and that of the Cultivar x Year interaction (*P*<0.05). Average values linked by shading were not significantly different. For the Cultivar x Year interaction, only the changes within cultivar through years is highlighted to assist the Discussion of these results.

monitoring of these cultivars at Yass revealed a main effect of cultivar over the three-year monitoring period with frequencies ranked: Leura (84%) > Avila (71%) > Margurita (50%) > Santorini (30%).

The result was sobering. Serradella cultivars being sold to farmers were not persisting well enough to be considered viable alternatives to subterranean clover at these sites, with the possible exception of yellow serradella cv. Avila which had maintained a level of groundcover that was a little below that of cv. Leura.

6.2.4.2 Persistence in newly sown experiments

A network of five newly sown field experiments was also established near Bombala, Yass, Goulburn, Gunning and Bigga, NSW to compare the persistence of a wider range of serradella cultivars with locally-adapted subterranean clover cultivars across the Monaro and Southern Tablelands of NSW where serradella is not used currently. Seasonal conditions during these experiments were generally drier than average across all sites in the network.

Serradella cultivars established at higher initial seedling densities compared to the subterranean clover varieties, primarily because they had been sown at higher densities as a consequence their smaller seed size. In year 2 many of the serradellas regenerated at higher densities than the subterranean clover; this was often (although not always exclusively) a characteristic of softseeded varieties. However, by year 3, the softseeded serradella cultivars were usually present in very low densities, unable to persist under drought conditions without hard seeds (e.g. Fig. 6.2 – 18).

Without exception, yellow serradella cv. Avila, was the most persistent of all serradella species and cultivars tested across all sites based on final year seedling regeneration densities (e.g. Fig. 6.2 – 18). At some sites (Goulburn and Gunning) it regenerated at densities that were significantly higher than the subterranean clover check cultivars. At the remaining sites it regenerated at densities similar to the check cultivars. The consistent performance of cv. Avila in this series of experiments was consistent with previous evaluations during the 1980’s (Oram 1990).

Some concern still exists with this cultivar because it can exhibit lower year 2 regeneration densities compared to other cultivars. This may be more of a concern in more favourable seasons when pressure from companion species and background weeds might be greater, but under the droughts experienced at this network of sites, cv. Avila demonstrated a capacity to persist for three years as well or better than any other cultivar.

Yellow serradella cv. King was another highly persistent serradella cultivar. This cultivar generally regenerated in year 3 at densities similar to cv. Avila. The relative productivity of these cultivars generally reflected their superior persistence although in year 1 when many of the serradella cultivars were present in relatively high densities, the herbage production from cv. King was significantly less than later maturing cultivars such as cv. Avila.

Cultivar Yellotas was flagged early in the program as a cultivar of interest. It regenerated in high densities in the early years of the experiment at multiple sites; it was also observed to exhibit a delayed regeneration of seedlings in year 2. These patterns of regeneration were backed by observations in the seed softening studies conducted at the research stations where Yellotas displayed a rapid rate of seed softening during the summer immediately after seed had been produced and ongoing hardseed breakdown throughout the growing season. This distinct pattern of seed softening would be beneficial for recovery from a false break but left the cultivar with a low soil seed reserve by winter. This may have been the reason that Yellotas declined to very low levels after droughts in years 2 and 3 and did not recover. It is postulated that under a wetter sequence of years

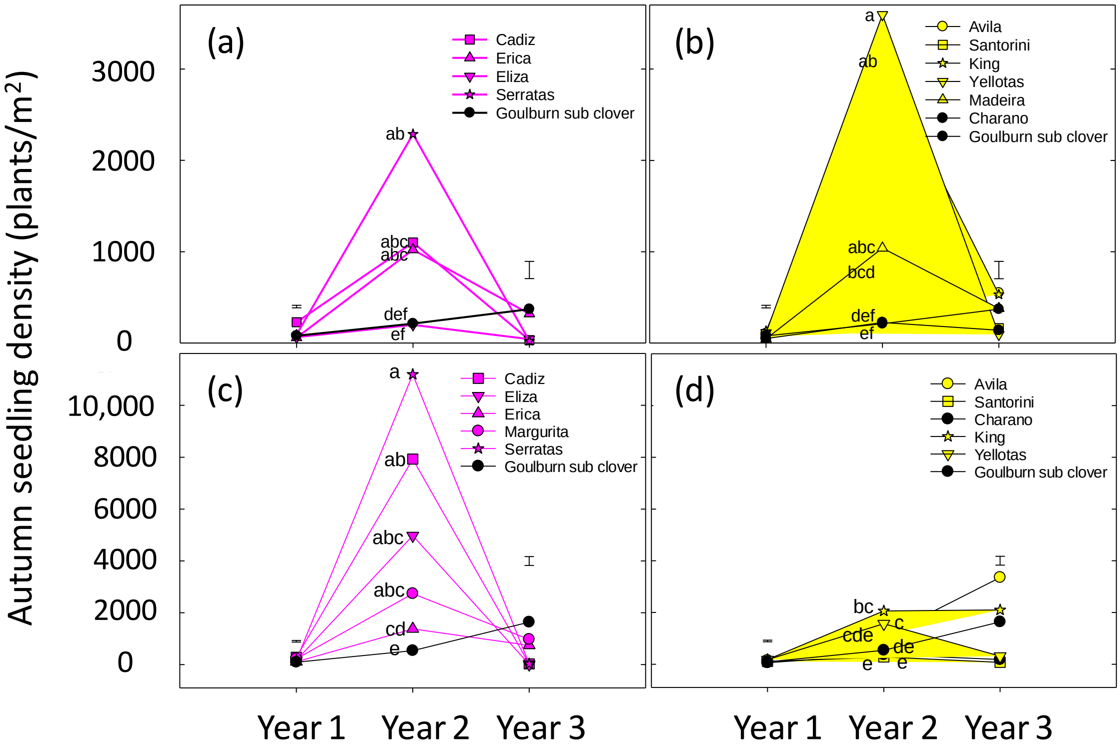


Figure 6.2 – 18. Densities of seedlings establishing during autumn each year at Bombala (a, b; Monaro district) and Goulburn (c, d; Southern Tablelands) in two of the five persistence experiments.

the performance of Yellotas may have been substantially better than was recorded. In particular, a more favourable year 2 may have allowed it to build up a sufficient seed bank to withstand the subsequent failed spring. It remains a cultivar of interest warranting further research; there may also be a novel application in which Yellotas is combined with Avila to ensure adequate year 2 regeneration densities in newly sown pastures.

None of the French serradella cultivars demonstrated persistence equal to that of cv. Avila (e.g. Fig. 6.2 – 18). The completely softseeded cvs Cadiz and Serratas failed to persist beyond year 2. The harder-seeded cvs Erica and Margurita, persisted better but generally regenerated in the final year at densities similar to or below the subterranean clover check cultivars; their viability as alternatives to subterranean clover in these NSW tableland environments is not yet established. The evaluation of Erica was further complicated by the very poor quality of (old) seed that had to be used in sowing the network of persistence experiments. Increasing sowing rates to adjust for poor seed viability proved unsuccessful in establishing this cultivar at reasonable densities in the field under drought conditions. This was an example of the challenges in acquiring seed when research novel species; this cultivar proved to be completely unprocurable from commercial sources throughout the project.

There are some suggestions in the data that Erica may be a better suited cultivar in permanent pastures than Margurita due to improved flowering time stability, later maturity and favourable seed production, but these findings are not conclusive and further testing is needed. Cultivar Erica remains a cultivar of interest for future research, but any future program of work would need to propagate its own fresh source of seed of this cultivar for testing on a scale relevant to farmers.

6.2.4.3 Maximum rooting depth of serradella pastures

Serradellas are known to develop very deep roots in sandy soil profiles in WA. The roots of serradella can reach up to 1.5 m depth, well in excess of subterranean clover roots. Rooting depth of serradella has only rarely been assessed in heavier and/or duplex soil profiles, but deeper rooting is, nevertheless, often promoted as an attribute of the serradellas.

Maximum rooting depth was assessed at Yass and Gunning in southern NSW, where serradellas are not used presently, and at Purlewaugh and Spring Ridge in northern NSW, districts where serradella is used.

- **Yass (November 2016)**: maximum rooting depth (average: 89 cm) did not differ among yellow serradellas (cvs Avila, Santorini), French serradella (cv. Margurita), and subterranean clover (cv. Leura) and other legumes measured.
- **Gunning (November 2017)**: French serradella (cv. Margurita) and yellow serradella (cv. Avila) had marginally deeper roots (84 and 86 cm, respectively) than subterranean clover (cv. Leura, 69 cm) when grown at high P , but not when grown in a low P treatment
- **Gunning (August 2019)**: French and yellow serradella again had marginally deeper roots (70 and 79 cm, respectively) than subterranean clover (59 cm). High soil P fertility promoted rooting depth (73 cm) relative to the low P treatment (64 cm) but there were no significant interactions between species, P and/or lime on rooting depth.
- **Purlewaugh (September 2019)**: yellow serradella (cv. King), French serradella (cv Margurita) and subterranean clover (cv. Dalkeith) had penetrated a clay layer at 44 cm depth) but only reached depths of 77±8.3 cm, 55±2.2 and 52±0.3 cm, respectively.
- **Spring Ridge (September 2019)**: yellow serradella (cv. King), French serradella (cv Margurita) and subterranean clover (cv. Dalkeith) had similar rooting depths to about 1.1 m. The two serradella species had higher scanned root length than subterranean clover in the surface soil (0-10 cm), but there were no differences below this depth.

The clear message from these studies was that it cannot be assumed that serradellas will achieve substantially deeper roots than subterranean clovers in non-sandy soil profiles. At the two sites where differences were recorded the serradellas did, however, achieve marginal to substantial improvements in maximum root depths (+5-49% deeper). Studies of water use under crops indicate that differences of this magnitude may prove to be important for prolonging end of season growth and seed production on stored water.

Further research suggested by the project activities: Comparative rates of root growth early in the growing season were not examined in the present project but may be worthy of investigation because the serradellas were often able to establish relatively well compared with subterranean clover under very dry starts to the growing season; on some occasions the serradellas survived dry conditions after sowing that killed subterranean clover seedlings.

6.3 Results and Discussion: Output 5(c) - Conduct a ‘watch and act’ regime for occurrence of any serradella leaf disease at all field sites to assess whether leaf disease is likely to occur when serradella pastures are used.

This work aimed to assess whether susceptibility to fungal leaf disease(s) may, or may not be an issue for serradella pastures, particularly in higher rainfall areas where the legume is not already used.

6.3.1 Monitoring for disease on serradella at the pre-existing field experiment near Yass

This experiment was last sown/oversown with various serradella varieties in 2014, with persistence of key legume species subsequently monitored from 2016 -2018. During the previous experiment, small patches of leaf disease were observed, but they had always been treated with a fungicide. During 2017, serradella treatments (cvs Avila, Santorini, Margurita) were monitored for leaf disease symptoms. Disease occurrence was low. However, samples of diseased shoots, along with the roots and soil around affected plants in the Avila plots and Margurita plots were sampled in spring and sent to two accredited pathology laboratories at Wagga Wagga and Menangle, to obtain a formal identification of pathogen(s) present on the serradella.

No pathogen was observed on the diseased samples submitted to the laboratories, although the Wagga Wagga laboratory confirmed the presence of S*clerotinia minor* in the soil of the samples that had been sent. No further disease symptoms were observed at the Yass site.

6.3.2 Observations at the disease-screening nursery sown adjacent to the Yass experiment

A new disease-screening nursery was sown into a cultivated seedbed at the Yass experiment site on 18 May 2017. Treatments included 22 serradella breeding lines and cultivars (10 lines of French serradella, 11 lines of yellow serradella, one French x yellow serradella hybrid, with two subterranean clover cultivar controls). This was all varieties for which seed was available at the time of sowing. Seed availability constrained the seeding rates (3-6 kg/ha) and the seed quality of some lines, especially breeding lines held in storage, was not high.

No disease symptoms were observed in the serradella nursery plots. It was assumed the unusually dry winter/early spring conditions were not conducive to development of leaf disease.

Observations of the nursery plants did, however, provide other information of use to the project. For example, a number of French serradella lines (including 145772, 145872, 145883, and Emena) were identified as late maturing and likely to be of interest for Tableland environments. No lines of yellow serradella were observed to be later flowering than Avila, Yellotas or Charano.

6.3.3 WA serradella grower alerts

There were no reports of leaf diseases in serradellafor the entire duration of the project. Assessment of varietal responses to Sclerotinia disease in a screenhouse nursery in Perth (a facility used to screen canola germplasm for leaf diseases) was postponed and ultimately abandoned because there were no disease outbreaks (i.e. no inoculum sources) identified in WA.

In summary, apart from one possible occurrence of disease at Yass in the first year of the project (the pathology laboratories were unable to confirm disease or a causal agent), there were no foliar diseases reported on serradella across the whole of the project network from 2016-2019.

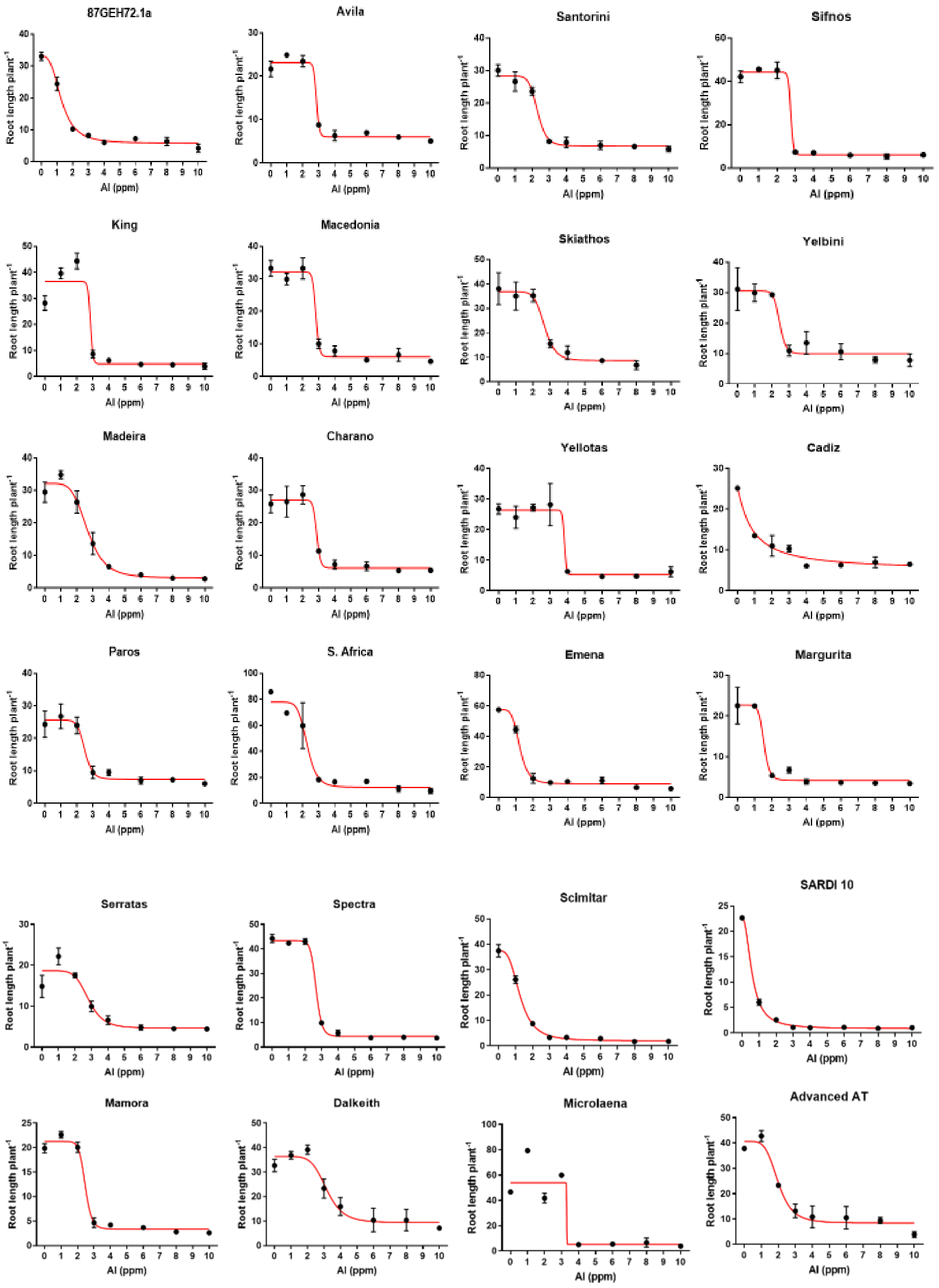


Figure 6.4 - 1. Response of root length to increasing concentrations of aluminium in nutrient solutions maintained at pH 4.5 with as low an ionic strength as practicable. Symbols represent mean ± s.e.; see Table 6.4 - 1 (below) for the species identity of each cultivar and statistics associated with the fitted dose-response functions.

Table 6.4 - 1. Cultivar ranking, from most Al tolerant to least Al tolerant, based on the concentration of Al at 95% maximum root length (calculated from fitted curves shown in Figure 6.4 - 1); values for the concentration of Al at 50% maximum root length and R^2 of the fitted curve also shown. Reference species are shown in bold type.

Species	Cultivar	Al_{RL95}	Al_{RL50}	R^2
Ornithopus compressus	Yellotas	3.83	3.82	0.91
Microlaena stipoides	**Microlaena**	**3.28**	**3.29**	**0.88**
Ornithopus compressus	King	2.62	2.84	0.91
Ornithopus compressus	Avila	2.61	2.86	0.96
Ornithopus compressus	Charano	2.56	2.87	0.89
Ornithopus compressus	91GRC33COM.1	2.55	2.75	0.98
Ornithopus compressus	GEH113COM-B1	2.53	2.82	0.95
O. compressus x O. sativus	Spectra	2.19	2.67	0.99
Trifolium subterraneum	**Dalkeith**	**2.00**	**3.17**	**0.84**
Ornithopus compressus	Yelbini	1.96	2.44	0.78
Ornithopus perpusillus	Mamora	1.94	2.46	0.98
Ornithopus compressus	DAM7COM-A	1.88	2.66	0.85
Ornithopus compressus	Paros	1.86	2.49	0.85
Ornithopus sativus	Serratas	1.77	2.82	0.88
Ornithopus compressus	Santorini	1.64	2.31	0.94
Ornithopus compressus	ZAF61COM	1.52	2.27	0.83
Ornithopus compressus	Madeira	1.48	2.68	0.93
Ornithopus sativus	Margurita	1.12	1.53	0.90
Phalaris aquatica	**Advanced AT**	**1.08**	**1.99**	**0.90**
Ornithopus sativus	Emena	0.68	1.22	0.98
Medicago polymorpha	**Scimitar**	**0.51**	**1.27**	**0.98**
Ornithopus compressus	87GEH72.1a	0.49	1.28	0.97
Medicago sativa	**SARDI 10**	**0.14**	**0.59**	**1.00**
Ornithopus sativus	Cadiz	0.06	0.86	0.84

6.4 Results and Discussion: Output 5(d) - Conduct glasshouse/controlled-environment research to identify and quantify the suspected soil and environment constraints to the serradella adaptation range.

6.4.1 Tolerance of toxic aluminium and manganese by serradellas

Aluminium (Al) and manganese (Mn) toxicity are soil constraints associated with very acid soils, and, in the case of Mn, with waterlogged soil. Previous studies have identified serradellas as tolerant of Al and moderately tolerant of Mn toxicity but with some variability in tolerance among cultivars. Anecdotally, serradellas are regarded as highly suitable for very acid soils, however, the limited research results and some anecdotal accounts indicate that the degree of tolerance may vary among the cultivars. Experiments to characterise the capacity for tolerance of Al or Mn toxicity by a wide range of cultivars were undertaken as part of the project to support appropriate use of serradella in acid soils.

6.4.1.1 Aluminium tolerance

Genotypes were screened in solution culture as a means of assessing their physiological tolerance of Al and were ranked by determining the Al concentrations that corresponded with critical thresholds in their root growth inhibition response to Al (Fig. 6.4 - 1; Table 6.4 - 1). There was substantial variation among and within serradella species and cultivars with regard to their Al tolerances. Many of the French serradella cultivars were relatively sensitive to Al and some had either the same or a greater sensitivity than the susceptible reference species, (cvs Scimitar and SARDI 10). The exception among French serradella was cv. Serratas which was less tolerant than subterranean clover (cv. Dalkeith), but more tolerant than phalaris (cv. Advanced AT). Most yellow serradella cultivars were moderately to highly-tolerant of Al but there were significant exceptions (e.g. breeders' line 87GEH72.1a (now released as cv. Regena) was ranked among the susceptible *Medicago* spp.

This was an unusual result because it is not common to find such a wide range in the apparent tolerance of Al within a species and to have commercially-available varieties that were as intolerant of Al as the most sensitive pasture species (e.g. *Medicago* spp.) used in Australia, whilst other varieties approached the tolerance of the most Al-tolerant pasture species (e.g. *Microlaena stipoides*). A bid was made for supplementary funding to validate these results by conducting an additional root growth bioassay in an Al-toxic soil. The experiment provided strong evidence that the Al-tolerance rankings derived in the solution culture experiments were valid (see **Supplementary Final Report**).

Table 6.4 - 2. Shoot dry mass (mg/plant) of cultivars in response to increasing concentrations of Mn at pH 4.5. Cultivars are ranked on shoot index which is determined as the percentage of (shoot DM at 1821 µM Mn)/(maximum shoot DM). Highly ranked cultivars are more tolerant. Reference species are shown in bold type.

		pH 6.0	pH 4.5			Manganese (µM)					
Species	Cultivar	18	18	91	182	364	546	911	1821	R^2	Shoot index
Ornithopus compressus	Santorini	45	39	42	37	40	34	36	28	0.80	66
Ornithopus compressus	Charano	26	28	26	25	19	19	19	17	0.62	60
Ornithopus compressus	Madeira	24	22	19	12	21	14	14	13	0.31	59
Phalaris aquatica	**Advanced AT**	**22**	**22**	**15**	**17**	**18**	**13**	**18**	**13**	**0.31**	**59**
Ornithopus compressus	King	43	39	32	43	38	30	36	26	0.48	59
Ornithopus sativus	Margurita	42	46	40	43	39	38	32	27	0.90	58
Ornithopus sativus	Bydgoska Asa	41	32	37	44	48	27	23	24	0.39	55
Ornithopus compressus	Tauro	32	38	33	37	30	25	27	21	0.75	54
Trifolium subterraneum	**Dalkeith**	**42**	**38**	**35**	**36**	**29**	**29**	**27**	**20**	**0.90**	**53**
Ornithopus compressus	Pitman	32	28	23	15	22	23	32	17	0.04	53
Ornithopus compressus	Uniserra	33	27	30	39	26	24	19	19	0.51	49
Ornithopus compressus	Yellotas	37	33	38	28	28	29	27	18	0.79	48
Ornithopus compressus	Yelbini	16	19	27	28	18	25	18	13	0.51	45
Ornithopus compressus	Avila	19	22	20	27	17	18	13	12	0.73	44
Ornithopus compressus	Paros	27	27	30	35	27	26	24	15	0.76	44
Ornithopus pinnatus	Jebala	20	16	16	18	18	13	9	8	0.75	42
Ornithopus sativus	Emena	40	25	26	29	23	21	38	16	0.12	41
Ornithopus sativus	La Coruna	36	42	27	36	24	25	18	15	0.65	37
O.compressus x O. sativus	Grasslands Spectra	49	68	38	46	62	51	45	24	0.50	36
Ornithopus sativus	Cadiz	13	20	13	15	20	13	10	7	0.63	33
Ornithopus sativus	Warta	32	39	39	29	29	30	24	13	0.89	32
Ornithopus sativus	Vinar	22	29	44	15	22	29	21	14	0.33	31
Ornithopus sativus	Biata	25	33	52	14	15	12	30	16	0.14	30
Ornithopus sativus	Erica	22	21	21	19	19	18	12	9	0.93	30
Ornithopus sativus	Eliza	25	41	40	34	57	39	31	17	0.55	29
Medicago sativa	**SARDI 10**	**27**	**20**	**19**	**20**	**13**	**9**	**9**	**5**	**0.76**	**26**
Medicago polymorpha	**Scimitar**	**28**	**27**	**26**	**22**	**13**	**11**	**6**	**7**	**0.67**	**25**
Ornithopus sativus	Aza	26	42	28	20	34	23	15	10	0.61	24
Ornithopus sativus	Serratas	36	50	23	36	18	16	24	10	0.43	20
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
LSD (P<0.05)		13	14	12	13	10	10	11	6		

6.4.1.2 Manganese tolerance

Serradella species have been reported to be less tolerant than subterranean clover to high concentrations of Mn, but more tolerant than lucerne or annual medics. When tested in solution culture, the yellow serradellas were found to be moderately to highly tolerant of Mn (similar or better than subterranean clover), while the French serradellas, with the exceptions of cvs Margurita and Bydgoska Asa, were moderately to highly sensitive to Mn (marginally better or similar to the medics).

Interestingly, all cultivars that ranked well for Mn tolerance are listed in seed company and government agency descriptions as sensitive to Mn. This raises a question that is yet to be resolved: are field (anecdotal) observations contributing to misconceptions? Manganese concentrations are often elevated in waterlogged soils and high tissue Mn levels will inevitably be linked to poor serradella performance if measured under these circumstances.

6.4.2 Intolerance of waterlogging by serradellas

Waterlogging tolerance was tested by transferring plants to stagnant (deoxygenated) agar solutions (a waterlogging mimic) for 14 days. Root growth was slowed by 40-70% under these conditions, but most striking was the very poor recovery by serradellas when the “waterlogged” conditions were alleviated (shoot growth was reduced by 50-90% relative to plants grown without waterlogging) (Table 6.4 – 2). All genotypes of serradella were adversely impacted, including slender serradella which enjoys an anecdotal reputation for being tolerant of waterlogged conditions. Slender serradella grows slowly during winter and has a large flush of late spring growth. These field observations, combined with the waterlogging experiment results, suggest the reputed “waterlogging tolerance” of slender serradella may instead be due to avoidance of waterlogging stress.

6.4.3 Tolerance of cool (winter) growth temperatures

In regions of southern Australia that experience cool winter temperatures (<15°C daily maximum), the adoption of serradellas is influenced by anecdotal reports of poor establishment and low winter growth rates compared to that of other pasture legumes. Indeed, yellow serradellas were not found to be as productive as French serradellas or subterranean clover in NSW tablelands environments (e.g. Sandral *et al.* 2019). Experiments were undertaken to assess whether cool (winter) temperatures were constraining the germination, emergence and growth of serradellas more than other pasture species.

The experiments provided no evidence that cool temperatures resulted in substantially slower germination rates for French and yellow serradellas compared to other pasture legume species such as lucerne and subterranean clover. Dry matter production of serradella cultivars under a cool temperature regime (15/10°C) was comparable to a winter-active lucerne (cv. SARDI 10) and subterranean clover (cv. Woogenellup).

These results indicated that cool temperatures may not be a significant factor limiting the productivity or adaptation of serradellas for pasture systems in the cooler regions of southern Australia.

Table 6.4 - 2. Average shoot and root dry mass (DM) per plant of aerated control and stagnant treatments following the waterlogged and recovery periods. Plants were 21-d-old when the treatments of an additional 14 d duration were imposed. Values are the average of four replicates (two plants per replicate). Numbers in brackets represent the standard error of the mean.

		Waterlogged period						Recovery period					
		Shoot dry mass (mg plant^{-1})			Root dry mass (mg plant^{-1})			Shoot dry mass (mg plant^{-1})			Root dry mass (mg plant^{-1})		
Species	Cultivar	Aerated	Stagnant	% Control	Aerated	Stagnant	% Control	Aerated	Stagnant	% Control	Aerated	Stagnant	% Control
Ornithopus compressus	Avila	427 (28)	358 (24)	84	171 (14)	102 (7)	60	2268 (113)	1379 (113)	61	548 (31)	377 (33)	69
	Santorini	549 (22)	458 (23)	84	212 (12)	115 (5)	54	2459 (136)	1512 (89)	61	501 (37)	376 (24)	75
	Yellotas	355 (18)	341 (19)	96	145 (9)	99 (7)	68	1568 (110)	1280 (102)	82	324 (23)	311 (23)	96
Ornithopus sativus	Margurita	365 (33)	340 (26)	93	146 (18)	84 (5)	57	2407 (274)	1143 (84)	47	480 (54)	274 (20)	57
	Serratas	360 (38)	324 (16)	90	149 (12)	81 (3)	54	1935 (142)	1234 (116)	64	385 (45)	326 (32)	85
O. sativus x O. compressus	Grasslands Spectra	315 (20)	283 (38)	90	117 (8)	70 (9)	60	1629 (231)	904 (95)	55	309 (41)	216 (27)	70
Ornithopus pinnatus	91FRA19PIN.1	26 (1)	24 (3)	93	13 (1)	8 (0.5)	56	149. (13)	100 (10)	67	40 (4)	38 (4)	95
	GEH114PIN	50 (7)	32 (5)	63	27 (3)	10 (1)	38	451 (56)	404 (46)	90	152 (18)	167 (20)	110
	Jebala	107 (11)	86 (15)	80	61 (8)	32 (4)	53	1091 (102)	543 (154)	50	311 (27)	205 (54)	66
Trifolium michelianum	Bolta	552 (50)	584 (33)	106	168 (19)	204 (15)	121	3208 (298)	3657 (577)	114	577 (65)	712 (112)	123
	Frontier	778 (64)	859 (96)	110	210 (20)	238 (20)	114	4553 (571)	6714 (417)	147	736 (116)	1182 (92)	161
	Taipan	732 (92)	786 (128)	107	178 (23)	237 (34)	133	4823 (616)	6488 (650)	135	686 (82)	1114 (155)	162
P- value (< 0.05)	Cultivar	< 0.001			< 0.001			< 0.001			< 0.001		
	Treatment	0.524			< 0.001			< 0.001			0.055		
	Interaction	0.843			< 0.001			< 0.001			< 0.001		
	LSD$_{interaction}$ (P < 0.05)	ns			*			169			ns		

* no LSD presented for root DM (waterlogged period) as data were log transformed

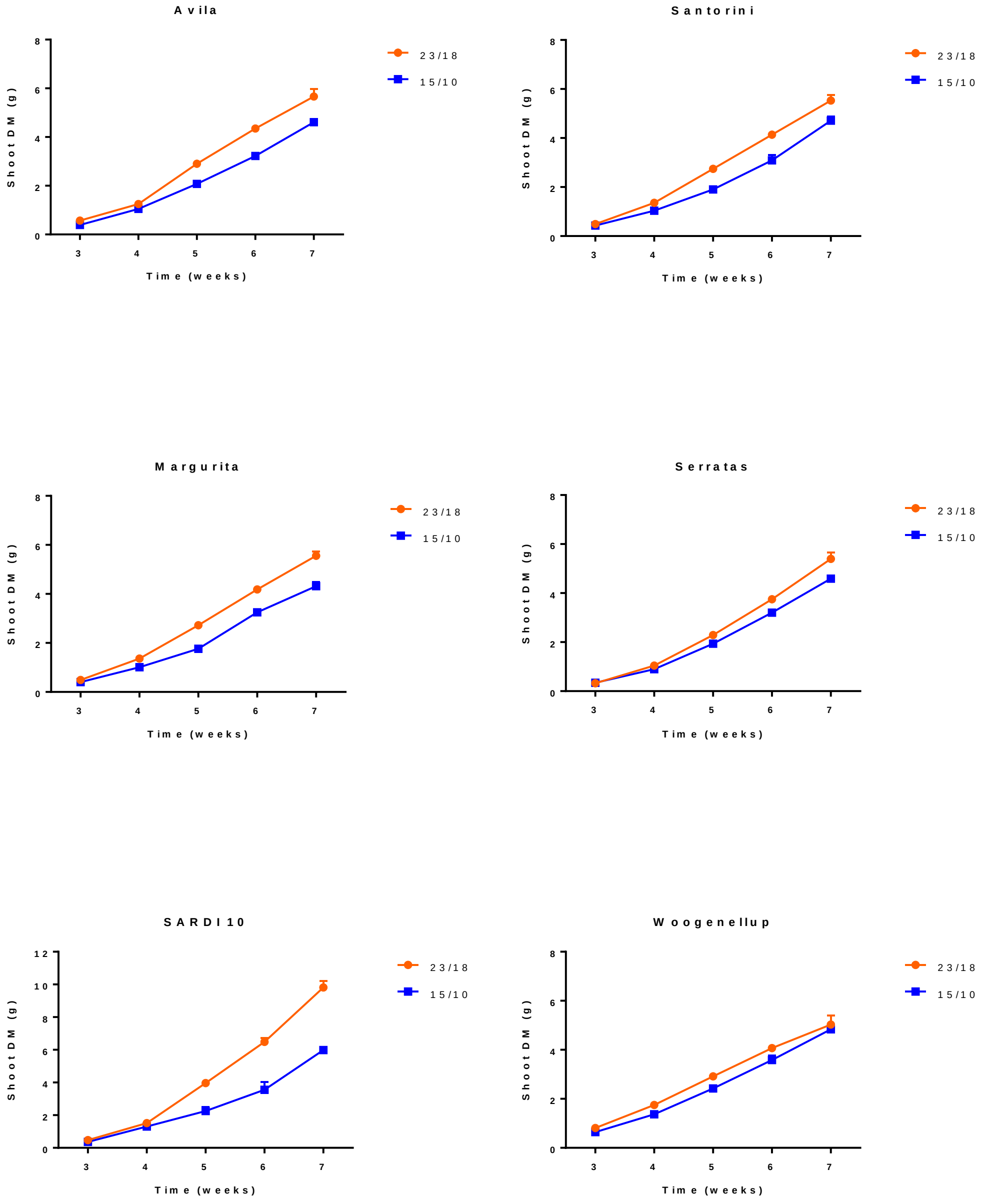


Figure 6.4 - 2. Shoot dry mass (DM) response of serradella and reference cultivars to temperature over time under controlled environment conditions (mean ±s.e., n=3). There was a significant interaction of treatment and time (P < 0.05) except for *Trifolium subterraneum* cv. Woogenellup. The species identity of the other cultivars can be found by reference to the preceding tables.

6.4.4 Implications for use of serradellas in the areas where they are presently not used

These experiments provided further evidence that the adaptive range of serradella is wider that the lower rainfall, infertile, sandy environments of southern Australia where it was first used. However, anecdotal perceptions concerning adaptation of serradellas to acid soils and cold environments must be modified because there were wide ranging differences among the cultivars in their tolerance of high Al or Mn, and growth in cool conditions was equivalent to that of other temperate pasture species.

Serradellas survived a two-week waterlogging period, but growth was significantly reduced (especially during "recovery" after waterlogging). This result reinforces the view that waterlogging is a significant edaphic limitation for the serradella species and that they will not perform well in areas prone to persistent winter waterlogging. Surprisingly, the view that slender serradella is tolerant of waterlogging was not supported. It is now surmised that slender serradella persists in waterlogged areas of paddocks by way of avoidance rather than tolerance of waterlogging.

6.5 Results and Discussion: Output 5(e) - Describe the soil and climatic zones where P efficient serradellas could be utilised and any key traits to be developed in varieties for better adaptation to these new areas of use.

6.5.1 Potential adaptation zone and current use of serradella in southern Australia

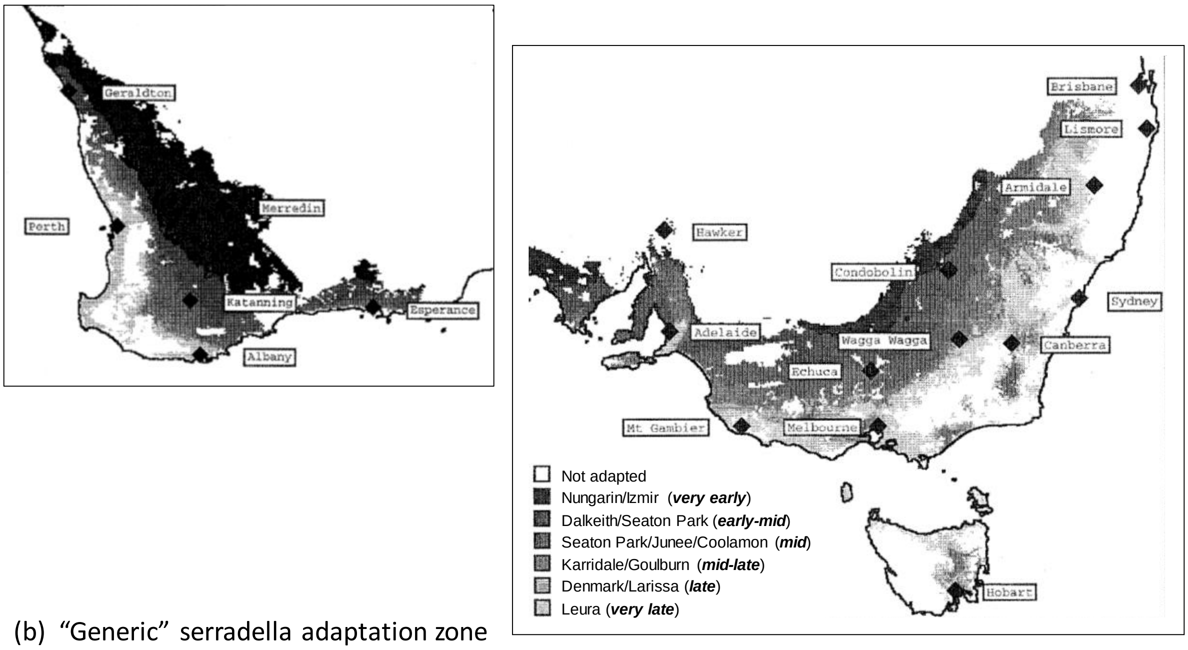
The climatic zone of adaptation for serradella in southern Australia has been predicted by Hill (1996). The analysis and other supporting evidence cited by Hill, recognise that serradellas have a wide adaptation potential; considerably wider than that of many other alternative temperate pasture legumes and temperate grasses. The predicted area of serradella use was determined by the following climatic boundaries:

- winter precipitation/evaporation ratio ≥0.5,
- summer precipitation/evaporation ratio <0.5 ● and ● summer precipitation – evaporation <-50,
- total rainfall ≥450 ● or ● spring precipitation/evaporation ratio >0.28,
- total rainfall <720 ● or ● spring precipitation/ evaporation ratio <0.25.

This essentially defined a zone of potential serradella use within a broad rainfall band between 300 and 720 mm average annual rainfall (Fig. 6.5 - 1b). The analysis demonstrated that serradella may be broadly suitable for use across most of the subterranean clover adaptation zone (Fig. 6.5 - 1a). However, some high rainfall areas (>720 mm) that are suited to very-late maturing subterranean clovers were excluded by this prediction. The predictive model understates the potential role of serradellas in at least some high rainfall areas. Records of serradella collections held in Australasian herbaria clearly indicate that serradellas have naturalised in coastal high rainfall areas (Fig. 6.5 - 2) and late-season yellow serradellas have been recommended for use in high rainfall, coastal sand-dune areas of south-western WA (e.g. Revell *et al.* 1994). Consultations conducted within the current project, indicate that serradella continues to be a viable alternative to subterranean clover in the high-rainfall areas of south-west WA, but its use is confined to soils that are not subject to waterlogging (e.g. A. Loi, *pers. commun*). More recent recommendations for serradella use in NSW also suggest an upper annual rainfall boundary of 800 mm (Hackney *et al.* 2013).

The major limitation of defining a serradella adaptation zone by climatic analysis alone, was that potential edaphic constraints to serradella use were not fully considered. Hill (1996) attempted to do this for NSW by determining a "suitability zone" for serradella use in which the potential distribution of serradella was further confined to soils with surface soil $pH_{(Ca)}$ <5 (Fig. 6.5 - 3). Comparisons of this

(a) subterranean clover adaptation zone



(b) "Generic" serradella adaptation zone
(from Hill 1996)

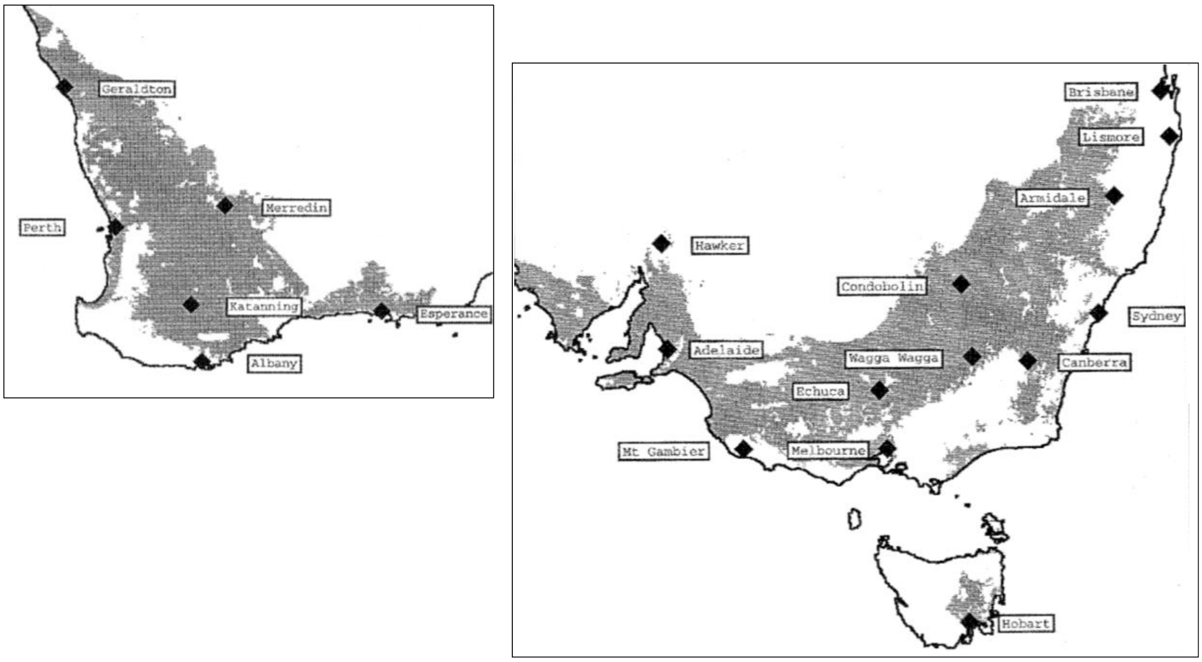


Figure 6.5 - 1. Predicted zones of adaptation for (a) subterranean clover in southern Australia with coverage shown for cultivars that differ in their maturity-type, and (b) the species of serradella used in southern Australia. (Adapted from Hill 1996). The distribution of serradella was predicted from climatic data by specifying: winter precipitation/evaporation ratio ≥0.5, and (summer precipitation/ evaporation ratio <0.5 and summer precipitation – evaporation <-50), and (total rainfall ≥450 or spring precipitation/evaporation ratio >0.28) and (total rainfall <720 or spring precipitation/ evaporation ratio <0.25). This essentially confined the potential distribution of serradellas to a broad rainfall band between 300 and 720 mm average annual rainfall and suggests that serradella may be broadly suitable for use across most of the subterranean clover adaptation zone; some high rainfall areas (>720 mm) that are suited to very-late maturing subterranean clovers are excluded by this prediction.

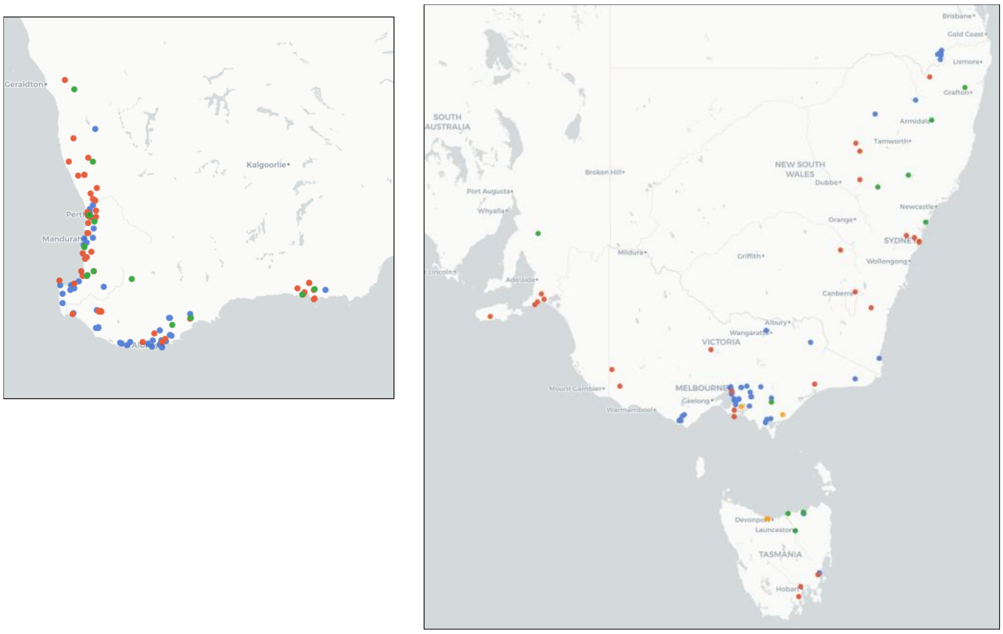


Figure 6.5 - 2. Location records of *Ornithopus* spp. collected for herbaria in Australia, from The Australasian Virtual Herbarium (https://avh.chah.org.au/). Key to species: (●) *O. sativus*, (●) *O. compressus*, (●) *O. pinnatus*, and (●) *O. perspusillus*.

Figure 6.5 - 3. The "suitability zone" suggested by Hill 1996 for use of serradella in NSW based on the intersection of his "generic climatic adaptation zone" and surface soil pH$_{(Ca)}$ <5.0.

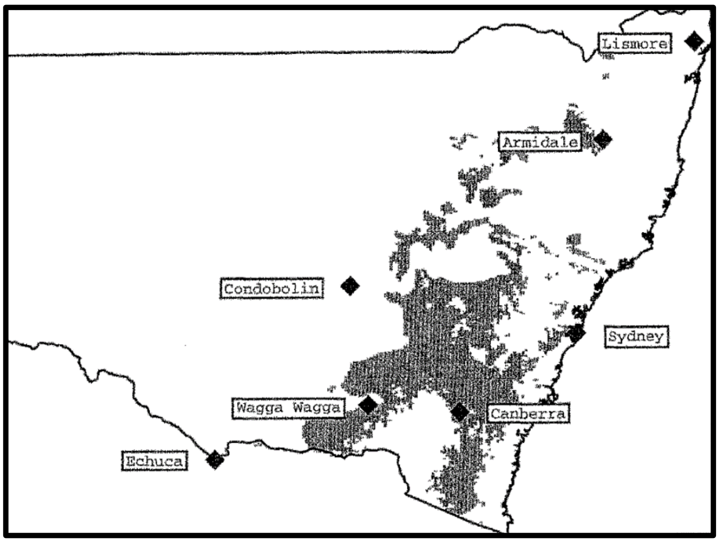
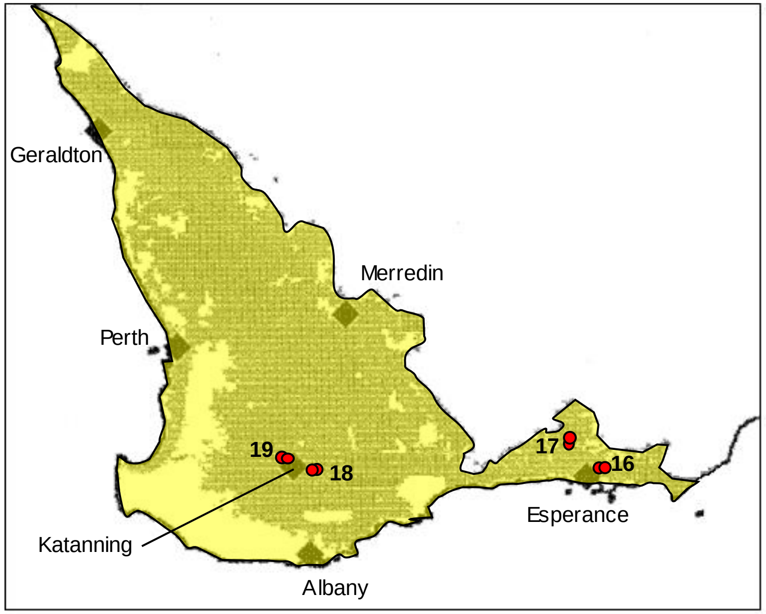
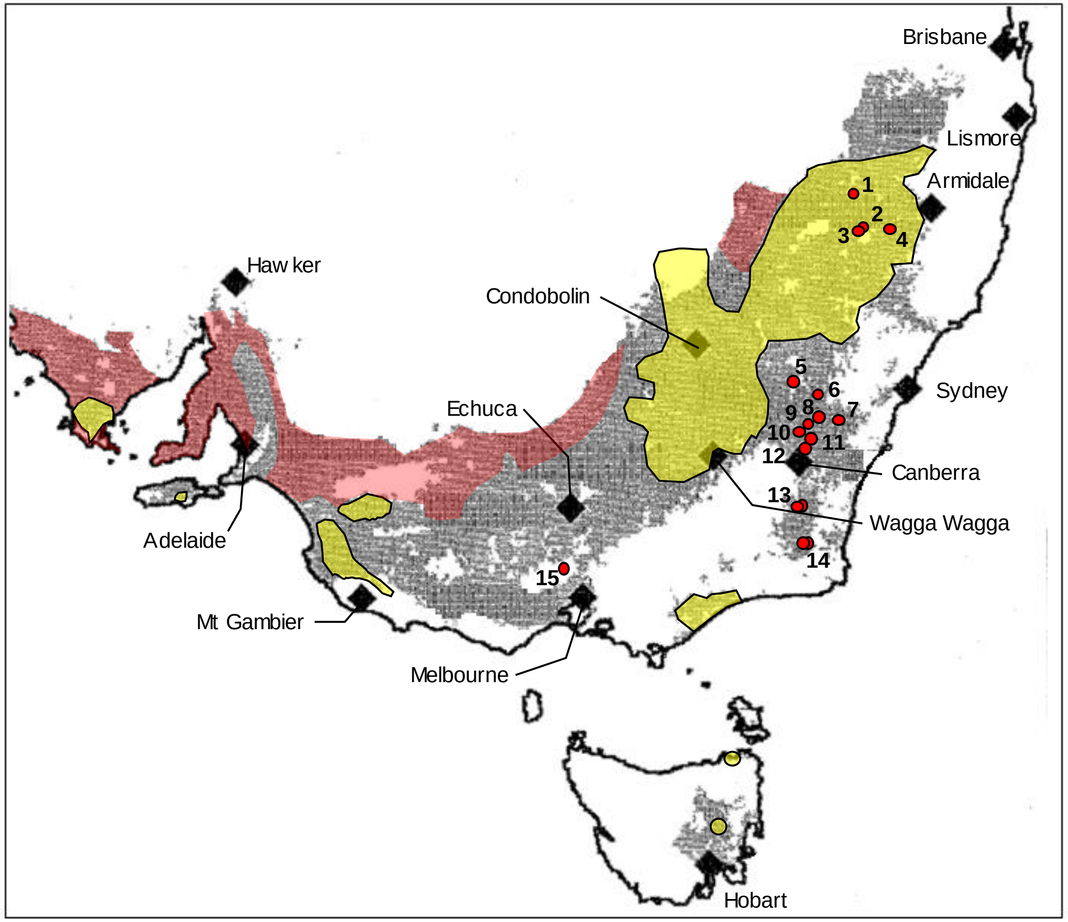


Figure 6.5 - 4 (*following page*). Areas of southern Australia in which serradella is used currently (yellow shading) superimposed on the climatic adaptation zone for serradella (grey shading) predicted by Hill (1996). Red dots indicate sites used for experiments in the *P efficient pastures* project. Serradella does not thrive in neutral-alkaline soils. The red shading indicates approximate zones with surface soil pH$_{(Ca)}$ >~7 where serradella is likely to grow relatively poorly compared to annual medics.
Acknowledgement: Bob Freebairn, Belinda Hackney, Graeme Sandral, Daniel Kidd, Angelo Loi, Clinton Revell, Ross Ballard, Lyn Dohle, Rowan Smith and Lisa Warn are thanked for their assistance in defining the zones in which serradella has been used with success.





Key to serradella & P-efficiency research locations:

1. Boggabri farm group participatory research site
2. Purlewaugh farm group participatory research site
3. Purlewaugh/NSW DPI participatory research site
4. Spring Ridge/NSW DPI participatory research site
5. Cowra core research site
6. TablelandsFS/NSW DPI co-located participatory and core research site (Bigga)
7. Goulburn core research site
8. Gunning core research site
9. Bookham Agric. Bureau (Yass) participatory research site
10. Yass core research site
11. Murrumbateman core research site
12. Canberra core research site
13. MonaroFS/NSW DPI co-located participatory and core research site (Glenfinnan)
14. MonaroFS/NSW DPI co-located participatory and core research site (Red Cliff)
15. Central Ranges Grassland Society participatory research sites
16. ASHEEP/Murdoch Univ co-located participatory research sites (Neridup)
17. ASHEEP/Murdoch Univ co-located participatory research sites (Grass Patch)
18. Southern Dirt/Murdoch Univ co-located participatory research sites (Broomehill)
19. Southern Dirt/Murdoch Univ co-located participatory research sites (Katanning)

map (Fig. 6.5 - 3) with a map that includes current successful use of serradella (Fig. 6.5 - 4) demonstrate the analysis was flawed because serradella use in current times is not confined to *very* acid soils. It is likely that this viewpoint was influenced by the prevailing perception at the time, that serradella was most suited as an alternative and better legume for pastures on very acid, light soils where subterranean clover failed to persist (e.g. Gladstones and McKeown 1977, Freebairn 1996, Freebairn *et al.* 1997).

The edaphic constraints that are more likely to define serradella use are:
(1) waterlogged soils
French and yellow serradellas are generally regarded as intolerant of persistent waterlogging, although (until this project; e.g. **Section 6.4**) most evidence is experiential/anecdotal (e.g. Revell *et al.* 1994). The exception is slender serradella (*O. pinnatus*), which is often included in serradella pasture mixtures because it populates areas of paddocks with shallow soil that become waterlogged during winter. Studies of waterlogging tolerance in the current project (**Section 6.4.1.3**) indicated that slender serradella does not display better direct tolerance of waterlogging than French or yellow serradellas. It was surmised that slender serradella is tolerant of waterlogging by way of avoidance. Its early season growth is very slow and is followed by a large flush of growth in late spring (Freebairn *et al.* 1997), when issues of waterlogged soil have dissipated.

(2) neutral to alkaline soil pH
The serradellas grow poorly in neutral to alkaline (sandy) soils where their performance can fall well behind that annual medics or vetch (a forage crop option) (e.g. Ballard *et al.* 2020; Fig. 6.5 – 5)

An attempt was made (Figure 6.5 – 4) to define the approximate edaphic boundary for use of serradella where $pH_{(Ca)}$ exceeds ~7. NB: There will be pockets of soils outside of this boundary where soil pH is lower than 7. Likewise, there will be paddocks within the climatic/edaphic boundaries that regularly experience winter waterlogging. The sporadic occurrence of localised edaphic constraints throughout the adaptation zone for serradella and cannot be mapped realistically.

Figure 6.5 – 5. Serradella does not thrive in neutral-alkaline soils. Yellow serradella (cv Santorini; left foreground), strand medic (PM-250; middle foreground) and vetch (cv. Studenica; right foreground) growing in a pasture evaluation experiment (2018) at Minnipa Agricultural Centre, SA as part of The Dryland Legumes Pasture Systems project[4]. This figure was supplied by Ross Ballard (PIRSA-SARDI) and is used with permission.

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[4] The Dryland Legumes Pasture Systems project is supported by funding from the Australian Government Department of Agriculture as part of its Rural R&D for Profit program, the GRDC, Meat and Livestock Australia and Australian Wool Innovation.

6.5.2 Constraints to wider use

On face value, Figure 6.5 - 4 indicates that there is a large potential for wider use of serradella. The recent history of serradella use in southern Australia is, indeed, one of very rapidly expanding use. Initially, yellow serradella varieties were considered mainly for use in light-textured, very-acid soils where subterranean clover struggled to survive. Their success in sandy soils entrenched the early view that they were a niche species and they were often excluded from wide ranging evaluations of annual legume species on heavier soils (e.g. Dear *et al.* 2003). However, serradella use has expanded rapidly in recent times, but almost exclusively in the crop-pasture zones of WA and NSW (Fig. 6.5 - 4) following the development of hard-seeded French serradella varieties (Nutt 2004a, Nutt 2004b).

This expansion is on-going and demonstrates that serradellas are not confined to sandy soils. However, they are not used in heavy clay soils, even in areas where widespread use is now common (e.g. northern NSW and Riverina). This is most likely because yellow and French serradellas are intolerant of prolonged waterlogging.

In contrast to the crop-pasture zones, the uptake of serradella for use in permanent pasture areas is stalled (Fig. 6.5 - 4).

Serradella persistence: A major issue is that the cultivars of serradella that are presently promoted for use in permanent pastures where serradellas have not been grown traditionally differ in their ability to persist (**Section 5.2.4**). This has been a recurring theme in discussions with farmers, advisors and pasture seed merchants. Their perception that persistence of serradellas is poor, was backed strongly by legume frequency data collected during this project at a site near Yass that was monitored 4-6 years after being sown in a previous research project. The experiment showed that high persistence by serradella is possible (e.g. yellow serradella cv. Avila) but may not be guaranteed when using varieties that are being sold to farmers presently (e.g. French serradella cv. Margurita and yellow serradella cv. Santorini).

The concern about persistence of serradellas is exemplified by experience conveyed to a project team member (Richard Hayes, *pers. commun.*) by a seed business that services farms on the NSW southern tablelands and the Monaro-Snowy region:

"*We never sell serradella to the same person twice ...the serradellas available to us* (cvs Cadiz, Margurita and Santorini) *are, in our opinion, too early maturing and flower in the frost period in this region. They don't persist because they don't set adequate seed*."

(The business was unaware of cv. Avila, or that it may be more persistent.)

The perception of poor persistence of serradellas has, most probably, been reinforced by use of cultivars that are commonly available but are inappropriate for use in permanent pastures. None of the cultivars listed above by the seed retailer were shown to be highly persistent in the network of serradella persistence experiments in the present project (**Section 6.1**). A second retailer in the NSW southern tablelands was asked to source serradella seed for a commercial pasture to be sown in 2020 and was only able to supply cv. Serratas (Richard Hayes, *pers. commun.*). Similar to cv. Cadiz, this cultivar is completely softseeded and demonstrated very poor persistence in the present project.

Perceptions of low yield: It is also commonly perceived that serradellas do not yield as well as subterranean clovers in permanent pasture areas (e.g. R. Ballard, *pers. commun.*); this may be contributing to poor adoption and use.

Results from this project and previous research indicate why this is a potentially misleading "perception", although it has possibly arisen from true observations. Early experiences with serradella often reflect an era when only a limited number of yellow serradellas were available for evaluation. In recent research, Sandral *et al.* (2019) found that spring yields of yellow serradella varieties grown near Yass, NSW and in the NSW Riverina were, indeed, less than locally-adapted subterranean clover varieties but, in the same experiments the French serradella (cv. Margurita) yielded as well as subterranean clover, and did so at a lower level of soil P fertility. Other research with French serradella has also reported high yields relative to clover (Hackney *et al.* 2013).

Farm group participatory research at a number of the southern NSW sites in the current project found that, after the establishment year, there was often no difference in legume (or total pasture) production by subterranean clover, French serradella (cv. Margurita) and yellow serradella (cv. Avila) when sown as grass-legume pastures on moderately P-fertile soils (e.g. **Appendix 6**). This seemed initially to conflict with the expectation that French serradella should out-yield subterranean clover at moderate levels of soil P (e.g. Colwell P = ~20 mg/kg) (Sandral *et al.* 2019). Closer inspection of the results from the experiments revealed that both the yellow (cv. Avila) and French (cv. Margurita) serradellas used in these experiments could out-yield subterranean clover when present at a similar plant density. However, this most often only occurred in the establishment year. This further highlighted the importance of identifying varieties that regenerate reliably and are persistent. The experiments demonstrated that regenerating legume densities varied among years and among the legume species (cultivars) tested. These attributes varied among the cultivars of serradella that are presently available to farmers (see further discussion below). The results obtained from the participatory experiments are in broad agreement with those reported by Sandral *et al.* (2019), but also indicated that yellow and French serradellas can yield well in these new environments, provided regeneration densities and persistence are good. Yellow serradella also out-yielded subterranean clover on three occasions at the core research site near Gunning, NSW.

Until issues of relatively poor persistence by some serradella cultivars in permanent pastures are overcome, the perception of poor yield by serradella in permanent pastures will remain unresolved. It is also usual to expect wide fluctuations in legume content of mixed subterranean clover-based pasture between seasons (Rossiter 1966). The yield and carrying capacity of the clover-grass system is, consequently, the result of longer-term contributions of legume yield and N-fixation to the pasture system.

Studies over a number of years using serradella cultivars, with proven persistence, will be necessary for confident assessments of the yield and carrying capacity of serradella-based pasture systems.

6.5.3 Factors associated with poor persistence by serradellas in permanent pastures in areas where serradellas are not used presently

6.5.3.1 Soft seeded varieties

Until the recent development of cvs Margurita and Erica (Nutt 2004a, Nutt 2004b) only soft-seeded French serradella varieties were available. Softseeded varieties do not persist more than a few years because they do not survive false breaks or prolonged droughts (Dear *et al.* 2002). This was also observed during drought in the current project (see **Section 6.2.4**).

6.5.3.2 Aerial seeding

Poor persistence of serradella is often considered to be associated with aerial seeding and inadequate grazing control during seed production, and/or intolerance of grazing by serradella varieties. Repeated grazing during seed production and overgrazing of mature pods on the soil

Figure 6.5 - 6. French serradella (line 150S-1C) beginning to flower on 31 March 2020; seven weeks from germination (~11 February), after early season rainfall at a site near Murrumbateman, NSW. Frosts commenced 4 May.

surface during summer will potentially have a negative impact on persistence of serradellas. However, yellow serradella cv. King persists well in grazed permanent pastures in northern NSW provided gazing pressure is reduced during the seed production period every 3-4 years (Freebairn 1996, and *pers. commun.*).

In addition, the moderately-poor (cv. Margurita) and very poor (cv. Santorini) persistence by particular cultivars of hardseeded French and yellow serradellas (described above) was observed, within 4-6 years after sowing, in the absence of grazing or mowing during seed production (**Section 6.2.4**). In the same experiment, however, one cultivar (Avila) exhibited similar persistence to a highly-persistent subterranean clover. This suggests that grazing of the aerial-seeded serradellas may not be the primary challenge to achieve longer-term persistence.

6.5.4 Key traits to be developed in serradella varieties for better adaptation and wider use

6.5.4.1 Flowering time and flowering date stability

Persistence by annual legumes in permanent pastures depends on having cultivars that exhibit reasonably stable flowering dates that ensure flowering and pod set will occur between (and thus avoid) high risk periods of frost and terminal drought. In southern Australia, rainfall is highly variable during autumn with opening rains occurring at any time from February to June depending on location and season. Pasture cultivars must be capable of flowering reliably after the high-risk frost period, irrespective of the date on which they germinate, as exhibited in this project by the subterranean clover cultivars (**Section 6.2.1**). Many of the serradella cultivars began to flower far too early (during frost periods) when sown at an early sowing date.

Regulation of flowering in temperate pasture legumes is expected to be controlled by photoperiod (long days) and vernalisation (cold winter temperatures). Vernalisation is a particularly important regulator of flowering date in subterranean clovers (Aitken 1955, Evans 1958, Salisbury *et al.* 1987). Unstable flowering, as exhibited by many of the serradellas, may be an indication that they have low vernalisation requirements. However, there has been no research to determine how flowering date and flowering date stability are regulated among cultivars of serradella.

Unstable flowering is expected to have negative consequences for:

(i) **seed production and persistence**,

Frost kills flowers and is also likely to damage early pod development. However, the sensitivity of serradella pod growth stages to frost damage has not been quantified. For annual legumes, high seed production underpins persistence and is achieved by flowering soon after the risk of frost has diminished so that there is a long period for seed development before growth is curtailed by terminal drought (e.g. Donald 1970). Because serradellas have indeterminate flowering there may be some latitude in the optimum timing of flowering relative to frost occurrence, but this has not been determined.

(ii) **grazing management,**

Serradellas are aerial seeding and grazing should be avoided periodically during flowering and seed production to ensure the maintenance of seed reserves. Unstable (unpredictable) flowering dates will complicate grazing management.

(iii) **forage yield and feeding value**.

Serradellas have similar feeding value to subterranean clover (e.g. Hackney *et al.* 2013). However, pasture growth rates and feeding value of annual species decline once flowering commences; pasture and animal production will not be assisted by premature, unstable flowering.

Among the serradellas, the flowering dates of yellow serradella cultivars were more stable than those of the French serradellas, and late-maturing varieties of both species tended to be more stable that early varieties (**Section 6.2.1**; Fig. 6.2 - 3). However, this depended on location. No cultivars of serradella exhibited relatively stable flowering dates in Perth. It was also observed regularly in experiments in NSW and Victoria, that French serradella varieties such as Margurita and line 150S-1C, exhibited extremely premature flowering after germinating on very early season rainfall (e.g. Fig. 6.5 - 6).

This issue of early and unstable flowering was discussed with a leading Australian serradella seed producer and retailer (Richard Simpson, *pers. commun*.). It was proposed by the producer that unstable and early flowering may not matter because serradellas have an indeterminate flowering habit and would be able to compensate for loss of seed potential if early flowers were frosted. The amount of research into flowering physiology of the serradellas is very limited. However, Revell *et al.* (1999) have examined flowering, pod and seed development in the very early and late-maturing yellow serradella cultivars: GEH72-1A and Avila, respectively. They found that while flowering was indeterminate, the setting of pods was finite and, after a characteristic period, flowers did not continue developing into pods. The mechanisms behind the cessation of pod set is unknown. The consequences, however, are very clear: indeterminate flowering does not mean indeterminate seed production. The timing of flowering in serradellas is likely to be as important for persistence, as it is in other pasture legumes.

It is notable that yellow serradella cv. Avila, which was found to have similar persistence to subterranean clover in the Yass-Canberra area (**Section 6.2.4**; Fig. 6.2 - 17), also exhibited relatively stable flowering dates in this environment and flowered at a time that would assist it to avoid high risk of frost at flowering. Cultivar King, which has a proven record in permanent pastures in northern NSW also exhibited stable flowering dates in the area where it is used. (**Section 6.2.1**; Table 7.2.1).

6.5.4.2 Seed production, hardseededness and rate of seed softening

Seed production: Seed production across a network of persistence experiments in southern NSW was generally low for all cultivars reflecting dry seasonal conditions. However, seed production by the serradellas was generally better than subterranean clover under these conditions (**Section 6.2.2**). In these experiments, there was often no difference between total seed yield of French and yellow serradella cultivars. The lower seed yield of some serradella cultivars in this network of experiments was generally a reflection of low seedling establishment in year 1 which is more a reflection of poor seed quality than it is of the seed yield potential of these cultivars.

Hardseededness: Entirely softseeded cultivars of serradella do not persist and should not be recommended for used in permanent pasture. A majority of the hardseeded cultivars of serradella have very high (>80%) levels of initially hard seeds (Figs 6.2 – 13 and 6.2 - 14). However, there are also a few cultivars (e.g. King, Margurita, line 150S-1C;) that exhibit intermediate (~50-60%) initial levels of hard seeds. The reasons behind these differences are not known.

A novel finding in the present project has been that there are wide differences in the rates of seed softening among some serradellas. At least one cultivar (cv. Yellotas) is often categorised incorrectly as having "low levels of hard seeds" or even as "soft seeded" (e.g. McCormack and Lattimore 2012). In fact, Yellotas has high initial levels of hard seeds, that soften relatively rapidly during late summer/autumn.

Seed softening: Research in this project found that there were three diverse patterns of seed softening exhibited among serradella cultivars:

(1) Cultivars with high proportions of initially hard seeds and slow to very slow seed softening.
A majority of the hardseeded yellow and French serradella cultivars reside in this category (including cv. Avila). It is anticipated that seed softening may span at least two years for these varieties.

(2) Cultivars with intermediate levels of initially hard seeds (or initially very rapid seed softening), followed by relatively slow seed softening.
A limited number of cultivars (including cvs Margurita, line 150S-1C and sometimes King). Typically, 40-60% of hard seed remain by June/July.

(3) Cultivars with high proportions of initially hard seeds followed by rapid seed softening.
A less-common pattern expressed in yellow serradella genotypes (including cv. Yellotas) which resulted in low to very low proportions of hard seed remaining by June/July.

None of the serradella seed softening patterns matched the proposed "idiotypic" pattern observed in well-adapted subterranean clover cultivars: i.e. a characteristic level of initial hard seeds with about a third of seeds soft and ready to germinate at the beginning of summer, a further third softening until June/July, and the remainder persisting as hard seed until seed softening recommences in the following summer. However, it must be recognised that this is an idealised ideotype description. In reality, the patterns of seed softening among subterranean clovers also varied between seasons and locations, so chasing a "close match" to the ideotype may not be realistic or entirely desirable.

6.5.4.3 "Best-bet" cultivars for persistence and production in permanent pastures

This project was commenced with a view to extending the use of serradella pastures into areas where subterranean clover is essentially the only annual legume used in pasture systems. The initial goal was to capture the P-efficiency advantage of serradellas to reduce the P-fertiliser cost of maintaining high production by up to 30% every year (e.g. Simpson *et al.* 2014). Farmer groups participating in the project have added to this goal, the objectives of capturing the "bloat-safe" characteristics of serradella pasture, the ability to diversify the legume base of their pastures with species that can avoid diseases of clovers, provide out-of-season growth in years when rainfall extends spring onto summer, deliver improved early- and late-season drought tolerance as a consequence of faster root growth and/or deeper root systems and deliver improved drought resilience associated with a smaller-seeded species improving seed-set in a dry spring compared to larger-seeded species such as subterranean clover.

Although a major conclusion from the research is that serradella persistence in permanent pastures is a significant barrier to the adoption of current cultivars, the research has informed development of a short list of "best-bet" serradella cultivars for further research and on-farm experiments in permanent pasture areas where serradellas have not been adopted.

Table 6.5 – 1. "Best bet" cultivars of serradella for further research and on-farm experiments in permanent pasture areas where serradellas have not been adopted.

Cultivar	**Species**	**Seed availability**	**Rating**	**Maturity and flowering date stability**	**Hardseededness and seed softening**	**Other comments**
Avila	Yellow serradella	Available from WA at present	Very promising	* mid-late season variety with good flowering date stability on the southern tablelands, NSW (Canberra) and at Cowra. * near-ideal flowering date for the target region and high seed yields	* produces high levels of hard seed that soften relatively slowly. This may inhibit its performance because this pattern of seed softening is associated with a year-2 germination problem which may check production in that year and may allow invasion of weeds into newly sown pastures. * Avila germinates in high numbers in year 3.	* outstanding persistence across a broad range of core and participatory research sites under generally drier-than-average conditions. In the longest running persistence experiment (~6 years), the frequency of Avila has been essentially equivalent to that of subterranean clover cv. Leura. * production and persistence in mixed pastures has not been evaluated
King	Yellow serradella	Presently available from single grower in northern NSW	Proven performer in northern NSW; (persistent & productive)	* early-season variety that exhibited stable flowering dates in its target environment (i.e. northern NSW; Tamworth); relatively stable in Perth and Cowra, but unstable in Canberra. * sets large quantities of seed	* high levels of hard seed * slow to soften, but not as slow as Avila.	* known to persist well in grazed permanent pastures in northern NSW provided gazing pressure is relaxed during seed production every 3-4 years to maintain the soil seed bank (Freebairn 1996, and *pers commun.*).
Yellotas	Yellow serradella	Available from Tasmania in limited quantities	Worthy of further investigation	* mid-late season variety when grown in NSW, has a relatively stable flowering date for the NSW tablelands (Cowra, Canberra, Tamworth) but was unstable in Perth.	* high proportions of hardseeds that soften rapidly. * Pattern of seed softening has parallels with that of some subterranean clovers; may protect against false breaks. However, can also result in low levels of residual hard seeds to carry it in droughts. * did not persist in adequate densities by year 3 of a drought period in the present project; as such was inferior to cv. Avila.	* often germinates in high densities in year-2 after establishment. * Yellotas should continue to be examined because it may perform significantly better in a run of more favourable seasons. * has seed softening characteristics that may make it an ideal component of a mixture with cv. Avila to address its year-2 germination problem.

Table 6.5 – 1. (*continued*)

Erica	French serradella	Seed not commercially available; seed growers prefer cv. Margurita	Promising	* mid-season variety *relatively stable flowering in Cowra, but unstable Canberra and very unstable Perth and Tamworth. (However, potentially more stable than cv. Margurita).	* Usually high proportions of initially hard seeds (but can be as low as ~50% depending on season). * Slow seed softening. * can have a year-2 germination problem (see Avila description).	* herbage yield considered equivalent to cv. Margurita (Hackney *et al.* 2013). * more prostrate growth habit than cv. Margurita (Nutt 2004a).
Line 150S-1C (to be released as Rosa)	French serradella	Seed not yet available commercially	Promising	* mid-late variety (Canberra); but mid-season (Perth & Tamworth). * unstable flowering (Canberra and Tamworth); very unstable Perth. (However, potentially more stable than cv. Margurita).	* intermediate proportions of initially hard seeds. * Slow seed softening. * less likely to have a year-2 germination problem (see Avila description)	* seed softening pattern has parallels to that of adapted subterranean clovers.
Margurita	French serradella	Available	Worthy of further investigation	* Early-maturing (Perth). * Early-mid (Tamworth). * Mid-season (Cowra and Canberra). * unstable flowering date (Cowra); very unstable elsewhere.	* intermediate to high proportion of initially hard seeds. * slow seed softening. * can have a year-2 germination problem (see Avila description).	* high yielding but only moderate persistence, (southern tablelands, NSW). * A useful benchmark variety against which improvements can be gauged.

No one variety has all of the putative traits that are thought to be desirable for persistence and high production, but each variety has sufficient merit to potentially kick-start adoption once performance in a mixed pasture is proven (Table 6.5 - 1). In the short-term, these cultivars are expected to add value to existing subterranean clover-based pastures in some new environments; in the longer term they have potential to replace subterranean clover in some environments.

7 Conclusions/recommendations – (Activity B5) Extending the adaptation range of serradella systems.

7.1 Output 5(a) - Conduct participatory research experiments with farmer groups in areas serradella is not used to examine productivity, persistence and potential use.

7.1.1 Growth and persistence of serradella on farms where it is currently not gown

- The participatory research experiments provided valuable insights into the performance of serradella in some diverse farm environments of southeastern Australia. The experiments also complemented core research activities, raised farmer awareness and have provided direction for future development of serradella pastures.Serradella yield was often greater than that of subterranean clover when grown at STP concentrations that were close to the critical STP requirement of serradella, provided that the legumes were present at comparable plant densities in the pasture swards. This level of soil P fertility should have been suboptimal for subterranean clover. These observations provided additional farm-level confirmation that serradellas are more P efficient than subterranean clover.

- However, comparable densities for serradellas and subterranean clover were mainly achieved in the year of pasture establishment pointing to potential serradella regeneration and/or persistence issues.

- Persistence by serradella in mixed pastures was flagged as probably the most important issue constraining the adoption of serradellas by farmers and seed retailers in permanent pasture areas.

There were a number of factors identified as potential contributors to this issue:

(i) Some of the serradella species/cultivars being sold to farmers may not be well adapted to the environments in which the participatory experiments were conducted. The comparison of yellow serradella cv. Avila with cv. Santorini demonstrated that particular cultivars can persist better than others (data from participatory experiments [MFS sites]and persistence research experiments [NSW DPI]).

(ii) There is a year-2 serradella regeneration issue which is a consequence of the very slow seed softening exhibited by many serradella varieties. This is also an acknowledged issue in phase farming applications of the serradellas. In phase farming systems, the issue is overcome by planting a cereal crop in year 2 (e.g. Revell *et al.* 1998). However, this is not a practical solution for permanent pastures where the gap in serradella regeneration is expected to allow the ingress of weeds and other competitors to the disadvantage of the serradella.

(iii) Flowering date research experiments (CSIRO and NSW DPI) also indicated that many serradella cultivars have poor flowering date stability which may also have contributed to seed production problems.

- The participatory research highlighted the fact that limited weed control options will present real challenges when serradella is adopted for use in permanent pastures. In particular, a wider range of weed species and the need to consider safe options for non-target perennial species warrant further research to address this problem if serradella is to penetrate these markets.

- Recurring issues with serradella establishment were reported in the central range's region of Victoria. Timeliness of sowing was crucial in a short window before cold, wet and sometimes waterlogged conditions occurred; slower serradella germination and weed and grass competition added to the problem, and the available cultivars of serradella were, most probably, not well-enough adapted to the region. In contrast, the farm group participants found subterranean clover was relatively easy to establish under the same conditions. Nevertheless, serradella was established at the last of the three experiment sites to be sown and has regenerated well in 2020. It is possible that successful use of serradella in this region may prove to be very site-specific. Good weed control and seedbed preparation may prove to be more critical when establishing serradella pastures compared with subterranean clover pastures.

- Positive observations in support of serradella uptake were:
  (i) Serradellas had better seed production and seedling regeneration success during very dry periods than subterranean clover.
  (ii) Serradellas had deeper root systems than subterranean clover in some soil types; but the extent to which this occurs varies from nil to substantial depending on the soil profile.
  (iii) Good regeneration and persistence were observed for some cultivars. The standout cultivar in this respect was yellow serradella cv. Avila, which may approach the persistence levels of subterranean clover cv. Leura. Other evidence indicated good persistence by cv. King. The performance of these cultivars should continue to be monitored for longer periods in more diverse environments. A wider selection of new cultivars that can match or exceed their performance should be sought.
  (iv) There was genuine farmer interest in diversifying the legume base of permanent pastures, but they need strong evidence of persistence to support adoption.

7.2 Output 5(b) - Conduct field experiments with serradella to quantify the range in serradella flowering time, seed production, hardseededness/ breakdown, persistence, seedling recruitment and survival traits that underpin cultivar development.

The sum of all of these experiments has enabled a definitive characterisation of the cultivars that are presently being marketed to farmers. The research results underpin a significant revision of maturity type classifications, issues with flowering date stability, an understanding of seed softening patterns and improved information concerning persistence attributes of these cultivars (see Summary Table 7.2 – 1). These data can be used to revise substantially the advisory information presently available to farmers.

7.2.1 Serradella persistence in permanent pastures

Persistence by many serradella varieties in permanent pastures is problematic: The persistence in permanent pastures at high frequencies by a number of the serradella varieties currently sold to farmers was found to be inadequate in comparison with subterranean clover. This was identified as the major factor needing a solution if there is to be adoption of serradellas for use in permanent pastures.

Ungrazed, alternative-legume monocultures (managed by occasional mowing) from previous experiments at three southern tableland locations were revisited two years after they had been last sown or resown. Many of the alternative legumes were already no longer present at levels that could be assessed; the serradella varieties that were commercially available at that time (French serradella cv. Margurita or yellow serradella cv. Santorini) were doing better but, were typically present at frequencies of 40-60% or less (**Section 6.2.4**). Yellow serradella cv. Avila which had been sown only at one of these sites and only in the final year of that experiment (due to low seed availability) was, however, present at frequencies that approached that of the locally-adapted subterranean clover cv. Leura. The comparatively high frequency of cv. Avila was maintained over the next two years of monitoring.

These experiments were not designed to test persistence and may be criticised as inadequate for this purpose, but the observations and a previous assessment of one of the sites (Hayes *et al.* 2015) provided the first clear indications to the project team that persistence by at least some of the serradella varieties being sold to farmers was inadequate for permanent pasture applications. The observations of persistence issues were backed by anecdotal accounts from a key seed retailer.

Persistence by self-regenerating annual legumes is largely a consequence of their seed production, seed protection and establishment characteristics; these are influenced, in turn, by competitiveness of the species in mixed pasture swards, grazing tolerance/management, legume yield, plant disease, etc. However, it was evident that serradella persistence was problematic even in ungrazed monocultures. Consequently, project activities were primarily focussed on seed production, seed protection and seedling establishment. A series of core research and participatory experiments on farms examined serradella maturity types, flowering dates, seed production, hardseededness and seed softening, and seedling establishment success to understand why serradella cultivars appeared to be vary in their ability to persist.

Factors identified as potentially impacting serradella persistence under these conditions were:
(i) cultivars of inappropriate maturity-type were being sold for use in high rainfall zone permanent pastures;
(ii) gaps in the availability of very-early and late maturing hardseeded cultivars of French serradellas;
(iii) the flowering dates of many cultivars being marketed to farmers were unstable leading to unpredictable flowering and flowering during periods of frost depending on the date of opening rainfall;
(iv) seed softening patterns among the hardseeded serradellas varieties were often too slow for the environments in which they were being grown, with no options offered to mitigate the risks that this incurred.
(v) advice to farmers concerning persistence traits of various serradella cultivars (e.g. maturity type, hard seed levels) was in some cases incorrect leading to incorrect cultivar selection.

Positive attributes of the serradellas were:
(i) high seed production potential relative to subterranean clover (particularly under dry seasonal conditions);
(ii) relatively good seedling regeneration and establishment under difficult, dry seasonal conditions;
(iii) deeper rooting capacity than subterranean clover, provided the soil profile permitted its expression.
(iv) capacity to continue growing on late season rainfall (when it occurred), after subterranean clover have senesced.

Table 7.2 - 1. Summary of flowering time, hardseededness, seed softening and persistence characteristics of serradella cultivars. The subterranean clover cultivars were included as “control” varieties for maturity type and flowering stability.

Species	Cultivar	Maturity[1]	Flowering date stability[2]	Hardseededness[3] (assessed over 2 years, Cowra and Canberra)	Persistence[4] in permanent pastures (initial assessment)
subterranean clover (*Trifolium subterraneum*)	Izmir	Very early	Stable (Perth, Tamworth, Cowra) Relatively stable (Canberra)	Initial hardseed proportions: ~75-95%. Relatively slow seed softening. Residual hardseed: ~60-85% depending on location.	
	Seaton Park	Mid-season	Stable (Perth, Tamworth, Cowra) Relatively stable (Canberra)	Initial hardseed proportions between ~55-85%. Relatively rapid seed softening. Residual hardseed ~20% - ~50% depending on location	
	Coolamon	Mid- season	Stable (all locations)	Initial hardseed proportions between ~65-95%. Relatively fast seed softening. Residual hardseed ~30-50% but could be as high as ~85% depending on location and year.	
	Goulburn	Mid-late (Tamworth, Cowra, Canberra) Very-late (Perth)	Stable (Perth, Cowra, Canberra) Relatively stable (Tamworth)	Initial hardseed proportions: ~80-90%. Relatively fast seed softening, but can also be slow depending on seasonal conditions. Residual hardseed ~10-75% depending on location and year.	Persistent in target area (e.g. Sthn tablelands, NSW)
	Leura	Very late	Stable (all locations)	Initial hardseed proportions ~70-80%. Fast to very fast seed softening depending on location. Residual hardseed ~5-45% depending on location.	Persistent in target area (e.g. Sthn tablelands, NSW)
yellow serradella *(Ornithopus compressus)*	King	Early	Stable (Tamworth) Relatively stable (Perth, Cowra) Unstable (Canberra)	Initial hardseed proportion ~45-95% depending on location. Generally, very slow seed softening but may increase in some seasons. Residual hardseed ~40-80% depending on location and year.	Persistent in target area (e.g. northern NSW)
	GEH 72.1A (released as Regena)	Early	Unstable (Perth, Cowra) Relatively stable (Tamworth) Very unstable (Canberra)	Initial hardseed proportions generally, >90% but occasionally as low as ~50% depending on year. Generally, very slow seed softening but may increase in some seasons.	

				Residual hardseed often only marginally below initial hardseed level (~90%), but can soften rapidly in some seasons (~50%).	
	Santorini	Early	Relatively stable (Tamworth, Cowra) Unstable (Canberra) Very unstable (Perth)	Initial hardseed proportions ~85-95%. Very slow seed softening. Residual hardseed only mrginallly lower than initial hard seed levels (~80-95%).	Not persistent (sthn tablelands, NSW)
	Elgara	Mid (Perth) Early-mid (Tamworth, Cowra, Canberra)	Relatively stable (Cowra, Canberra, Tamworth) Very unstable (Perth)	Initial hardseed proportions >90%. Very slow seed softening. Residual hardseed only mrginallly lower than initial hard seed levels.	
	Yellotas	Mid (Perth) Mid-late (Tamworth, Cowra, Canberra)	Relatively stable (Cowra, Canberra, Tamworth) Unstable (Perth)	Initial hardseed proportions: ~70-95% Relatively fast to very fast seed softening. Residual hardseed: ~20-25%, but can be as low as ~5% depending on location and season.	Not persistent during drought (sthn tablelands, NSW)
	Avila	Mid-late	Stable (Canberra, Cowra) Relatively stable (Perth, Tamworth,)	Initial hardseed proportions >90%. Slow seed softening. Residual hardseed typically between ~75-90%.	Persistent (sthn tablelands, NSW)
French (aka pink) serradella *(O. sativus)*	Eliza	Early (Perth, Tamworth) Early-mid (Cowra, Canberra)	Very unstable (all locations)	Soft seeded	Not persistent
	Margurita	Early (Perth) Early-mid (Tamworth) Mid (Cowra, Canberra)	Unstable (Cowra) Very unstable (Perth, Canberra, Tamworth)	Initial hardseed proportions ~50-80%; variation depending on location. Slow seed softening. Residual hardseed ~40% - ~60% reflecting initial hardseed level.	Moderate persistence (sthn tablelands, NSW)
	Cadiz	Early (Perth) Early-mid (Tamworth) Mid (Cowra, Canberra)	Very unstable (all locations)	Soft seeded	Not persistent
	Erica	Mid	Relatively stable (Cowra) Unstable (Canberra) Very unstable (Perth, Tamworth)	Initial hardseed proportions ~80-90%; occasionally as low as ~50% depending on season. Slow seed softening. Residual hardseed typically ~70-80% but can be as low as ~45% if initial level is also low.	
	150S-1C	Mid (Perth, Tamworth) Mid-late (Canberra)	Unstable (Canberra, Tamworth)	Initial hardseed proportions: ~50-60% Slow seed softening	

	(to be released as Rosa)		Very unstable (Perth)	Residual hardseed: ~40-45%.	
	Serratas	Mid-late (Perth, Tamworth) Very-late (Cowra, Canberra)	Relatively stable (Cowra, Canberra) Unstable (Tamworth) Very unstable (Perth)	Soft seeded	Not persistent
	Grasslands Koha	Late	Relatively stable (Canberra) Very unstable (Perth)	Soft seeded	Not persistent
hybrid **(Frenchxyellow)** **serradella**	Spectra	Late (assessed Canberra only)	---	Initial hardseed proportions: ~40%. Slow seed softening Residual hardseed: ~20% (assessed one year, Canberra only)	
slender serradella ***(O. pinnatus)***	Jebala	Mid (Perth, Tamworth) Mid-late (Cowra, Canberra)	Stable (Perth, Cowra, Canberra) Unstable (Tamworth)	Initial hardseed proportions: ~80-95%; but can also start as low as ~60% and increase hardseed proportion as season progresses depending on location. Slow seed softening. Residual hardseed: ~70-85%	
biserrula ***(Biserrula pelecinus)***	Casbah	Early-mid (assessed Canberra only)	---	Initial hardseed proportions: ~90%. Little or no seed softening. Residual hardseed does not vary from initial hardseed levels (~85-90%).	

1. Assessed at Perth, Tamworth, Cowra and Canberra by plotting flowering dates observed in 2017 after a “late” sowing were plotted against those observed in 2018 to obtain a “typical” maturity rank (Figs 6.2 – 5a and 6.2 - 5b). Maturity type of each serradella variety was assigned by comparison with the maturity types assigned previously to the subterranean clover “control” varieties (Lattimore and McCormick 2012). This avoided the complication of unstable flowering dates for maturity-type classification purposes. However, it must be recognised when using these rankings that unstable flowering dates among the serradella varieties will substantially confound the actual flowering dates experienced in different growing seasons.
2. Flowering date stability describes the ability of a cultivar to resist change in flowering date despite germinating on an early opening seasonal rainfall.
   Key: “stable” – flowering date is not advanced or is advanced ≤1 week after germination in late March cf. germination in early May; “relatively stable” – flowering date is advanced by 1-2 weeks; “unstable” – flowering date is advanced by 2-3 weeks; “very unstable” – flower date is advanced by >3 weeks. (see Fig 6.2 - 4)
3. Hardseededness assessed in pods or pod fragments (serradellas) or naked seed rubbed gently from burrs (subterranean clover) that had been produced in flowering time experiment in the preceding year. Seed softening was assessed for pods and seeds laid on the surface of bare soil in micromesh bags. Locations were Cowra and Canberra in 2018 and 2019 (see Figs 6.2 - 13 and 6.2 - 14)
4. Persistence notes are summaries of expectations from experiments conducted on the southern tablelands and Monaro regions of NSW during the project. The experiments were of limited duration and span, at most, experience over up-to-6 years. Soft seeded varieties are automatically classed as non-persistent based on published data (Dear *et al.* 2002) and experience during droughts in the current project.

Where to next?: Development of viable serradella-based pastures for the permanent pasture areas needs to move to research and evaluation of particular serradella cultivars (now tagged as "best-bet" options) for production, botanical composition and persistence in mixed pastures under grazing. These "best-bet" cultivars (see Table 6.5 - 1) have been identified based on their relatively stable flowering dates and/or more appropriate patterns of seed softening. No one variety has all of the putative traits that are thought to be desirable for persistence and high production, but each variety has sufficient merit to potentially kick-start adoption once performance in a mixed pasture is proven.

In addition, there is reasonable evidence to suggest that a simple agronomic solution to the year-2 poor regeneration problem can be found (options are listed below and in **Section 7.2.2)**.

7.2.2 Maturity types and flowering date stability

Maturity type classifications: The national flowering time experiments demonstrated that a wide range in maturity types (similar in range to early- through late-maturing subterranean clovers) are available among the yellow and French serradella cultivars. However significant gaps exist in the French serradella maturity types that are suitable for use in permanent pastures because only cvs Margurita, Erica and breeder's line 150S-1C have any level of hardseededness.

Poor flowering dates stability: A major and unexpected finding was that many of the serradella cultivars lacked flowering date stability (**Section 7.2.1**) and began to flower too early (during frost periods) when sown at an early sowing date to simulate an early break to the growing season. This is expected to have negative consequences for seed production, forage yield and feeding value and may be a major factor in poor persistence. Persistence by annual legumes in permanent pastures depends on cultivars flowering on dates that ensure seed production will avoid high risk frost and terminal drought periods. In southern Australia, rainfall is highly variable during autumn with opening rains occurring at any time from February to June depending on location and season. Pasture cultivars must be capable of stable flowering dates irrespective of the date on which they germinate, as was exhibited in this study by the subterranean clover cultivars.

The instability in flowering dates observed in some cultivars was so large that it is entirely possible that early rain, which normally leads to exceptional years for pasture production, may result in poor seed production and persistence by these cultivars. It may be significant that Avila and King, cultivars with proven persistence in permanent pastures, also exhibited relatively stable flowering dates in their target environments.

Largely because of this issue, the maturity-type of many serradella cultivars varied depending on the location at which they were grown. The common practice specifying maturity type based on phenology recorded in Perth was not appropriate.

7.2.3 Hardseededness and seed softening

The importance of hard seeds: It is not yet known what constitutes an ideal initial proportion of hard seeds or the ideal pattern of seed softening to ensure serradella persistence in permanent pastures. However, it is clear from other studies in southern NSW that some level of hardseededness is essential for persistence of serradella (Dear *et al.* 2002). This was verified in the present experiments. In addition, very rapid seed softening patterns that left few residual hardseeds (e.g. Yellotas) proved to also be unsustainable under persistent drought conditions.

Year-2 regeneration issues and a solution: Most of the hardseed serradellas had a very high initial level of hardseeds with very slow seed softening. Once established, it is feasible that this pattern of seed softening may prove highly resilient. However, it does create a year-2 regeneration problem which can potentially open a window for weed invasion in a newly sown permanent serradella pasture. There is no current solution to this problem, but the research has indicated three options for research that could deliver practical solutions to this problem: (i) planting combinations of softened naked seed and hard seed in the pod; (ii) planting a 'hard, slow softening' variety (e.g. Avila) with a 'fast softening' variety (e.g. Yellotas); (iii) selecting among the diversity in serradella seed softening patterns to develop new cultivars with more ideal seed softening patterns (**Section 7.2.2**).

An ideal pattern of seed softening: An underlying premise of the present experiments was that the seed softening patterns of adapted subterranean clover may provide an "ideotype" for hard seed production and softening under Australian pasture conditions. Based on the results obtained at two sites over two years, it is surmised that "well-adapted" clovers exhibit three characteristics in their seed softening patterns: (i) a high initial proportion of hard seeds, (ii) ~30% seed softening between February and June, and (iii) a residual proportion of seeds (e.g. ~30%) that remains hard after the end of June and replenishes the bank of seed in the soil. This is seed that will remain viable until at least the next growing season. None of the serradellas mimicked this idiotypic pattern of seed softening particularly well. The serradellas that most closely resembled the putative ideotype exhibited intermediate levels of initially hard seeds or initially very rapid seed softening, followed by relatively slow seed softening and a moderate level of residual hard seeds. This group included Spectra (a yellow and French serradella hybrid), King (early-season yellow serradellas), Margurita and breeder's line 150S-1C (mid-season French serradellas). However, a large investment in improving the seed softening pattern of serradellas may not be necessary if a simple agronomic planting strategy can be demonstrated to avoid the year-2 gap in serradella regeneration.

7.2.4 Seed production and seedling establishment

Seed production by serradellas in the present experiments was generally regarded as adequate to good, and better than subterranean clover under dry seasonal conditions (**Section 6.2.2**). Seedling establishment in the year after pasture had been sown typically reflected seed production and the rate at which seed softened over the summer-autumn period.

7.3 Output 5(c) - Conduct a 'watch and act' regime for occurrence of any serradella leaf disease at all field sites to assess whether leaf disease is likely to occur when serradella pastures are used.

Although leaf disease was observed to cause collapsed necrotic patches in serradella monocultures in a previous experiment near Yass, NSW, there were no observations of confirmed leaf disease on serradella nationwide in the present project. Nevertheless, it would be prudent to continue to be alert to the possibility of any disease on a species that is essentially novel for the permanent pastue zone in any future research.

7.4 Output 5(d) - Conduct glasshouse/controlled-environment research to identify and quantify the suspected soil and environment constraints to the serradella adaptation range.

7.4.1 Aluminium (acid soils) and manganese tolerance

Aluminium tolerance of a wide range of serradella cultivars was assessed in solution culture and revealed a very wide range apparent Al-tolerance range within both yellow and French serradellas (i.e. some varieties was as intolerant of Al as the most sensitive pasture species (e.g. *Medicago* spp.), whilst other varieties approached the tolerance of the most Al-tolerant pasture species (e.g. *Microlaena stipoides*)) (**Section 6.4.1.1**). A subsequent root growth bioassay in a very Al-toxic soil (reported in the Supplementary Final Reports) confirmed the validity of these ranking but also provided a soil-based perspective: i.e. many serradella varieties will be tolerant of the Al in very acid soils. However, promotion of serradellas as universally suited to Al-toxic soils is wrong as there are notable exceptions among the cultivars that are sensitive to Al toxicity. These data should help to clarify the descriptions of serradella varieties in this regard.

Serradella species have been reported to be less tolerant than subterranean clover to high concentrations of Mn, but more tolerant than lucerne or annual medics. When tested in solution culture, the yellow serradellas were found to be moderately to highly tolerant of Mn (similar or better than subterranean clover). However, French serradellas (except cvs Margurita and Bydgoska Asa) were moderately to highly sensitive to Mn (**Section 6.4.1.2**).

7.4.2 Intolerance of waterlogging

All serradellas were found to be sensitive to waterlogging. Root growth was suspended when waterlogging was imposed, and shoot growth was severely constrained in the “recovery period” after waterlogging had been alleviated (**Section 6.4.1.3**). This is in agreement with the general view that serradellas are not suited to soils where waterlogging is a regular occurrence. Surprisingly, slender serradella (which is often included in a seed mix to provide serradella cover in waterlogged areas of paddocks), was no more tolerant of waterlogging than any other serradella. Its growth pattern suggests that it succeeds by avoiding growth during waterlogged periods.

7.4.3 Growth under cool conditions

Serradella growth was not unduly constrained by cool conditions that were typical of winter periods in permanent pasture areas; the cultivars tested grew as well as subterranean clover.

7.5 Output 5(e) - Describe the soil and climatic zones where P efficient serradellas could be utilised and any key traits to be developed in varieties for better adaptation to these new areas of use.

Readers are referred to a full discussion of this topic in **Section 6.5**. In essence the serradellas are well suited to the same climatic zone as subterranean clovers (29M ha) but are constrained within this zone by edaphic constraints: (i) inability to tolerate soils with neutral to alkaline soils, and (ii) intolerance of waterlogged soils.

Uptake in Australia was initially constrained by the perception that the serradellas were best suited to deep sandy and infertile soils. Yellow serradella (*O. compressus*) was the first serradella species to be released commercially in Australia (late 1950’s) and it was intended for use on acid, infertile sandy soils where their deep roots, high tolerance of acid soils and hard seeds (transiently impermeable seed coats that protect the seeds against premature germination after summer storms) allowed them to be productive and persistent where *T. subterraneum* was failing (Bolland and Gladstones 1987, Loi *et al.* 2005, Freebairn 1996). Cultivars of French serradella (*O. sativus*) have been released for use since the mid 1990's, but also with the intent that they be used mainly on light textured soils. However, as was the case for subterranean clover in the first 30 years after its initial release (Donald 1970), further use has also been constrained by lack of suitably adapted cultivars. For example, serradella use was initially constrained by the relatively high cost of seed. However, development of improved yellow and French serradella cultivars and seed processing technologies has underpinned their expanded use in rotation with crops since about 2009 (Nichols *et al.* 2012). A further breakthrough in cultivar suitability came with the development of the first hardseeded cultivars of French serradella (e.g. cv. Margurita; Nutt 2004a, Nutt 2004b). This fuelled a rapid expansion in the use of French serradellas in phase farming systems (in WA and the NSW Riverina), and on heavier soils never previously considered suitable for serradella (Nichols *et al.* 2012; see Fig.6.5 – 4).

It is contended in this report, that uptake of serradellas for use in permanent pasture areas is also constrained by the suitability of current cultivars, many of which do not exhibit adequate persistence when used in a mixed, permanent pastures (especially in the cooler and higher rainfall zone of southeastern Australia). An attempt to map current potential use of serradellas against the zone of likely adaptation (Fig.6.5 – 4) indicates there remains an area of ~6M ha where adoption is stalled by the need of persistent cultivars. The issues considered to be contributing to poor persistence are already outlined.

7.5.1 How close are we to achieving more P-efficient pastures based on serradellas?

Sandral *et al.* (2019) found that French serradella (cv. Margurita) produced peak spring yields that were equivalent to, or exceeded that of subterranean clover. However, they found that yellow serradella cultivars had lower yields. Nevertheless, yellow serradella has been found to yield as well as the clover in other soil type/climatic districts (e.g. Bolland and Paynter 1992) and at a number of the participatory research sites in the current project, once legume density had been taken into account (**Section 6.1.4**). This highlights a continuing need to consider the regional performance of pasture legumes; but also indicates potential for replacing subterranean clover-based pasture with serradella-based pasture.

In early experiments, the lower P-fertiliser requirement of yellow serradella relative to subterranean clover was also noted (e.g. Bolland 1986, Paynter 1990, Bolland and Paynter 1992). However, this did not lead to changed recommendations for soil P management because critical STP concentrations were not determined until the experiments of Sandral *et al.* (2019). Phosphorus efficiency can only be captured by managing soil P fertility at the lower critical STP concentration suitable for the serradella species.

In areas where serradella is already grown successfully, there is sufficient evidence to indicate that P efficiency can be captured by adopting the lower, species-specific STP benchmark for serradella production (e.g. Sandral *et al.*, 2019) (e.g. **Section 4.4.2**). This information is also included in an updated version of the "Five Easy Steps" P management tool (see **Section 11**) and is ready for release. The project has also shown, however, that these principles cannot be easily applied to serradella pastures growing on sandy soils with very low PBI which permits P to move readily to depth in the soil profile. New soil sampling protocols, strategies to use "legacy" P at depth in these soils and potentially a rethinking of how to specific critical P requirements in these soil types are required to ensure effective use of P fertilisers (see **Sections 4 and 5**).

Achieving P efficient pastures in those areas of the permanent pasture zone where serradella is not already used, now hinges on resolving the issue of serradella persistence. It should be remembered that serradella is already used as the primary legume component of permanent pastures in some districts (e.g. northern NSW pastures based on yellow serradella cv. King; Freebairn 1996). This is a feasible task; however, the contention of this report is that the main issue is limited cultivars that adapted for use in the permanent, mixed pasture areas of southeastern Australia (see **Sections 6 and 7**).

7.6 Recommendations: Activity B5 - Extending the adaptation range of serradella systems.

7.6.1 Recognising larger potential benefits from wide adoption of serradellas

- This project was commenced with a view to extending the use of serradella pastures to capture (i) their **P-efficiency advantage** (up to ~30% lower P-fertiliser cost of maintaining high pasture production). Farmer groups participating in the project have added to this goal, their objectives of capturing: (ii) the **"bloat-safe"** characteristics of serradella pasture, (iii) **risk mitigation** by diversifying the legume base of pastures with species that can (iii) **avoid diseases of clovers**, (iv) provide **out-of-season growth** in years when rainfall extends spring onto summer, and (v) deliver **improved early- and late-season drought tolerance** as a consequence of faster root growth and/or deeper root systems.

7.6.2 Lack of persistent cultivars for mixed, permanent pastures is stalling adoption of serradellas in the areas where they are not being used.

- Serradellas should no longer be promoted primarily for use in light, sandy, or acid and infertile soils. They clearly have wider edaphic adaptation; however, they are unsuited to neutral-alkaline soil types and will not tolerate soils that are subjected to persistent waterlogging.

- Although the serradellas were often highly tolerant of toxic aluminium, some cultivars were sensitive to toxic aluminium and may prove unsuited for use in very acid soils.

- The phenology, hardseededness and seed softening characteristics of many serradella varieties can now be described more accurately in literature to be made available to the seed industry and farmers (e.g. Table 7.2 – 1).

- There needs to be more attention paid to the phenological suitability of cultivars for the districts into which they are being sold. Experimental and anecdotal observations indicate very poor persistence by many serradella cultivars when used in higher rainfall areas suited to permanent pastures. However, yellow serradella cvs Avila (late maturing) and King (early maturing) provide experimental and experiential evidence, respectively, that serradellas can persist in permanent pastures. It was surmised that poor persistence is due to inappropriate cultivar adaptation for used in mixed, permanent pastures.

- Factors considered to be contributing to poor serradella persistence included:
  (i) use of cultivars with inappropriate flowering dates for the growing season in areas of proposed used,
  (ii) unstable flowering dates that confound maturity-type selections among many serradella varieties,
  (iii) continued promotion of soft seeded varieties to farmers,

(iv) non-ideal seed softening patterns among the hardseeded serradella varieties; one manifestation of which was poor seeding regeneration in the year following pasture establishment.

- The project results indicated at least <u>three potential solutions</u> to the problem of year-2 seedling regeneration when establishing permanent serradella pastures. These options should be tested in experimental pasture establishment trials:
  (i) plant new pastures using a combination of softened naked seed and hard seed in the pod; the latter will germinate in year 2.
  (ii) plant new pastures with contrasting serradella cultivars – a 'hard, slow softening' variety (e.g. Avila) with a 'fast softening' variety (e.g. Yellotas); the latter will germinate in year 2, but may not persist.
  (iii) high diversity in seed softening patterns suggests that it may be feasible to select new varieties with more ideal seed softening patterns that avoid poor regeneration in year 2; clearly a longer-term solution.

- Serradella persistence in mixed, permanent pastures will only be resolved by: (i) field testing genotypes selected to address the factors (see above) considered most likely to be contributing to poor persistence, and (ii) by researching how successful cultivars (e.g. Avila and King) avoid loss of ground cover in pastures managed under "farm" conditions. The current research has informed development of a short list of "best-bet" serradella cultivars (Table 6.5 – 1) for further research and on-farm trialling in permanent pastures in the areas where serradellas are not being adopted. No one variety has all of the putative traits that are thought to be desirable for persistence and high production, but each variety has sufficient merit to potentially kick-start adoption once performance in a mixed pasture is proven. This list includes cvs Avila and King.

7.6.3 Further research into the physiology, phenology and agronomy of serradellas.

- Further research into the physiology, phenology and agronomy of serradellas is required. This is a relatively new species at a stage of development equivalent to subterranean clover in the 1920-30's when cultivar development was constraining wider use of the clover.

  The following areas of research need were indicated by observations and results from the current project:
  (i) Only a few late-maturing serradella cultivars displayed reasonable levels of **flowering date stability**. There is a need to understand how the photoperiod and vernalisation responses of pasture legumes (e.g. subterranean clover and serradella) ensure flowering date stability and to the pursue the development of cultivars that will flower reliably at optimum dates for seed production, irrespective of the timing of the break to the growing season.
  (ii) The **optimum time of flowering** for a pasture legume with an indeterminate flowering habit is unknown. Presently it is assumed to be similar to that of subterranean clovers which have a "more" determinate flowering pattern. However, it is conceivable that indeterminacy may allow a wider flowering window, provided that early flower loss to frost is compensated by later flowering.
  (iii) Comparative **rates of root growth** early in the growing season were not examined but may be worthy of investigation because serradellas were able to survival very dry starts to the growing season that occasionally killed subterranean clover seedlings.
  (iv) **Competitive outcomes in mixed, grazed pastures**. It is inevitable that serradella pastures in areas sown traditionally to subterranean clover will be invaded by subterranean clover germinating from the seed bank. Exploratory pot experiments (reported in the Supplementary

Final Report) indicate a strong competitive interplay between clover and serradella will occur depending of soil P fertility, grazing management and the relative fecundity of the alternative legumes. This, in turn, will influence the P efficiency of the pasture system. Competition will undoubtedly become even more complex in pasture mixtures with grasses. Research that examines soil P, pasture and grazing management to support high serradella content in pastures should now be conducted in the field with the objective of developing guidelines for farmer management of serradella-based pastures.

(v) **Yield of serradellas**. Serradellas must yield and persist as well as locally-adapted subterranean clovers to be viable alternative legume options for permanent pastures. In some locations, one or other of the yellow or French serradellas has shown superior yield. The best serradella has always been equal to, or better than subterranean clover. Further experience in differing farm environments is needed to understand why the relative yields of the serradellas has varied in some locations.

8 Results and Discussion – (Activity B6) Identifying P efficient subterranean clover cultivars.

8.1 Results and Discussion: Output 6(a) - Conduct field and glasshouse experiments to rank commercially-available subterranean clover cultivars for P-efficiency and to quantify the yield advantages of using them in pastures on moderately P-deficient soils.

8.1.1 Plant growth conditions for "P-efficiency" screening.

The logistics of screening large numbers of subterranean clover cultivars and 'core collection' genotypes (a set of lines that represent ~80% of the variation in the subterranean clover genome) for P efficiency and detailed P-efficiency traits presented a number of challenges:

- The collection of clover genotypes was diverse and included maturity types that range from very-early to very-late flowering (Nichols *et al.* 2013), a factor that was certain to confound comparisons of growth rate and trait expression unless managed to ensure genotypes were compared at an equivalent phenological stage.
- For relevance to the objective of reducing P fertiliser requirements, high "P-efficiency" was defined by having a low critical P requirement for near-maximum growth (Simpson *et al.* 2014). This is usually measured by examining response to P over a wide range of soil P fertility levels (e.g. Haling *et al.* 2016a) but an assessment such as this is logistically impossible when comparing numerous genotypes.
- The total number of genotypes to be compared (~150) was challenging even for comparison at only two levels of P supply.
- All subterranean clover genotypes fail to express their P-efficient potential in *very* low P soil (e.g. Haling *et al.* 2016a, Haling *et al.* 2018; Section 3.1). Genotypes must be screened in moderately P-deficient soil (i.e. at P levels above the threshold at which nutrient foraging by roots "fails" to occur).
- When the work was begun it was strongly suspected that the key to differences among subterranean clover genotypes would be differences in the intrinsic root morphology traits important for P acquisition, plus the capacity to proliferate root length for nutrient foraging (Haling *et al.* 2016b). Experiments that did not permit expression of these root system traits were likely to be misleading.
- Core-collection lines have not been selected for agronomic merit and growth of some lines was often compromised because they were very susceptible to root disease(s) when grown in field soils.

These issues were dealt with by comparing genotypes grown as microswards (i.e. mimicking canopy constraints and light conditions in pasture swards) under 12h days (controlled-environment conditions) to ensure genotypes remained in the vegetative phase of growth for the duration of the test (Fig. 8.1 - 1). Soil profiles were constructed using steam pasteurised soil to reduce soil pathogens and to reflect the stratification of available-P concentrations in the surface layer of the soil profile as occurs under pastures in the field. The soil configuration that was adopted also permitted nutrient foraging roots to be harvested separately from the rest of the root system and analysed for root morphology traits. Comparisons of critical P requirements among various pasture legumes (e.g. Haling *et al.* 2016a, Sandral *et al.* 2018), indicated that herbage yield in moderately P-

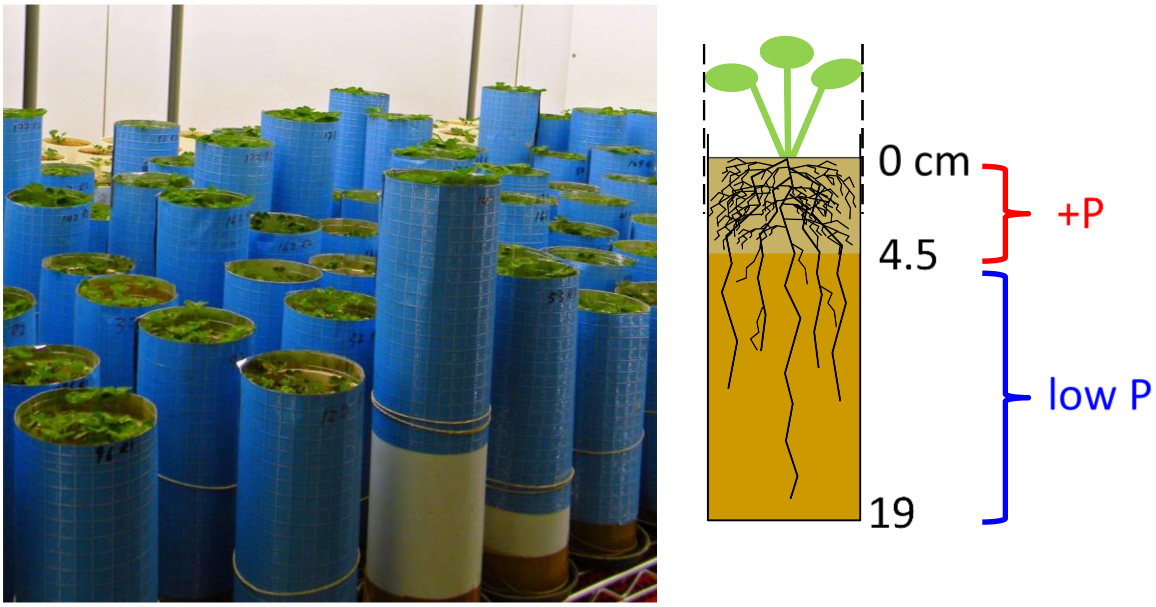


Figure 8.1 - 1. Subterranean clover grown as micro-swards in a controlled environment experiment with a soil profile that reflected the stratification of P in soil under a pasture. The topsoil and subsoil layers were harvested separately to allow the characteristics of nutrient foraging roots to be determined.

deficient soil provided a reasonable proxy for the critical P requirement of a plant. Genotypes were, therefore, screened using shoot yield (sometimes also P uptake) at a single, moderately-deficient P supply level (typically 40 or 60 mg P/kg topsoil). When logistically feasible, growth under P-deficient conditions was also compared with growth when P supply was sufficient for maximum growth. Initially, microswards were established by planting the same mass of seed per pot (50 mg/pot).

Although the seed size of subterranean clover can vary substantially, evidence from previous studies (e.g. Davidson and Donald 1958, Silsbury and Fukai 1977) indicated that yield of a fertilised clover pasture was independent of plant density once canopy closure was achieved. Indeed, under P-sufficient conditions, canopy closure under these growing conditions occurred at about 3 weeks after emergence (accounting for ~20% of the final harvested shoot yield) and then the plants entered a rapid (essentially linear) phase of vegetative growth until harvested at ~ 5 - 6 weeks from emergence (Haling *et al.* 2016b). P-efficiency differences were expressed predominantly during the rapid phase of growth.

Research examining the root morphology traits that contributed to high P-efficiency (i.e. relatively fast growth in low P soil and a low critical P requirement) were conducted alongside screening activities. The logistics and timelines required for screening and a fixed project duration demanded that these activities proceed concurrently. Consequently, each genotype screen for P-efficiency was conducted using the best currently-available knowledge of how subterranean clover achieved high P-acquisition efficiency. Many growth conditions considered potentially influential for P uptake were examined systematically: e.g. canopy size, pot size, presence of mycorrhizal fungi, and planting density. All had some influence, but the impact of planting density required the results of P-efficiency screening to be reanalysed, as outlined below.

8.1.2 Variation in “P-efficiency” among subterranean clover cultivars and lines.

Some subterranean clover genotypes had been screened by the commencement of the project (B.PUE.0104) for their ability to yield in soils with low to moderately-low P fertility. Further analysis

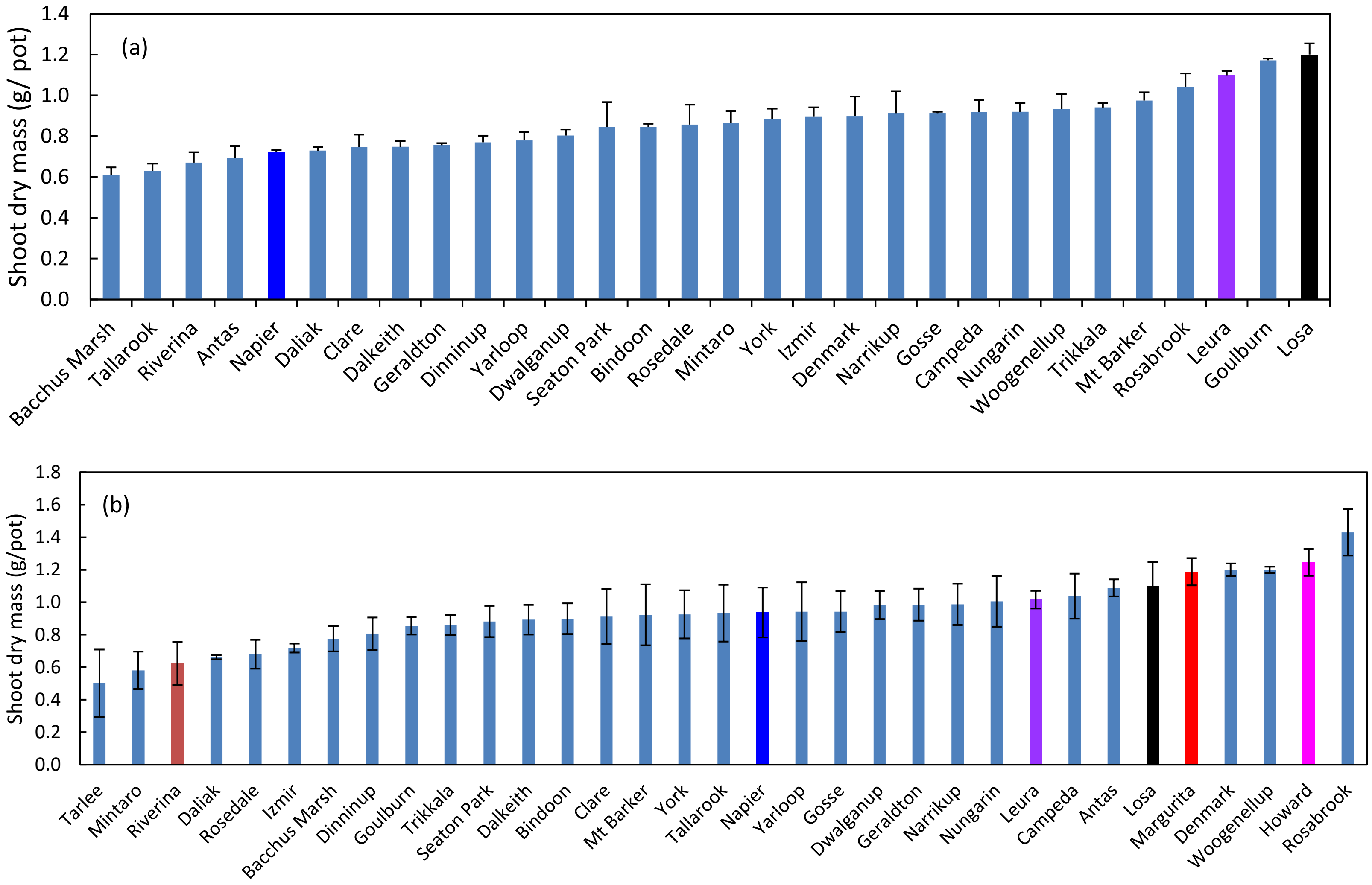


Figure 8.1 - 2. Shoot dry mass of cultivars of subterranean clover grown at (a) 50 mg viable seed per pot (screened as part of project B.PUE.0104) and (b) 3 plants/ pot with a moderate level of P applied to the topsoil of pot. Plants grown at 50 mg viable seed per pot. Values show average ± 1 error (n=3).

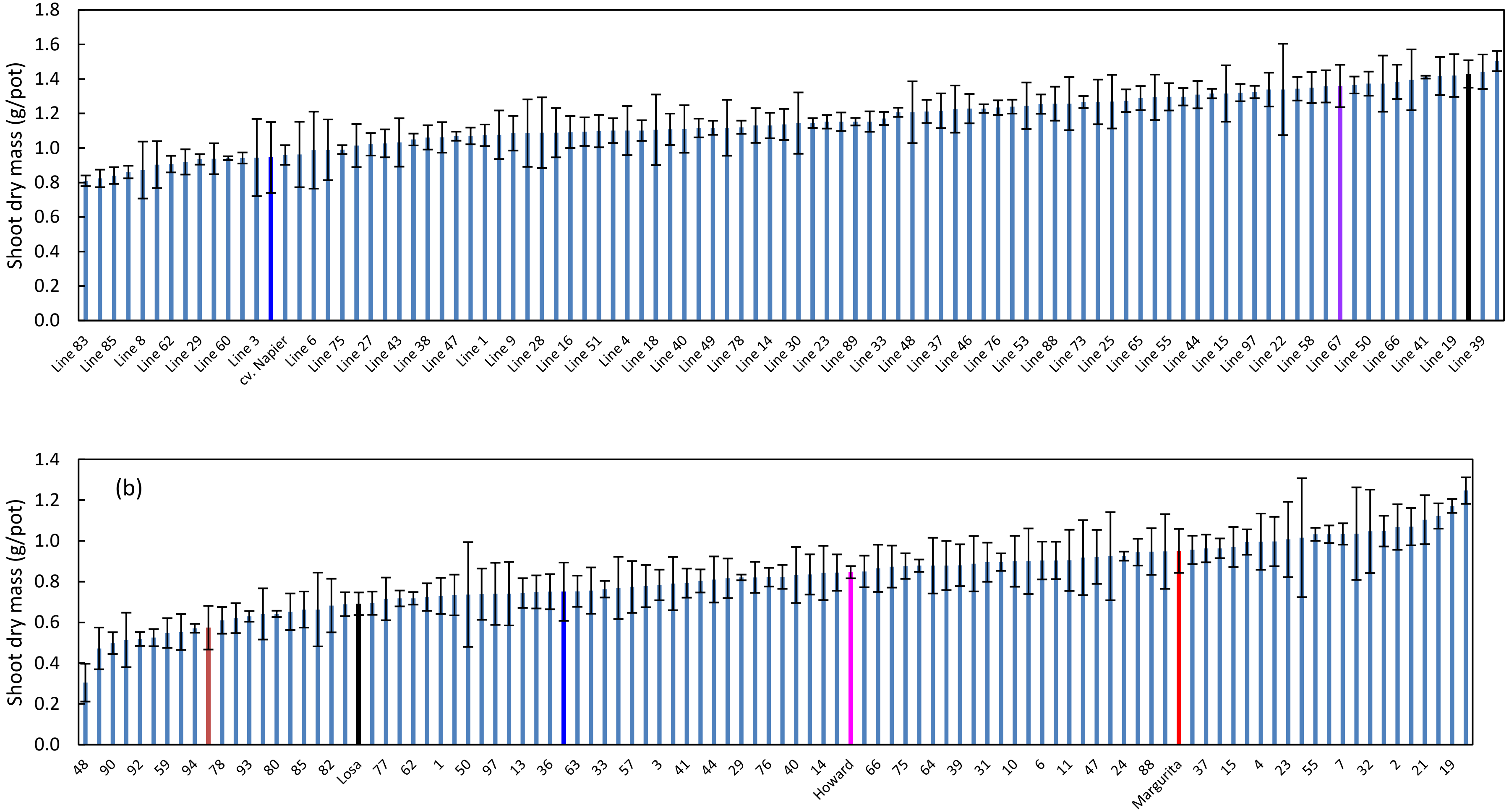


Figure 8.1 - 3. Shoot dry mass of cultivars of subterranean clover grown at a moderate level of P applied to the topsoil of pot at (a) 50 mg viable seed per pot (screened as part of project B.PUE.0104) and (b) 3 plants/ pot with. Includes control lines: *Ornithopus sativus* cv. Margurita in red; subterranean clover cv. Howard in pink; cv. Napier in blue; cv. Losa in black, cv. Riverina in orange and cv. Leura in purple. Values show average ± 1 standard error (n=3).

of historic and current cultivars of subterranean clover was also undertaken (Fig. 8.1 – 6a). A two-fold variation in shoot yield (and P uptake) was commonly measured among the cultivars (Fig. 8.1 - 2a) and among the core-collection (Fig. 8.1 - 3a) of subterranean clover genotypes.

8.1.2.1 Impact of planting density

In the current project, the growth conditions used when screening for P efficiency were being checked concurrently with screening experiments. The experiments exploring growth conditions confirmed the importance of decisions to mimic field sward conditions in the controlled-environment (pot) experiments. Common growth conditions used in P-efficiency research elsewhere, e.g. unconstrained leaf canopies, small pot volumes, and the use of "rootboxes" (rather than pots with microswards), were found to influence the expression of root traits and/or changed the relative rankings for P efficiency among subterranean clover genotypes. These issues were avoided in this project.

Initial assessment of the impact of planting density on P efficiency using a subset of clover cultivars did not raise any concerns. However, it was noted that cv. Napier (generally regarded as P-inefficient) had altered its root morphology and shifted its P-efficiency rank in some experiments. This led to a detailed study of the interaction between P efficiency and response to planting density by cv. Napier (putatively P-inefficient) and cv. Losa (P-efficient) (McLachlan *et al.* 2020a). The impact of planting density revealed by this work was unexpected and novel. The study demonstrated that some clover genotypes (e.g. Losa) have the capacity to proliferate roots into soil "space" in search of P when growing at a relatively low density (i.e. 150-500 plants/m^2), whereas other genotypes (e.g. Napier) do not do this (Fig. 8.1 - 4).

This novel nutrient-foraging "trait" has not been reported previously. Both cultivars also proliferated root length in response to low P supply, a P-efficiency attribute of roots that has been previously recognised. The nutrient-foraging response in soil space by cv. Losa was larger than its apparent response to a low-P stimulus alone. These combined root acclimation responses ensured that cv. Losa maintained a low critical P requirement irrespective of planting density, whereas cv. Napier was P-inefficient (i.e. grew poorly in low P soil) when growing at low density, but improved its P efficiency as planting density was increased (Fig. 8.1 - 5).

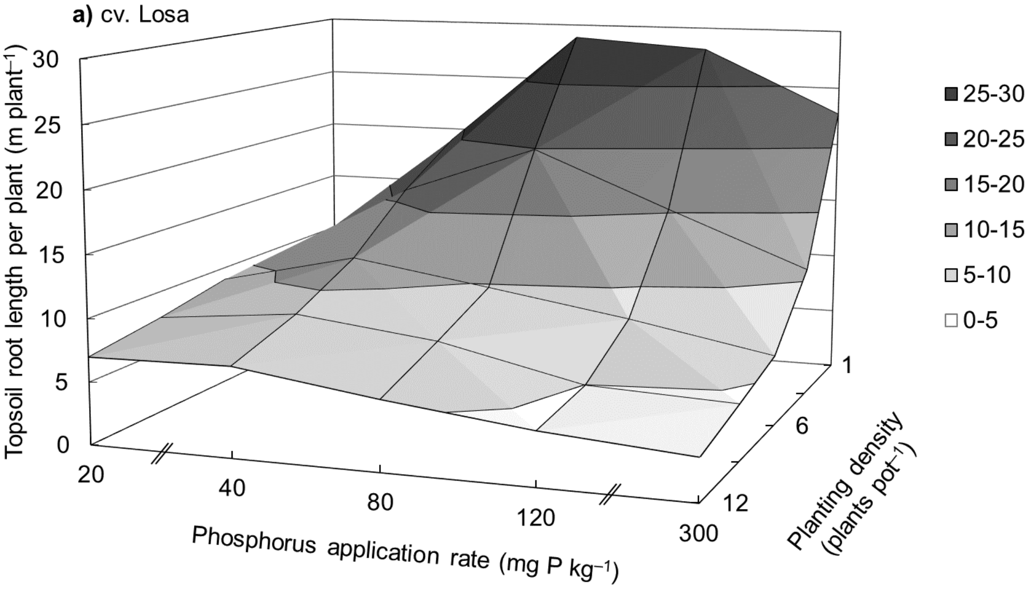
8.1.2.2 Magnitude and consistency of P efficiency differences among subterranean clover genotypes

The discovery of the novel nutrient foraging response to "space" in some subterranean clovers triggered an immediate reassessment of all P-efficiency screening data collected to that point in time. Additional screening experiments at a uniform, moderately-low planting density were also instigated (Fig. 8.1 - 2b and 8.1 - 3b).

Reassessments of the existing data confirmed that the magnitude of difference in P efficiency between highest-ranked and lowest-ranked subterranean clover genotypes was also about two-fold when they were compared at similar planting densities (Fig. 8.1 – 6b).

It was clear that the confounding effect of planting density increased the difficulty with which P efficient genotypes could be screened because:

(i) the P-efficiency rankings of some varieties were not expressed consistently when planting density was varied,
(ii) screening for a nutrient foraging response to "space", which underpinned consistently high P efficiency in cv. Losa, was logistically demanding (i.e. it could not be done at one plant density and P level), and
(iii) the "right" planting density at which to screen genotypes was not easily determined because plant establishment densities in pastures vary depending with seasonal conditions that, in



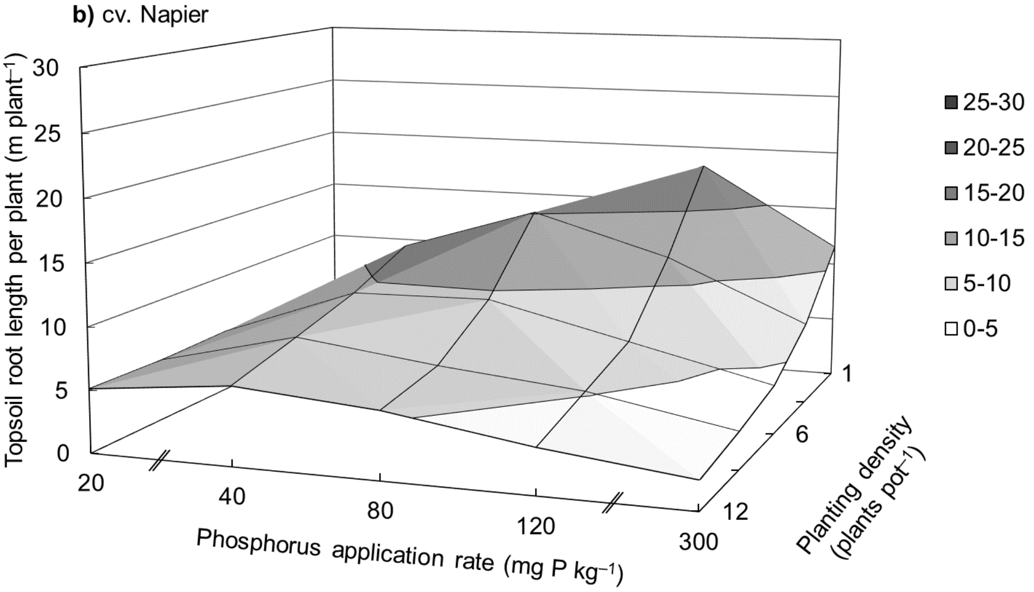


Figure 8.1 - 4. Topsoil root length per plant of two cultivars of *Trifolium subterraneum* grown at five planting densities in response to five rates of P applied in the topsoil layer of a pot. The 3D surface figures depict the root length response (y-axis) to both P application rate (x-axis) and planting density (z-axis). Phosphorus application rate is not linear along the x-axis; breaks in linearity occur between 20 and 40 mg P kg^{-1}, and between 120 and 300 mg P kg^{-1}. The cultivar*P treatment*density interaction was significant for topsoil root length per plant ($P = 0.007$) after log-transformation to meet the assumptions of normality. (McLachlan *et al.* 2020a)

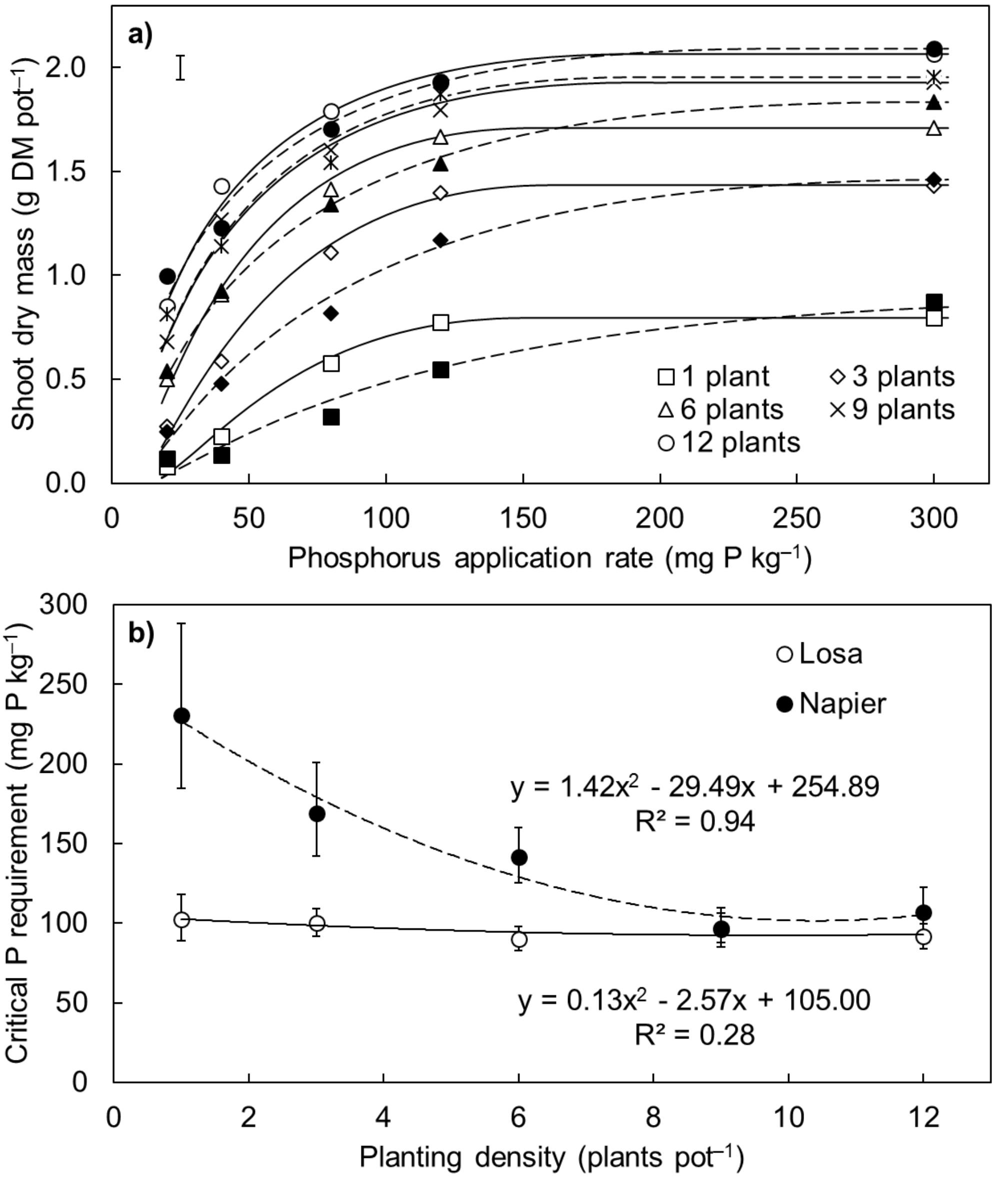


Figure 8.1 - 5. Shoot dry mass (a) and the critical external P requirement (b) of two cultivars of *Trifolium subterraneum* grown at five planting densities in response to five rates of P applied in the topsoil layer of a pot. Values in (a) show the mean (n = 5) and the bar shows the LSD ($P = 0.05$) for the cultivar*P treatment*density interaction ($P = 0.014$). Open symbols and single crosses represent cv. Losa and are joined by solid curves, whereas closed symbols and double crosses represent cv. Napier and are joined by dashed curves. These curves show the Dyson and Conyers (2013) yield response back-fitted to the data. The Dyson and Conyers equation addresses yield response to P application below the maximum yield, but the curves were continued along the implied maximum yield plateau. Values in (b) show the calculated critical external P requirement (i.e. the amount of P applied to achieve 90% maximum yield) for both cultivars at each planting density, based on the Dyson and Conyer curves back-fitted to the shoot yield data. Bars show the 95% confidence intervals. Open symbols represent cv. Losa and are joined by a solid trendline, whereas closed symbols represent cv. Napier and are joined by a dashed trendline (McLachlan *et al.* 2020a).

turn, determine seed production, seedling establishment success, and competition for scarce light, nutrient and water resources. High establishment densities also lead to self-thinning of the plant community during the growing season (Donald 1963, Puckridge and French 1983, Stern 1965, Taylor *et al.* 1984).
[Note: By comparison, a decision on the appropriate planting density for the assessment of crop genotypes would be relatively easy because crops are sown at predetermined densities to ensure final yield is maximised (e.g. Shi *et al.* 2016).]

Consistency in expression of P efficiency was explored by assessing growth in low P soil at a moderately-low plant density (556 plants/m^2) and compared it with growth in low P soil at a sowing rate of 50 mg seed/pot (Fig. 6). This comparison demonstrated that some genotypes rank consistently above-average regardless of the plant growth conditions, whilst other genotypes rank consistently poorly for shoot growth in P-deficient soil. Among the subterranean clover cultivars tested (Fig. 8.1 - 7a) about 50% consistently achieved average or above-average yield in low P soil, about 25% were consistently P-inefficient and the remaining 25% did not consistently exhibit a characteristic level of P efficiency. Among the core collection of lines (Fig. 8.1 - 7b), about 40% consistently expressed average or above-average P efficiency as measured by yield in moderately P-deficient soil.

8.1.2.3 Field verification of P efficiency rank among subterranean clovers

Field experiments were commenced in three consecutive growing seasons (two with irrigation) to verify the P efficiency rankings of a subset of subterranean clover cultivars but none of the experiments could be harvested as a consequence of various adverse impacts associated with severe droughts (e.g. desiccation of plants in the non-irrigated experiment, insect attack followed by suspected viral disease due to creation of a “green island”; poor establishment followed by out-of-season dicot weed emergence (for which there was no herbicide options).

Field verification of P efficiency ranking among subterranean clover cultivars is important for a number of reasons:
(i) significant differences in the critical P requirement of pasture legume species detected in glasshouse experiments (Sandral *et al.* 2018) are not always able to be confirmed in the field (Sandral *et al.* 2019),

(ii) if it is to be recommended that some cultivars can be managed at lower soil test P concentrations, there needs to be a measurable difference in the critical P requirements of the P efficient and P inefficient cultivars under field conditions. Soil test monitoring on farms can only effectively measure a difference of about five mg Colwell P per kg topsoil, because of inter-annual variation in soil test results. For realistic management, the P requirements of “efficient” and “inefficient” cultivars must be separated by this soil test P margin, at least.

Without field verification of suspected P-efficiency differences among subterranean clover cultivars, it would be unwise to promote to farmers the idea that cultivars differ in this respect.

8.2 Results and Discussion: Output 6(b) - Conduct experiments to identify the root traits and trait combinations that confer P-efficiency in subterranean clover and the diversity of these root traits to underpin identification of variation (genes) useful for plant improvement.

8.2.1 Key root traits for P efficiency

8.2.1.1 Root length proliferation

Under controlled environment conditions, cultivars that were found to yield relatively well and consistently at intermediate levels of soil P availability (i.e. had a lower critical external P requirement) were found to be able to:

(i) preferentially allocate root dry matter to nutrient foraging roots (i.e. in the P-enriched topsoil) in response to low levels of available soil P and to explore soil space for P when intra-specific competition was low. Both are *root acclimation traits* that were activated in response to P stress.

(ii) develop high specific root length (SRL; length of root per unit root dry mass), which ensures greater length of root per unit of root mass partitioned to the nutrient foraging roots and can assist a plant to capture scarcer P with less investment of dry mass and energy expenditure in nutrient foraging roots. This was a key *intrinsic root trait* which, in some genotypes, was also modified by acclimation to P stress.

The net result of these traits is an ability to develop higher root length density in the soil (i.e. proliferate roots) and therefore achieve a larger root-soil interface for P uptake. In the experiments this was measured as the “surface area of the root hair cylinder” (SARHC). The “root hair cylinder” (RHC) is the volume of soil encompassed by the root and root-hair zone.

8.2.1.2 The importance of root hair length

Earlier work had shown that among pasture legume species, species that achieved large RHC volumes (e.g. serradellas) were able to capture more P and achieve a lower critical external requirement for P (Haling *et al.* 2016, Sandral *et al.* 2018). Results from the current work indicated that among subterranean clovers the same principles applied. However, all subterranean clover genotypes had relatively short root hairs. Consequently, differences in the proliferation of root length were often sufficient to explain differences in P acquisition efficiency among subterranean clover genotypes (McLachlan *et al.* 2019).

Short root hairs stand out as the major impediment to achieving high P acquisition (i.e. equivalent to that of the serradellas) among subterranean clovers.

8.2.1.3 Colonisation of roots by mycorrhizal fungi

When mycorrhiza colonised the roots of clovers and serradella, a large mycorrhizosphere interface with the soil is created. An experiment examining the interaction of mycorrhizal colonisation and P efficiency, demonstrated that P uptake per unit of colonised root length was, consequently, enhanced. However, the host plant reacted to improved P acquisition by allocating less dry matter to growth of its nutrient-foraging roots. This reduced root length proliferation and essentially cancelled out much of the mycorrhizal benefit. Host legumes only gained a net P uptake benefit from mycorrhizal colonisation when grown in very low P soil at growth rates less than 50% of the plant’s potential growth rate (McLachlan *et al.* 2020b).

The experiment found that mycorrhizal colonisation did not override the value for P acquisition of having intrinsically P-efficient root morphology and it was concluded that selecting genotypes for

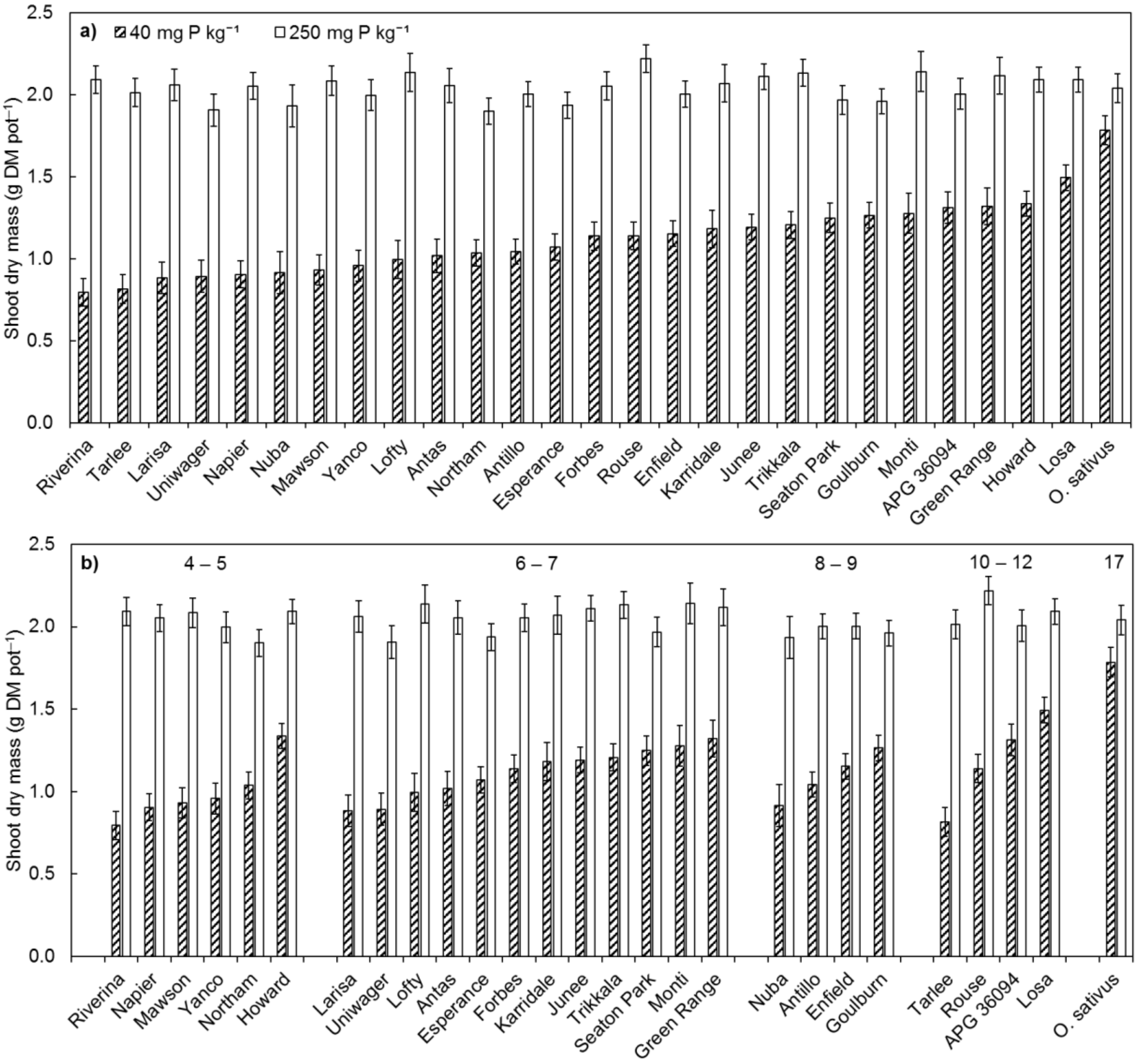


Figure 8.1 - 6. Shoot dry mass of 26 genotypes of *Trifolium subterraneum* and one cultivar of *Ornithopus sativus* grown with either 40 or 250 mg P kg^{-1} applied in the topsoil layer of a pot. Genotypes are ordered according to shoot dry mass when 40 mg P kg^{-1} was applied to the topsoil layer (a) and when grouped according to planting density (b). Values show the mean ± standard error (n = 4). Numbers in (b) indicate the five planting density groupings (plants pot^{-1}). Analysis of variance results were: genotype $P < 0.001$, P treatment $P < 0.001$, genotype*P treatment interaction $P < 0.001$. (McLachlan *et al.* 2019)

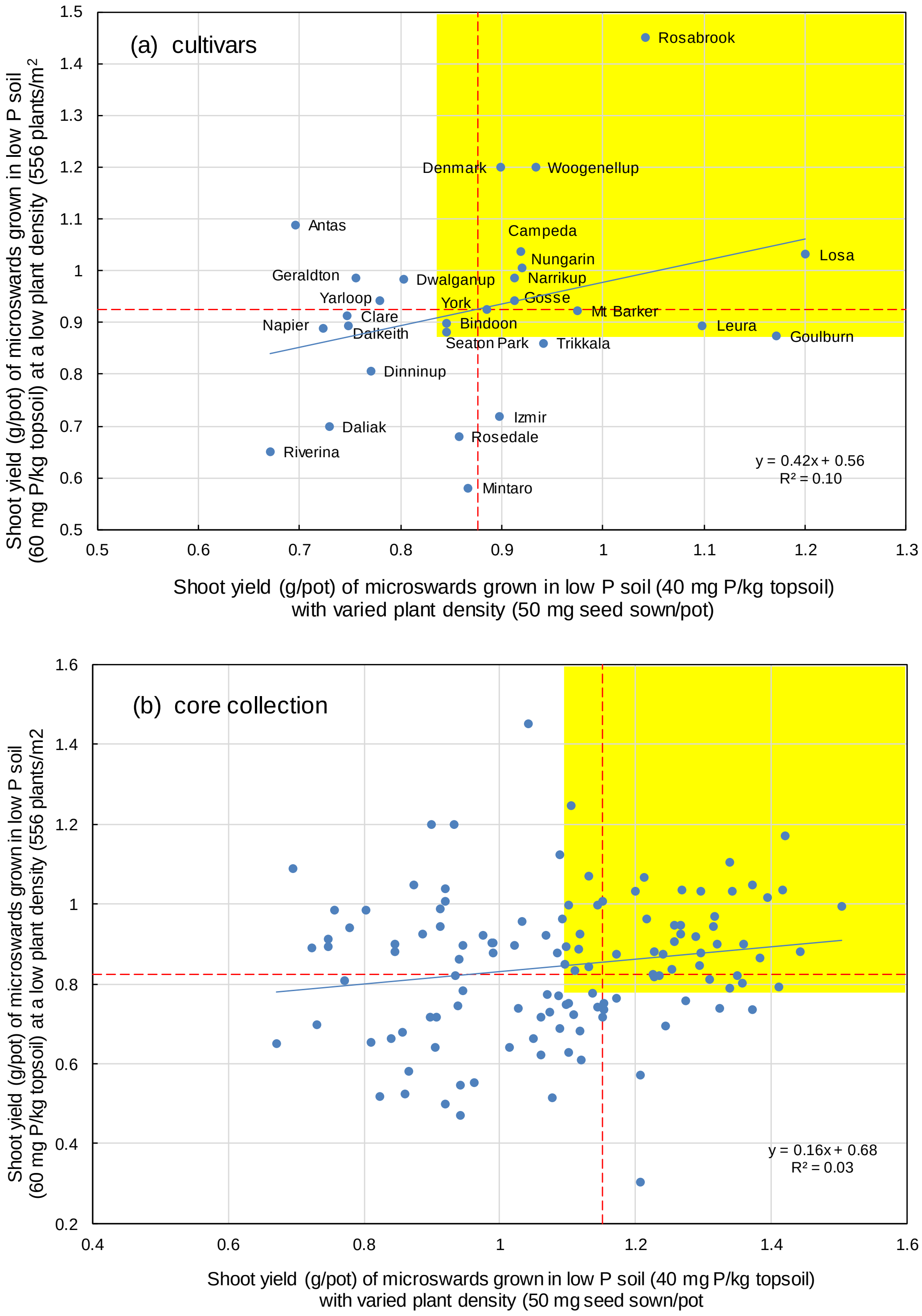


Figure 8.1 - 7. Shoot yield of (a) cultivars (from Figs 8.1 - 2a and 8.1 - 2b) and (b) genotypes in the core collection of subterranean clover (Figs 8.1 - 3a and 8.1 - 3b) grown under different conditions when screening for growth in a moderately P-deficient soil (P efficiency). Genotypes in the top right-hand sector of each graph were ranked consistently as “P-efficient” regardless of the growing condition. For example, about 50% of the cultivars tested consistently achieved average or above-average P efficiency (i.e. the area shaded yellow). Genotypes with consistently “poor” performance occur in the bottom left-hand sector of each graph, whereas genotypes in the remaining two sectors performed inconsistently between the experiments.

improved root morphology would improve P acquisition irrespective of whether or not the plant was colonised by mycorrhizal fungi.

8.2.2 Diversity of P efficient root traits among subterranean clovers

Screening of clover genotypes for differences in their P-efficient root traits demonstrated that among the 97 diverse genotypes of the core collection of subterranean clovers (a collection estimated to represent ~80% of the genetic diversity of the species) and 50 cultivars, that there were two-fold differences in the size of the intrinsic P-efficient root traits (SRL and RHL) from shortest to longest genotypes. The range in root trait sizes were similar among the cultivars and the core lines. Disappointingly, there was no further phenotypic diversity identified in the core lines for SRL or RHL than was observed among the subterranean clover cultivars. However, there were clover genotypes that had SRL that approach that of P efficient benchmark species (i.e. the serradellas), and clover genotypes that had root proliferation responses that were greater than that of serradellas.

However, the root hairs on subterranean clovers were always short (range: 0.2 – 0.4 mm) relative to the serradella species (0.7 – 0.8 mm) and many temperate grasses. Short root hairs prevented the clovers from achieving the high P efficiency equivalent to that of the serradellas. In addition, most subterranean clovers did not increase RHL in response to low soil P, as is observed in other plant species (Bates and Lynch 1996, Datta *et al.* 2015).

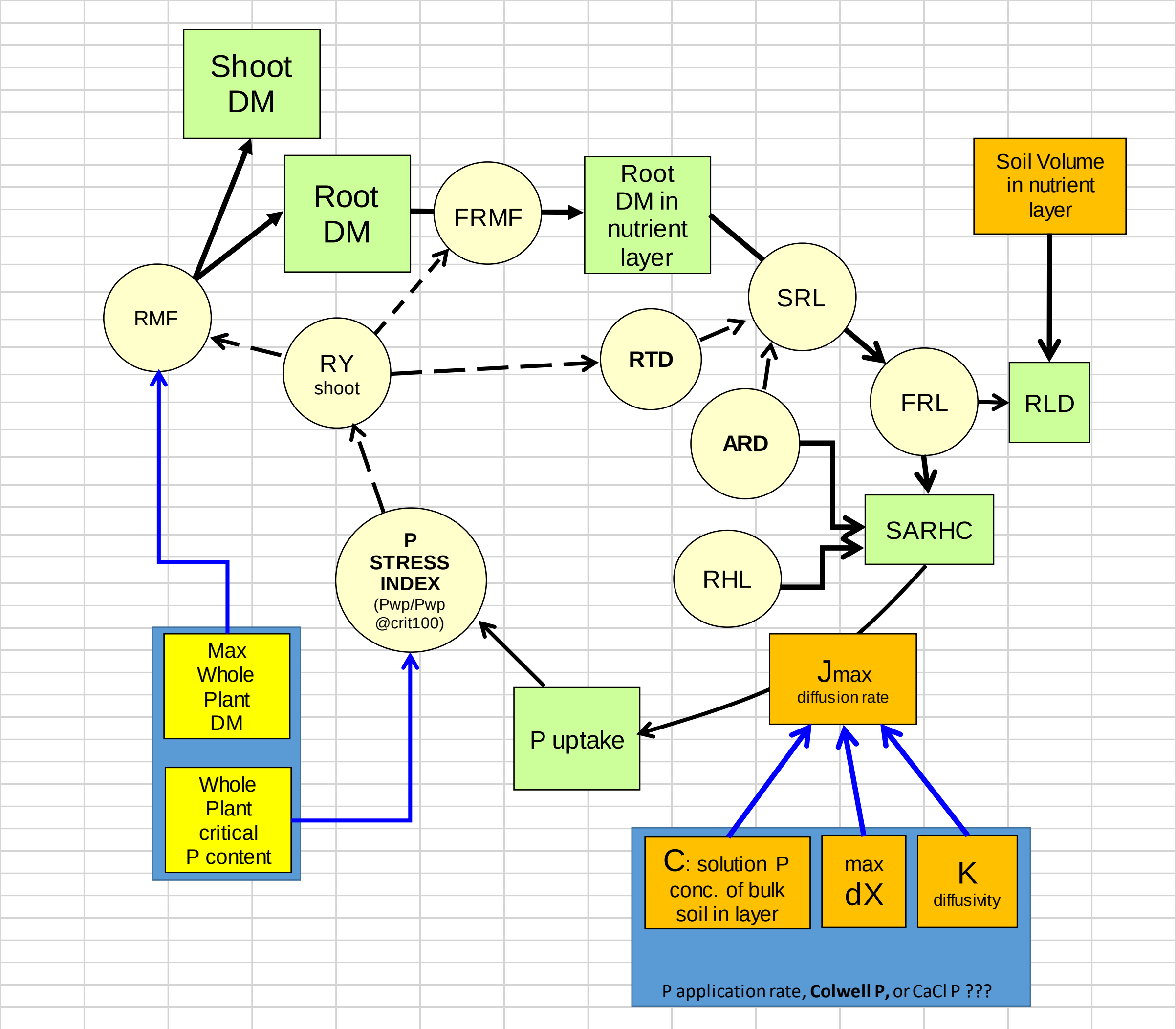
8.2.3 P-efficient root trait interactions

A model was developed to test the benefit of increasing RHL or specific root length in subterranean clover (Fig. 8.1 - 8). A central tenet of the model was our hypothesis that root acclimation in low P soil is regulated in relation to the degree of P stress being experienced by the clover plant (e.g. Haling *et al.* 2018). Modelling was used to determine if developing longer root hairs on a clover would be negated by a reduced root acclimation response when the plant was less P stressed. This was exactly the sort of response observed when mycorrhiza colonised clover roots (McLachlan *et al.* 2020b). Such a response, for example, may make breeding for increased RHL and/ or specific root length in subterranean clover a futile exercise.

The model predicted that an increase in either RHL or specific root length of a subterranean clover plant would, indeed, be counteracted to some extent by a reduced overall root acclimation response. However, a net positive P-acquisition benefit was predicted to result from improved root morphology. The magnitude of the reduction in a plant's critical P requirement was dependent upon the magnitude of the increase in RHL or specific root length.

Provided the assumptions underpinning the modelled interactions of root traits are correct, the results indicate how plant breeding for improved P efficiency should proceed. Essentially all of the pasture legume studies have indicated that acclimation of clover roots to low P stress is triggered when a plant encounters P supply levels that are close to or declining below the plants critical P requirement (Haling *et al.* 2016b, Haling *et al.* 2018). In this way, root acclimation responses may be likened to a homeostatic response in which the plant is diverting carbon to nutrient foraging roots and, in some cases, adjusting root morphology to re-attain a high growth rate. With the possible exception of yellow serradella (Haling *et al.* 2016b), we have not observed pasture legume (and certainly no clovers) that adjust their root morphology to achieve a lower critical P requirement. Despite this, the literature abounds in papers describing attempts to identify genes that regulate root acclimation responses with the objective of improving P efficiency (e.g. Misson *et al.* 2005).

The model outcomes indicate that a more effective approach will be to breed for root system morphology that is intrinsically more P-efficient (i.e. longer specific root length and/or longer root



RMF = root mass fraction (all roots) (g/g)
FRMF = topsoil (foraging) root mass fraction (g/g)
SRL = topsoil specific root length (m/g)
RTD = is root tissue density (g/cm3)
ARD = average topsoil root diameter (mm)
RHL = average topsoil root hair length (mm)
RLD = density of root length in the topsoil layer (cm/cm3)
FRL = length of nutirnt foraging roots (cm)
SARHC = surface area of topsoil root hair cylinder (cm2)
Jmax = surrogate measure of P diffusion to RHC = total plant P uptake (shoot +topsoil root+subsoil root) per unit SARHC (mg P/cm2)
P stress index = P content of whole plant (micro-sward) /lowest P content of whole plants needed for max yield (Pcrit100)

Figure 8.1 - 8. Overview of the model of root morphology and P acquisition by subterranean clover roots constructed for the purpose of understanding interactions of root efficiency traits, the value of root traits for P-efficiency, and the gains that may be achieved by selecting clover root systems for improved P acquisition in low P soils.

hairs); selection among plants that have evolved in very low P soils is the most likely route to identifying clovers with these root attributes and, consequently, a lower critical P requirement.

All of the studies of subterranean clover root morphology indicate that a step change in the P efficiency of the clover will require it to have substantially longer root hairs. Presently the range in observed RHL among subterranean clovers ranges from about 0.2 to 0.4 mm, whereas the RHL of serradellas are typically 0.7-0.8 mm. Knowledge concerning the genetic control of RHL has advanced markedly and it is possible to substantially increase RHL by molecular genetic interventions (e.g. Kim and Dolan 2016, Kim *et al.* 2017, Weber *et al.* 2018). In some cases, however, longer root hairs produced in this manner have not always delivered improved P uptake from low P soil (Zhang *et al.* 2018, Weber *et al.* 2018, Klamer *et al.* 2019). This may be associated with adverse pleiotropic effects of novels genes, but there is also evidence that the expected P acquisition benefits of longer root hairs may be counteracted by relaxation of root acclimation responses to P stress (Weber *et al.* 2018, Klamer *et al.* 2019), as was predicted by the root morphology model.

8.3 Results and Discussion: Output 6(d) - Examine the diversity of P-efficient root traits in species of *Trifolium* that are genetically-allied to subterranean clover and investigate the potential for introducing genes by inter-specific hybridisation.

Clover species in the *Tricocephalum* Section are a potential genetic resource for plant improvement in subterranean clover (*T. subterraneum*). Hybridisation among some species from the *Tricocephalum* Section can be achieved using traditional crosses (e.g. *T. subterraneum* x *T. eriosphaerum* and x *T. pilulare*; Katznelson 1967). However, crosses with *T. israeliticum, T. globosum,* and *T. pauciflorum* failed to produce viable seed. Nevertheless, it is likely that modern approaches to interspecific hybridisation using embryo culture and chromosome doubling may overcome these barriers (e.g. Williams 1978). Six clover species phylogenetically-allied with *T. subterraneum* in the *Tricocephalum* Section were compared with *T. subterraneum* and French serradella (*Ornithopus sativus*) to assess variation in their nutrient foraging root traits. Subterranean clover was found to be the best, or rank among the best of the allied clover species, in terms of its root proliferation response and specific root length.

However, genotypes of two species (*T. pauciflorum, T. meduseum*) were consistently found to have longer root hairs than subterranean clovers (0.5 - 0.6 mm cf. ~0.2 - 0.4 mm, respectively; Fig. 8.1 - 9). Root morphology modelling predicted that root hairs of this size could potentally reduce the critical P requirements of subterranean clovers. However, RHLs equivalent to those of the serradellas (typically 0.7-0.8 mm) would probably be necessary to justify investing the cost and time in a plant improvement program for this trait alone because successful production of hybrids *with T. subterraneum* would be a demanding task.

Very short root hairs on subterranean clover have been consistently identified as a major factor limiting the potential for a step-change in P-efficiency of the species. Further options that could be explored to identify genotypes with longer root hairs include:

(i) Further examination of the diversity in currently unscreened genotypes of subterranean clover (several thousand accessions held in the Australian Pastures Genebank; by definition, there is ~20% of variation within the subterranean clover genome [i.e. variation outside of that represented by the core collection] that has not been explored by the current experiments).

(ii) Proven molecular approaches that target genes controlling root hair elongation and can produce substantially longer root hairs.

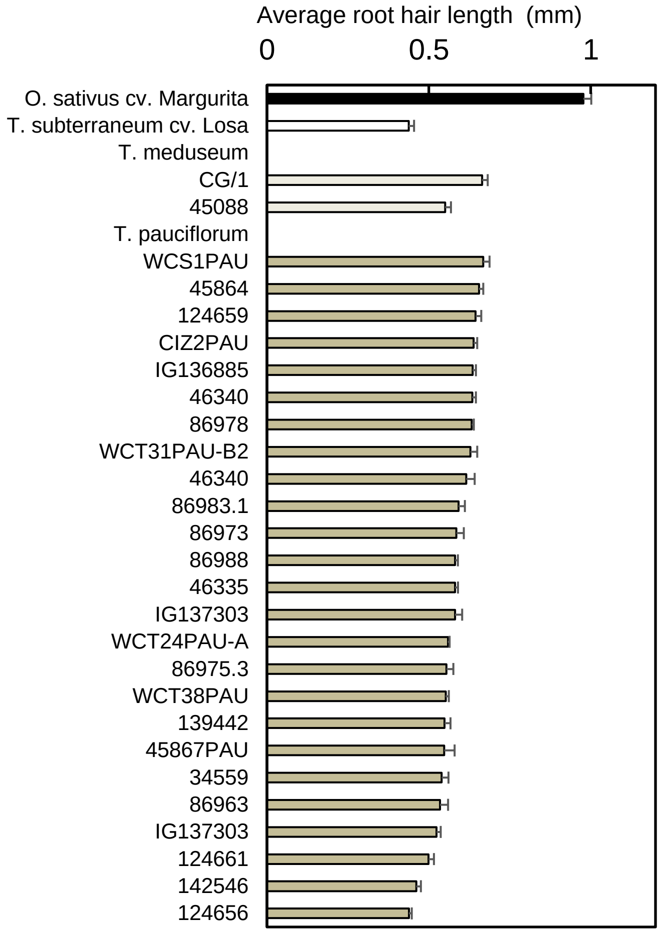


Figure 8.1 - 9. Average RHL of two lines of *T. meduseum* (these are the only lines of this species available from the Australian Genebank) and 25 lines of *T. pauciflorum* when grown at an 'intermediate' level of soil P deficiency. A single cultivar of *T. subterraneum* and *O. sativus* were included as controls of species with short and long root hairs, respectively. Values are ± standard error (n=5).

8.4 Results and Discussion: Output 6(c) - Develop molecular tools for breeding nutrient-efficient subterranean clover cultivars.

8.1 Genome wide association study (GWAS)

A Genome-Wide Association Study (Table 8.1 - 1) found single-nucleotide polymorphisms (i.e. variations in the genetic sequence of subterranean clover; aka SNPs) that were associated with P-efficient traits including: yield indices that indicate consistently higher yield in low-P soil, root mass and length, root diameter, and the plants ability to shift growth towards roots in low P soil (Table 8.1 - 1).

Unfortunately, SNP's were often only significantly associated ($P = 0.05$) with plant traits in particular experiments. In the case of root diameter, the candidate genes identified differed between two experiments. These inconsistencies may be a result of differences between experiments in plant age, plant density and soil type. Regardless of the cause, lack of consistency between experiments in associating candidate genes with plant traits was not encouraging in terms of finding robust molecular markers for key P efficient root traits.

SNPs were not associated with the key root traits (see **Section 8.2.1**) considered important for P efficiency such as RHL and specific root length (root length per unit root mass). This may

Table 8.1 - 1. The data set of phenotypic traits used in the GWAS. DW=dry weight, RL=root length, SRL=specific root length, ARD=average root diameter, RLD=root length density, RMF=root mass fraction, SA=surface area, TD=tissue density.

Experiment	P levels	Root phenotypic traits	Other phenotypic traits	Covariates used[1]	Data source
Experiment 1. UWA screen	Low	Seed P, Nodulation, Root DW, RL, SRL, ARD, RLD, RMF, Rhizosheath carboxylates (total and individual)[2], Mycorrhizal colonisation level	Development stage, Seed P content and concentration, Seed weight, Shoot DW, Total DW Shoot P concentration	Seed weight, nodulation and seed P content (on selected traits)	Previous MLA project
Experiment 2. Low-density screen	Low	Root DW, RL, ARD, SRL, RMF, Root SA, Root TD, Root SA/DM	Shoot DW	Planting time	This project
Experiment 3. Root proliferation	Low	SRL, ARD, RLD, RTD, Root DW, RMF, Root SA, Root SA/DM	Shoot DW		This project
	High	SRL, ARD, RLD, Root TD, Root DW, RMF, Root SA, Root SA/DM HIGH P	Shoot DW		This project
Experiment 4. Root hair length	Low	Root hair length			This project
Experiment 5. Root angle	Low	Root angle (0–5 cm) Root angle (5–10 cm) ARD, RMR			This project

[1] Subspecies (*subterraneum, yanninicum, brachycalycinum*) was used as a covariate in all analyses

[2] Acetic, citric, fumaric, lactic and malic acids

Table 8.1 - 2. Traits that were significantly ($P = 0.05$) associated with particular genes included the Yield Indices that summarised yield in low P soil across three independent experiments and some plant traits. DW=dry weight, RLD=root length density, RMF=root mass fraction, SA=surface area.

Gene	Associated traits		
	Root	Other	Low-P yield indices
g15396	Low-P root DW (Exp 2)	Low-P shoot DW (Exp 2)	Exp. 1+2
g16789	Low-P RLD (Exp 1) High-P root DW (Exp 3)	Low-P shoot DW (Exp 1)	Exp. 1+2+3
g31011	Low-P RMF (Exp 1) High-P root SA/DW (Exp 3)	Low-P shoot DW (Exp 2)	Exp. 1+2+3

mean that these traits are not controlled by a simple set of genes, or that there was just not enough trait variation within the genome to enable correlation with genomic variation. Regardless of the reason, these results indicate there is little opportunity to support breeding for root traits using genetic markers.

Collectively, the results indicated that it may be possible to use genetic markers to assist breeding for some aspects of improved P efficiency in subterranean clover (e.g. indices for yield in low P soil). However, the lack of SNP's associated with key root traits for P efficiency (e.g. RHL, specific root length) indicated that there was little opportunity to use genetic markers to assist breeding for improved root systems.

9 Conclusions/recommendations – (Activity B6) Identifying P efficient subterranean clover cultivars.

9.1 P efficiency among subterranean clover genotypes.

9.1.1 Two-fold range in P-acquisition efficiency in controlled-environment studies

Subterranean clover cultivars exhibited roughly a two-fold range in the growth rates achieved by the "best" and "poorest" cultivars during vegetative growth in moderately low-P soil. A two-fold range in P efficiency was evident when clovers were grown with similar planting densities or at a similar sowing rate. However, all of the evidence for the range in P efficiency comes from studies in controlled environments/ glasshouses because drought prevented the completion of experiments intended to verify the expression of P efficiency in the field.

A similar two-fold range in P efficiency was observed among the core collection of subterranean clover lines which represents ~80% of the variation in the subterranean clover genome. The best clover cultivars were as good as the best core lines with respect to growth rate in low-P soil. The P efficiency achieved by the best clover cultivars was similar to that of the best core collection lines.

It was clear from the study of growing conditions that the density of plants in a pasture sward can potentially alter the expression of P efficiency by some clover genotypes. This is an uncontrollable variable in pasture swards and may confound the identification of superior genotypes if not considered. Nevertheless, there was reasonable evidence that some clover genotypes (epitomised by cv. Losa, Rosabrook, etc.) express high P efficiency consistently, others appear to be consistently P-inefficient (e.g. Riverina, Daliak, etc.) and some (epitomised by cv. Napier) can exhibit variable expression of P efficiency depending on growth conditions (particularly at different planting densities).

9.1.2 Current STP benchmarks for soil P fertility management

The average to above-average P efficiency of cv. Leura (used as a key cultivar in the P benchmarking study of Sandral *et al.* 2019) indicates that current critical soil test P guidelines for the fertiliser management of subterranean clover pastures (Gourley *et al.* 2019) are pitched towards the requirements of average performing cultivars. Unfortunately, no cultivar or core line of subterranean clover achieved the high P-efficiency benchmark of the serradella species.

There is good field and controlled-environment evidence that current subterranean clovers have significantly higher P requirements than that of the serradellas (e.g. critical soil test P for subterranean clover ~33 mg Colwell P/kg soil cf. ~21 mg P/kg for serradellas; Sandral *et al.* 2019). The difference between the critical STP requirements of subterranean clover and serradella is large enough that farmers can realistically fertilise to the different benchmarks of each legume and, in fact, must do this to achieve the P efficiency benefits discussed elsewhere in this report (**Section 4.7.1; Section 3.1.3**).

9.1.3 Potential implications for farm productivity

The importance of knowing that there can be differences in the P efficiency of clover cultivars should not be underestimated. Low P paddocks exist on many farms because they have terrain that is difficult to fertilise, have shallow soils that are intrinsically less productive and are, therefore, fertilised less, and for various other reasons. Choosing a more P-efficient cultivar for use in these areas of farms could boost production with no other change to management. At the extreme, low production levels could potentially be doubled depending on cultivar choice. However, it is not sensible, and could be potentially misleading, to promote cultivars on the basis of their putative P efficiency in controlled environments, without field verification. Field verification was not achieved in the current project because of persistent drought conditions.

9.2 P efficient root traits and the potential for developing more P-efficient cultivars.

9.2.1 Key root traits that confer P-acquisition efficiency

Differences in P efficiency among subterranean clover genotypes were mainly attributed to differences in P-acquisition efficiency (the ability of a plant to acquire P from P-deficient soil) and not P utilisation efficiency (i.e. the P concentration of plant material). High P-acquisition efficiency among pasture legumes is associated with a lower critical P requirement (Sandral *et al.* 2018). This means that P efficient cultivars will achieve maximum yield at lower STP concentrations than less P efficient cultivars.

Pasture legumes that achieved high P-acquisition efficiency did so by: (i) **allocating dry matter preferentially to nutrient foraging roots**, and by combining this with (ii) **high SRL** (length per unit root mass), and (iii) **long root hairs**. The net result of these traits was development of a larger root-soil interface for P uptake (measured as a large SARHC). The rate of P supply to a root is determined by the rate at which phosphate diffuses from the bulk soil to the root-soil interface. Consequently, a larger surface for P uptake intercepts more P and allows more uptake at a lower STP concentration, when diffusion is slower.

9.2.2 Potential to improve the P-acquisition efficiency of subterranean clovers

Subterranean clover **preferentially allocated dry matter to nutrient foraging roots** when experiencing P stress; this was a root *acclimation* trait. When P stressed, some cultivars allocated even more dry matter to nutrient foraging when growing with space around them. This assisted their ability to find scarce P resources. Cultivars appeared to differ in the P stress threshold at which root mass was allocated to nutrient foraging; the more P-efficient plants continued to do this even when highly P stressed. *This trait is very difficult to measure and select for.*

High SRL or **long root hairs** were *intrinsic* root traits characteristic of each genotype that would potentially be amenable to selection for plant improvement. The SRL of the best clovers approached that of serradella species. However, despite varying by as much as two-fold, the RHL of all subterranean clover genotypes tested were universally short. (Consequently, the length of nutrient foraging roots was an equally good predicter of P efficiency as the SARHC among subterranean clovers.)

If breeding subterranean clovers for improved P efficiency is to be worthwhile a step change that equals the P-efficiency of the serradella is required. Very short root hairs were the reason why no clover has been found that can emulate the very high P efficiency of the serradellas. There was no evidence of longer root hairs among the core collection of subterranean clover lines. Longer root hairs on two phylogenetically-allied *Trifolium* species were still short of the long root hairs found on serradellas.

Changes in RHL of the magnitude required have been achieved using molecular genetic technologies (e.g. Kim and Dolan 2016, Kim *et al.* 2017, Weber *et al.* 2018). There is also ~20% of the variation within the subterranean clover genome that (by definition) has not yet been explored.

9.2.3 A role for molecular breeding tools?

Among the subterranean clover cultivars tested, about 50% consistently achieved average or above-average yield in low P soil, about 25% were consistently P-inefficient and the remaining 25% did not consistently exhibit a characteristic level of P efficiency. Among the core collection of lines, about 40% consistently expressed average or above-average P efficiency as measured by yield in moderately P-deficient soil. An index reflecting “consistent P-efficiency” was devised for use in the Genome-Wide Association Study. SNPs associated with the index were identified as well as for some other P efficiency traits, but not for the key intrinsic P efficiency traits: specific root length or RHL. This appears to limit the potential for using this technology to assist breeding for P-efficient root traits.

9.2.4 Interactions with mycorrhiza

Arbuscular mycorrhizal fungi always colonise clover roots in the field and are expected to deliver a P-acquisition benefit in P deficient soils. The fungal hyphae achieve increased P uptake for the plant because the size of the colonised root interface with soil is larger than that of a root system that is not colonised. The interaction of mycorrhizal colonisation and root trait expression was examined to determine whether the importance of the P-efficiency root traits was modified when roots were colonised by mycorrhiza.

Mycorrhizal roots did capture more P in very low-P soil, but the full benefit of mycorrhizal colonisation was not realized because the mycorrhizal plants reduced their allocation of plant dry mass to nutrient foraging roots (i.e. their root acclimation response to low soil P). The differing responses of alternative legumes to mycorrhiza were unified by assuming that plant P stress was a regulating influence, as hypothesised in the root morphology modelling.

Mycorrhizal colonisation did not override the value for P acquisition of having intrinsically P-efficient root morphology and it was concluded that selecting genotypes for improved root morphology would improve P acquisition irrespective of whether the plant was colonised by mycorrhizal fungi.

9.3 Recommendations: Activity B6 - Identifying P efficient subterranean clover cultivars.

- A two-fold variation in subterranean clover yield among cultivars and among the core-collection genotypes growing in P-deficient soil was observed in controlled-environment studies, but **verification of P efficiency in the field** was thwarted by persistent droughts. Field proof is logistically and technically difficult to achieve, but it is essential before any apparent difference among clover varieties is to be promoted to farmers. Differences in P-efficiency among other pasture legumes detected in glasshouse experiments are not always able to be detected under field conditions. Without field proof, it is recommended that the current benchmarks for fertilising subterranean clover be followed.

- **Short root hairs on subterranean clover** was the major factor limiting achievement of a step-change in P-efficiency of this species. In some experiments subterranean clover developed longer nutrient foraging root length than serradella but still failed to acquire as much P because its root hairs were so short. RHL that are equivalent to those on serradellas are required to deliver a clover with a step-change improvement in P efficiency. Further options that can be explored to identify or develop genotypes with longer root hairs include:
    - (i) In theory, ~80% of the genetic variation in the subterranean clover genome has been explored by examining RHL among the core collection of subterranean clover lines. The range in RHL among the core lines was similar to that among the clover cultivars; all were well short of the RHL on serradellas. However, this leaves about ~20% of the variation in the subterranean clover genome as yet unexplored. Because the Australian Pastures Genebank holds several thousand subterranean clover accessions, a highly targeted approach to screening is needed. The most obvious approach would be to examine accessions that have evolved under very low soil P conditions.
    - (ii) The RHL of a number of plant species has now been doubled or tripled using molecular genetic technologies. Benefits for P acquisition have not always been proven. However, the experience developed in the current project and the better understanding of P acquisition developed through modelling root trait interactions could be used to guide which cultivars of subterranean clover should be used for genetic transformation to ensure combinations of root traits that deliver a step-up in P efficiency.

- The current experiments did not provide any hope that genetic markers can be used to select for P-efficient root traits in subterranean clover. However, **SNP's were associated with yield indices** that reflected consistently better yield in moderately P-deficient soil. Further work would be required to validate this result. However, on face value, it indicates that it may be feasible to develop genetic markers for early elimination of low P-efficiency lines during subterranean clover breeding.

10 Results and Discussion – (Activity B7) "Five Easy Steps" P management tool.

10.1 Output 7(a) - Review and revise P fertiliser rate calculations and spreadsheets for on-farm fertiliser management planning and update the "Five Easy Steps" P application tool.

10.1.1 Phosphorus fertiliser rate calculations

In the 10-year interval since the original "Five Easy Steps" tool was released (2009) there has been a limited amount of feedback from advisors noting inconsistency in the outputs from fertiliser rate calculations based on the Colwell soil P test and the Olsen soil P test. Usually advisors and farmers use one or other of these tests and would not normally notice if there were inconsistencies. However, the research team were also aware of instances when the calculations gave "ideal" outcomes, and other instances where fertiliser amounts seemed to under- or over-shoot soil P fertility targets. The fertiliser rate calculations are based on published research by Burkitt *et al.* (2001).

A number of approaches were used to understand and then resolve this issue:
(i) **A desktop assessment of the P-fertiliser application calculations**: There was limited data available for these assessments. Dr L Burkitt (Massey University, NZ) provided the research team an unpublished dataset to check against her own published data. The data demonstrated that there are wide confidence intervals associated with P rate calculations. The work strengthened the team's view that the impact of seasonal conditions on soil test P values is the most likely reason for variation in the estimates.

(ii) **P rate experiments on farms** participating in the project (particularly members of the Monaro Framing Systems group): Estimates of P fertiliser amounts required to increase soil P fertility were made based on data collected in these experiments and were often within the confidence intervals from published research, but they also varied between years and sites. This confirmed the results and conclusions from the desktop assessment.

(iii) **Assessments of other data collected in the project**: For fertiliser rate calculations based on Olsen P and Colwell P to be consistent, there must be an implied relationship between these soil tests that will be modified by soil Phosphorus Buffering Index. Data from the soil testing in the project (particularly that from central Victoria where Olsen P is the preferred test) demonstrated that the Colwell P/Olsen P ratio varied typically from ~2- to 3-fold between sites and years in the low P buffering range. (The implied average ratio in the tool is ~3.3 in the low P buffering range.) This amount of variation in the Colwell P/Olsen P ratio, would alone contribute to a wide confidence interval in fertiliser calculations based on Colwell P or Olsen P. However, it was also noted that the Colwell P/Olsen P ratio was consistently 6 on one of the farms, for reasons that could not be established.

3.2.5.2 A pragmatic solution

The only practical solution to this issue (without a dedicated research effort) was to continue using the current fertiliser rate calculations with increased emphasis on the expectation that they provide a 'ball-park' estimate of fertiliser rate that should be checked and modified (if necessary) by establishing a soil fertility monitoring program. This advice was a standard feature of the "Five Easy Steps" P tool, but the emphasis is now strengthened in the revised tool.

3.2.5.3 Early extension and adoption success

- An article that covered aspects of this work and promotes soil test P monitoring on farms was written for the NSW Grasslands Society Newsletter.
- A major Australian fertiliser manufacturer, retailer and soil testing business (Incitec-Pivot Pty Ltd) adopted this message and has promoted a “new approach” to soil testing which includes new packaging of soil tests for farmers adopting soil test monitoring.
- This tool continues to be used by farmers, 10 years after its initial release in 2009. For example, in the last 12 months it was still ranked in the top 10 most popular tools/calculators in the Tools and Calculators section of MLA’s website. Since 2017 (when records began being collected) it has been downloaded 1200 times.
- The revised technical booklet and tool contains new information, case studies and improved recommendations for farm fertiliser practice; this is expected to revitalise interest in use of the P management tool.

10.2 Output 7(b) - Revise targets for soil P fertility management of pastures in the “Five Easy Steps” P application tool.

10.2.1 Revised targets for soil P fertility management

Work conducted in the project:

- Encouraged and supported publication of the science behind Australia’s critical soil test benchmarks for Colwell P, Olsen P, Colwell K, KCl40-S and CPC-S by Gourley *et al.* (2019; Crop and Pasture Science 70, 1065-1079). The publication was made OPEN ACCESS and can be downloaded for free by farmers and advisors (https://www.publish.csiro.au/cp/cp19068).

- Enabled publication of field-based assessments of the critical Colwell P and Olsen P soil test benchmarks for a range of alternative pasture legumes and two grasses. The publication confirmed the use of the Australian critical STP benchmarks for most clover-based pastures and the evidence for lower critical STP benchmarks for serradellas (Sandral *et al.* 2019; Crop and Pasture Science 70, 1080-1096). The publication was made OPEN ACCESS and can be downloaded for free by farmers and advisors (https://www.publish.csiro.au/cp/CP19014).

Revised soil P management guidelines were subsequently included in the “Five Easy Steps” technical booklet and a facility was added to the “Five Easy Steps” computer tool to allow STP targets for fertiliser management to be varied when legumes with different critical P requirements are being used. In addition, results from a recent review of the P management of Australian native grass pastures by Mitchell *et al.* (2019) (https://www.publish.csiro.au/cp/CP19217) were included in the technical booklet to provide guidance for farmers managing native grass and subterranean clover pastures.

The project team collated research data, and worked with the participating farm groups to develop examples and extension materials that can assist a wider cohort of farms to adopt simple practices that result in more effective P fertiliser use. These included recommendations to:

- setting a pragmatic target range for soil P management that is guided by the critical P requirement of the soil-pasture system,
- to testing pasture soils at the same time each year (typically spring),
- commencing an annual soil-fertility monitoring program.

Two examples of managing and monitoring soil STP levels to a target were expanded. An unexpected outcome of this exercise was evidence that targeted fertiliser management can deliver a slow, but steady reduction in the fertiliser cost of production over time. The reduction in P costs was associated with (i) the shift from building to maintenance of soil P fertility, (ii) improved confidence in P fertiliser decisions enabling decisions to skip fertiliser applications after dry seasons when soil P-fertility had been conserved and was also (iii) surmised to be due, in part, to the "P-sparing effect" of continuing P fertiliser applications that is known from laboratory studies of P sorption to soil.

10.3 Output 7(c) - Release updated version of the "Five Easy Steps' P management tool.

The revised "Five Easy Steps" technical booklet (*Five Easy Steps to ensure you are making money from phosphorus fertiliser*) and computer tool for P management of pastures is ready for loading into the new web format being developed by MLA. A draft layout for the revised technical booklet can be found attached to **Appendix 10**.

New features of the revised P management tool include:

- Strengthened advice about how to use calculations of P fertiliser application rates.
- New case study examples of how soil testing can be applied on farms.
- Analysis of the long-term P efficiency benefits of following 'Five Easy Steps' P management protocols.
- Revisions to text, the name of the tool, and the order of the "steps" based on feedback from several rural consultants.
- A revised 5-step sequence that elevates he need to check for other nutrient deficiencies and promotes a more "holistic" approach to soil fertility management.
- Guidelines regarding fertilisation of native perennial grass pastures have been added.
- New cautions regarding P application and soil P testing in potential "leaky" soils (PBI <35) have been added.
- Updated critical soil test values supported by published scientific literature.
- Advice regarding P conservation during drought was strengthened

11 Project key messages

11.1 Activity B4 - Lowering P costs of serradella systems.

- The key P-efficiency paper: *Field benchmarking of the critical external phosphorus requirements of pasture legumes for southern Australia*, by Sandral *et al.* (2019) was completed and published after peer review. This paper:
  (v) provides further field data from four locations over three years confirming the validity of STP benchmarks for clover-based pastures in southern Australia (Gourley *et al.* 2019),
  (vi) extends the use of these benchmarks for soil P management to nine alternative pasture legumes,
  (vii) indicates that lucerne requires a higher P supply for maximum production (critical STP concentrations were unable to be determined as they were higher than detectable by the experiment treatments), and
  (viii) sets new lower STP benchmarks for pastures based on five alternative legumes, of which three were forage crop legumes (*Trifolium incarnatum, T. purpureum*, *T. vesiculosum*) and two were pasture legumes (*Ornithopus compressus* [yellow serradella], *O. sativus* [French serradella])

- Farmers adopting a targeted approach to P fertiliser use that is based on these benchmarks soil P management can achieve highly effective use of their fertilisers and a steadily declining fertiliser cost of production. Case studies detailing this approach to fertiliser use and the cost savings are now included in a revised version of the “Five Easy Steps” P management tool (https://www.mla.com.au/extension-training-and-tools/tools-calculators/phosphorus-tool/)

- Experiments designed to check the critical P requirements of serradellas growing in areas where serradellas are already used were not able to achieve their objective because of persistent droughts. However, indirect evidence (faster growth rates in P deficient soil) demonstrated that the serradellas were more P efficient that subterranean clover when rainfall was sufficient for some pasture growth to be recorded.

- The movement of P in soil profiles should routinely be checked when surface soil Phosphorus Buffering Index (Burkitt *et al.* 2008) test values are <35. In the current research, all deep sandy soils used for serradella pastures in Western Australia exhibited very low P buffering capacities and were highly “leaky” for P. The research demonstrated that under these circumstances:
  (iv) traditional surface soil testing (0-10 cm depth) provided a misleading assessment of the soil’s P status,
  (v) P applied as fertiliser moved readily to depth within the soil profile,
  (vi) “legacy P” (from previous P fertiliser applications) at depth was sometimes sufficient to eliminate the need for P fertiliser applications in soil incorrectly diagnosed as P deficient using surface soil testing.

- The fastest way to improve P efficiency in soils types with low P buffering (low P retention) will be to recognise and use legacy P as part of the farm’s soil P management plan. This will also reduce the potential for loss of P to the wider environment where it can be a significant pollutant.

- Alternative soil sampling/testing protocols need to be developed for use in soil types with low P retention. Promotion of more routine assessments of soil P profiles and “deep” soil sampling to account for more of the P that is available within a soil P profiles is supported by the current project results. A soil test depth of 0-60 cm (as used in the present work) may be appropriate, but soil test depths should be examined critically. The logistic problems and costs of deep soil sampling soils are large and will be major impediments.

- The true "availability" of legacy STP at depth in these soil profiles needs to be understood and requires investment in systematic research that considers the likely impacts of: (i) soil P buffering capacity, (ii) P accumulation (P-fixation) rate, and (iii) the rooting depth of the crop or pasture being grown.

11.2 Activity B5 - Extending the adaptation range of serradella systems.

- This project was commenced with a view to extending the use of serradella pastures to the **P-cost pf pasture production.** It is estimated that serradella pastures can be maintained with up to ~30% less P-fertiliser than subterranean clover pastures with equivalent production (Simpson *et al.* 2014). Farmer groups participating in the project have added to this goal, their objectives of **diversifying the legume base of pastures** (risk mitigation), capturing the **"low-bloat"** characteristics of serradella pasture, **out-of-season growth** in years when rainfall extends spring onto summer, **improved early- and late-season drought tolerance** as a consequence of faster root growth and/or deeper root systems, and **avoiding clover diseases** and loss of production.

- Serradellas should no longer be promoted primarily for use in light, sandy, or acid and infertile soils. They clearly have wider edaphic adaptation; however, they are unsuited to neutral-alkaline soil types and will not tolerate soils that are subjected to persistent waterlogging.

- The use serradella of in southern Australia is expanding rapidly. Most of the expansion is in phase farming systems where serradellas are often grown as monocultures and where long-term persistence is not always required. However, the uptake of serradellas for use in permanent pastures (~6M ha) is stalled. The major factor underpinning reluctance to adopt serradellas in these areas is lack of persistence by many current serradella varieties in permanent pastures.

- Factors considered to be contributing to poor serradella persistence include:
  (v) promotion of cultivars with inappropriate flowering dates for the growing season in areas of proposed used,
  (vi) unstable flowering dates that confound maturity-type selections among many serradella varieties,
  (vii) continued promotion of softseeded varieties to farmers,
  (viii) non-ideal seed softening patterns among the hardseeded serradella varieties; one manifestation of which was poor seeding regeneration in the year following pasture establishment.

- Serradella persistence in mixed, permanent pastures will only be resolved by:
  (i) field testing genotypes selected to address the factors contributing to poor persistence, and
  (ii) by researching how successful cultivars (e.g. Avila and King) avoid loss of ground cover in pastures on farms.

  The current research has informed development of a short list of "best-bet" serradella cultivars for further research in the permanent pasture areas where serradellas are not being adopted. No one variety has all of the putative traits thought to be desirable for persistence and high production, but each variety has sufficient merit to potentially kick-start adoption once performance in a mixed pasture is proven. This list includes cvs Avila and King, which (in the areas to which they are suited) exhibit the best levels of persistence among the current serradella cultivars.

11.3 Activity B6 - Identifying P efficient subterranean clover cultivars.

- The project has not identified any subterranean clover genotypes that can emulate the high P efficiency of the serradellas.

- However, a two-fold range in subterranean clover yield in P-deficient soil (i.e. P efficiency) was observed consistently in controlled-environment studies. The yields were similar among subterranean clover cultivars and among core-collection genotypes.

  Differences among clover varieties in P efficiency was not verified in the field because of persistent droughts. Field proof is logistically and technically difficult to achieve, but it is essential before any apparent difference among clover varieties is to be promoted to farmers. Differences in P-efficiency among other pasture legumes demonstrated in glasshouse experiments are not always able to be detected under field conditions (e.g. Sandral *et al.* 2019).

  Without field proof, it is recommended that the current benchmarks for fertilising subterranean clover be followed (e.g. Gourley *et al.* 2019).

- Short root hairs on subterranean clover was the major factor limiting achievement of a step-change in P-efficiency of this species. In some experiments subterranean clover developed longer nutrient-foraging roots than serradella, but still failed to acquire as much P because their root hairs were very short. RHL that are equivalent to those on serradellas are required to deliver a clover with a step-change improvement in P efficiency. There are reasonable options that may yet identify or develop clover genotypes with longer root hairs:
  (iii) In theory, ~80% of the genetic variation in the subterranean clover genome has been explored in the present experiments by examining RHL among the core collection of subterranean clover lines. The range in RHL among the core lines was similar to that among the clover cultivars; all were well short of the RHL on serradellas. However, this leaves about ~20% of the variation in the subterranean clover genome as yet unexplored. Because the Australian Pastures Genebank holds several thousand subterranean clover accessions, a highly targeted approach to screening is needed. The most obvious approach would be to examine accessions that have evolved under very low soil P conditions.
  (iv) The RHL of a number of plant species has now been doubled or tripled using molecular genetic technologies. Benefits for P acquisition among RHL mutants have not always been proven. However, the experience developed in the current project and the better understanding of P acquisition developed through modelling root trait interactions could be used to guide the selection of subterranean clover cultivars for genetic transformation to ensure combinations of root traits that can deliver a step-up in P efficiency.

- The current experiments did not provide any hope that genetic markers can be used to select for P-efficient root traits in subterranean clover. However, SNP’s were associated with yield indices that reflected consistently better yield in moderately P-deficient soil. Further work is required to validate this result. Taken at face value, these results indicate that it may be feasible to develop genetic markers for yield indices to eliminate low P-efficiency lines during early-stage subterranean clover breeding.

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13 Appendices

13.1 Appendix 1: draft Final Milestone Report prepared for DoAWE

13.2 Appendix 2: Final Internal Milestone Report (#8) – Graminus Consulting

13.3 Appendix 3: Final Internal Milestone Report (#8) – ASHEEP

13.4 Appendix 4: Final Internal Milestone Report (#8) – Southern Dirt

13.5 Appendix 5: Final Internal Milestone Report (#8) – Central Ranges Grassland Society

13.6 Appendix 6: Final Internal Milestone Report (#8) – Bookham Agricultural Bureau / Tablelands Farming Systems

13.7 Appendix 7: Final Internal Milestone Report (#8) – Monaro Farming Systems

13.8 Appendix 8: Final Internal Milestone Report (#8) – Purlewaugh National Farmers / Boggabri Grazing Group

13.9 Appendix 9: Final Internal Milestone Report (#8) – NSW Department of Primary Industries

13.10 Appendix 10: Final Internal Milestone Report (#8) – CSIRO Agriculture and Food

13.11 Appendix 11: Final Internal Milestone Report (#8) – University of Western Australia

13.12 Appendix 12: Final Internal Milestone Report (#8) – Murdoch University

13.13 Appendix 13: Project communication materials

Year	Date	Communication type	Description	Scope	Reported by:
Field days and farm walks					
2016	2-Jun-16	field day/farm walk	Dr. Richard Simpson visited the Baynton research site and spoke at a MLA/GSSA Pasture Update field day held at Sutton Grange on the topic of Phosphorus Efficient Legumes.		Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2016	19-Oct-16	field day/farm walk	A small group field day was held at Purlewaugh were the highlights and updates on the experiment were discussed and the field site was inspected.	10 attendees	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2017	1-Feb-17	field day/farm walk	Phil Graham (NSW DPI) provided talks to members of both the Bookham Agricultural Bureau (BAB) and Tablelands Farming Systems (TFS) farmer groups about the project aims and scope, and their participatory research role	farmers groups --> Bookham Agricultural Bureau (BAB) and Tablelands Farming Systems (TFS); ~ 60 participants in total	Jim Virgona and Graminus consulting P/L
2017	20-Mar-17	field day/farm walk	A farmer seminar was held at Singleton, in which the progress and highlights of the project were discussed.	40 attendees	R. Freebairn, Boggabri grazing group
2017	18-Jul-17	field day/farm walk	A farmer seminar was held at Leadville, in which the progress and highlights of the project were discussed.	25 attendees	R. Freebairn, Boggabri grazing group
2017	27-Aug-17	field day/farm walk	A field walk for local farmers examined early spring growth at Southern Dirt experimental sites.		Melissa Jardine, Southern dirt
2017	12-Sep-17	field day/farm walk	This project was discussed in detail at a field day conducted in the Purlewaugh area with highlights relating to progress in 2017.	85 attendees (producers and advisors)	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2017	12-Sep-17	field day/farm walk	The purpose and progress of the project was presented at Southern Dirt's TECHSPO event.		Melissa Jardine, Southern dirt
2017	21-Oct-17	field day/farm walk	A field day to be held at Boggabri involved a discussion of the project, including progress made up to that point.	80 attendees	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2017	7-Nov-17	field day/farm walk	A field day held at Coolah informed local farmers on the purpose and progression of the research.	40 attendees	R. Freebairn, Boggabri grazing group
2017	Dec-17	field day/farm walk	A presentation of the experiments progress occurred at a MFS summer field day.	30 attendees	Doug Alcock and Graz Prophet consulting
2017	4-Dec-17	field day/farm walk	The Tablelands Farming Systems (TFS) group held a twilight “farm walk” at the Bigga site. Local farmers and members of TFS were introduced to the sites as well as the co-located legume evaluation site	15 attendees with Richard Hayes, Richard Simpson and Jim Virgona from the project team	Jim Virgona Graminus Consulting Pty Ltd.

2017	7-Dec-17	field day/farm walk	Monaro Farming Systems, Soils Club day – Richard Simpson discussed: *2017 soil test highlights, S-management experiments, soil P fertility experiments, soil testing strategies*, P-efficient pastures project. (Nimmitabel, 7 Dec 17)	~60 farmers attending	Richard Simpson and Rebecca Haling, CSIRO
2017	20-Dec-17	field day/farm walk	A twilight farm walk was held at the Red cliff site where Phosphorus efficient pastures were discussed. The research sites were inspected, speakers were Dr Richard Simpson (CSIRO), Richard Hayes (NSW DPI) and Doug Alcock (Graz Prophet).	15 attendees	
2017	20-Dec-17	field day/farm walk	An initial field day was held on site at Redcliff to inspect both the producer experiments and the pure sward experiments managed by Richard Hayes. Attendees observed the ongoing growth of the serradellas and also received preliminary results from the first year's measurements as well as a background to the reason for the work on P efficient legumes.	approx. 12 attendees	Doug Alcock and Graz Prophet consulting
2017		field day/farm walk	Updates on experiment progess and highlights were given at ASHEEP field days and AGM meetings	ASHEEP members and field day participants	ASHEEP
2018	30-Jan-18	field day/farm walk	A number of local landholders and members of the Bookham Agricultural Bureau visited the Blackburn sites to inspect the plots and become familiar with the progress of the project.	10 attendees, with Drs Richard Simpson and Jim Virgona from the project team and Fiona Leech from Local Land Services, Yass.	Jim Virgona Graminus Consulting Pty Ltd.
2018	23-Feb-18	field day/farm walk	A field day was conducted at the Purlewaugh site, with emphasis on covering issues like soil fertility and species like serradella.	20 attendees	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2018	12-Mar-18	field day/farm walk	A field day at Boggabri included a presentation that covered issues like species choice (serradella) and soil fertility, including P4P progress results	100 attendees	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2018	14-Apr-18	field day/farm walk	Jim Virgona presented at the Tablelands Farming Systems Soils Club day. An update of results from the project was presented as well as advice on soil phosphorus testing and management.	~40 farm businesses	Jim Virgona and Graminus consulting P/L
2018	Sep-18	field day/farm walk	At the Southern Dirt annual Spring field day, Brad Nutt presented at the Katanning P4Pasutures site, presenting the aims and results of the experiment and project. The group had special interest in the amount of phosphorus that was available deep in the profile of these poor sands.	50 farmers and industry professionals	Melissa Jardine, Southern dirt Brad Nutt
2018	6-Nov-18	field day/farm walk	A seminar was held at Coolah Seminar to survey the knowledge of farmers of the project.	~45 attendees	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2018	27-Nov-18	field day/farm walk	Suzanne Boschma and Robert Freebairn spoke of the project at a workshop held at Purlewaugh		Suzanne Boschma, NSW DPI
2018	Dec-18	field day/farm walk	A presentation was given at an MFS Soils Club field day which gave an update of results to date. Included experiment plot sowings, photos, seedling counts 2018 sowing and biomass measurements at the 2 Redcliff sites.	~45 farm businesses represented	Doug Alcock and Graz Prophet consulting

2018	Dec-18	field day/farm walk	Interim results of the research were presented to the MFS meeting to MFS members and others.	approx. 50 producers (MFS members and others)	Doug Alcock and Graz Prophet consulting
2018	5-Dec-18	field day/farm walk	Richard Simpson participated in a joint Bookham Agricultural Bureau and Tablelands Farming Systems group field day at the Merrill (via Gunning, NSW) research site. Richard Hayes (NSW DPI), Richard Simpson & Richard Culvenor (CSIRO) and Jim Virgona (Graminus Consulting) made presentations on various aspects of P efficient pastures project to attendees and the research plots inspected. Results from the RnD for Profit Phosphorus Efficient Pastures project were presented and plots inspected. In addition results from the participatory sites at Bigga and Blackburn were also presented and discussed	25 attendees	Richard Simpson and Rebecca Haling, CSIRO Jim Virgona and Graminus consulting P/L
2018	7-Dec-18	field day/farm walk	Richard Simpson discussed project results and management of sulphur at the Monaro Farming Systems soil club meeting. Doug Alcock (Grazprofit Consulting) and Jim Virgona (Graminus Consulting) also made presentations at this meeting.	approx. 50 attendees (FARMERS)	Richard Simpson and Rebecca Haling, CSIRO
2018	29-Aug-19	field day/farm walk	The research project was highlighted at Dowerin Field days in a display area shared by DPIRD and other research institutions. Daniel Kidd presented a research poster "Phosphorus use efficiency in dryland pastures" and had some serradella and subterranean clover plants on display. The display was visited by the WA Minister for Regional Development, Agriculture and Food.	>25,000 visitors to field day	Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2018		field day/farm walk	Discussion of the experiment progress and developments occurred at events including the 2018 & 2019 AGMs and field days	ASHEEP members and field day participants	ASHEEP
2019	Feb-19	field day/farm walk	Results from the experiments and an overall rundown of the project was highlighted in talks given by the Jim Virgona at a Tarcutta Landcare Group meeting	20 Attendees	Jim Virgona Graminus Consulting Pty Ltd.
2019	28-Feb-19	field day/farm walk	Results from the project were provided to producers and advisors at presentations at a nitrogen fixation workshop (28th February) organised by the South West Catchment Council, Toolbrunup with Southern Dirt group and at a personal development day of the ConsultAg consultancy group		Brad Nutt and Murdoch University
2019	Mar-19	field day/farm walk	A snapshot report of progress in the project was communicated to the Southern Dirt farm group by Emma Russell		Bronwyn Copestake, Southern Dirt
2019	Mar-19	field day/farm walk	Results from the experiments and an overall rundown of the project was highlighted in talks given by the Jim Virgona at a Kyeamba Landcare Group meeting	15 Attendees	Jim Virgona Graminus Consulting Pty Ltd.
2019	3-Apr-19	field day/farm walk	Richard Hayes presented "Sowing new pastures" at a Local Land Services Drought recovery workshop in Tarcutta	20 attendees	Richard Hayes, Suzanne Boschma, NSW DPI
2019	16-Apr-19	field day/farm walk	Updates on progress of the project were provided at meetings of the TFS Soils Club	~40 farm businesses	Jim Virgona Graminus Consulting Pty Ltd.

2019	26-Jun-19	field day/farm walk	The Central Ranges GSSA branch held a farm walk/dinner meeting where a local P4P project update was given.	25 attendees	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2019	22-Aug-19	field day/farm walk	A group of undergraduate students studying agricultural science at UWA attended a field day at the UWA Shenton Park research station. Daniel Kidd spoke to the students regarding P-efficient pastures and the environmental implications of fertiliser overuse. He emphasised the role that P-efficient plants such as serradella can play in improving the profitability and sustainability of future mixed farming systems.		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019	Sep-19	field day/farm walk	The northern NSW project team participated in a field day conducted at the Purlewaugh site. Highlights of the experiment were presented and a visit to the research station occurred.		Richard Hayes, Suzanne Boschma, NSW DPI
2019	Oct-19	field day/farm walk	A field day was held which included a plot inspection at the SIDN STH site and a presentation by Dr Brad Nutt, Murdoch University. An evaluation sheet was completed by those attending, which included the key question for the project.	approx. 40 attendees	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2019	23-Oct-19	field day/farm walk	Annual Legume Breeding Australia Launch. - University of Western Australia P-efficient Pastures team members, Megan Ryan, Daniel Kidd and Parwinder Kaur presented to invited delegates (researchers, students and industry) at the opening of the Annual Legume Breeding Australia (ALBA) joint venture between PGG Wrightsons and UWA. Parwinder discussed the molecular breeding techniques for the improved P efficiency of subterranean clover and Daniel provided a summary of major findings from the project and how they can feed into future annual legume breeding objectives.		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019	12-Nov-19	field day/farm walk	Bookham Ag Bureau and Tablelands Farming Systems (TFS) participated in Field Days held at the Merrill research site (managed by Richard Hayes) in which they were provided with updates and highlights on the experiment.	~25 attendees	Jim Virgona Graminus Consulting Pty Ltd.
2019	Dec-19	field day/farm walk	Completed research results were delivered to the MFS meeting highlighting the results from 2019.	approx. 60 producers	Doug Alcock and Graz Prophet consulting
2019	Dec-19	field day/farm walk	Completed research results were delivered at the 2019 MFS summer field day	55 attendees	Doug Alcock and Graz Prophet consulting
2019		field day/farm walk	Bigga (TFS) and Blackburn (BAB) site manager gave a series of talks to promote the progress and results of the project in 2019.		Jim Virgona and Graminus consulting P/L
Farm group newsletter articles					
2016	2-Jun-16	farmgroup newsletter	Following the visit and presentation by Richard Simpson (CSIRO) at the MLA/GSSA Pasture update field day, articles from the field day were featured in the GSSA newsletter.	circulation ~500	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland

2017	Mar-17	farmgroup newsletter	Project updates and progress was included in the Southern Dirt Autumn 2017 Quarterly Newsletter Project Report	circulation ~900	Emma Russell, Southern Dirt
2017	Apr-17	farmgroup newsletter	An article appeared in the GSSA newsletter relating to the presentation Lisa Warn gave at the MLA Pasture Updates run by the Albury-Wodonga branch of the Grasslands Society. The presentation included information on improving phosphorus use efficiency and the potential role of more P-efficient species like Serradellas.	circulation 500 people	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2017	Jun-17	farmgroup newsletter	New P fertiliser management experiments on the Monaro, Monaro Farmng Systems Newsletter June 2017, by Simpson R, Stefanski A, Haling, R.	~73 farm businesses	Richard Simpson and Rebecca Haling, CSIRO
2017	Jun-17	farmgroup newsletter	Project updates and progress was included in the Southern Dirt Winter 2017 Quarterly Newsletter Project Report	circulation ~900	Emma Russell, Southern Dirt
2017	1-Jul-17	farmgroup newsletter	A newsletter article was distributed to all MFS members relating to new P Fertiliser management experiments on the Monaro.	71 MFS member farm businesses	Doug Alcock and Graz Prophet consulting
2017	Oct-17	farmgroup newsletter	A newsletter article was distributed to all MFS members containing a P Efficient pasture Experiments update.	71 MFS member farm businesses	Doug Alcock - Graz Prophet consulting
2017	Dec-17	farmgroup newsletter	An introduction to the project was included in the Southern Dirt summer 2017 Quarterly newsletter		Melissa Jardine, Southern dirt Emma Russell
2017		farmgroup newsletter	An introduction to the project was included in the Southern Dirt Spring 2017 Quarterly newsletter	circulation ~900	Melissa Jardine, Southern dirt Emma Russell
2018	Apr-18	farmgroup newsletter	A report was included in the Southern Dirt Annual experiment booklet, an annual newsletter to members highlighting activities and projects they are involved in.	circulation ~900	Emma Russell, Southern Dirt
2018	May-18	farmgroup newsletter	ASHEEP Newsletter Article published May 2018 "Phosphorus-efficient pastures Project" newsletter #48	~160 recipients (92 farm businesses)	ASHEEP
2018	Jun-18	farmgroup newsletter	A newsletter article was published with the most up to date observations in the MFS June 2018 newsletter which was distributed to the entire membership base.	71 farm businesses	Doug Alcock and Graz Prophet consulting
2018	Jun-18	farmgroup newsletter	An article (written by Lisa Warn with assistance from Richard Simpson) creating awareness about the *Phosphorus efficient Pastures* project appeared in the GSSA newsletter. The article included information on strategies to improve phosphorus use efficiency, the potential role of more P-efficient species like Serradellas, findings from the root morphology work and an update on the local Central Ranges sites. "THE QUEST FOR MORE PHOSPHORUS EFFICIENT PASTURES"	Circulation 500	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2018	Jul-18	farmgroup newsletter	Monaro Farming Systems, July 2018 Newsletter article http://www.monarofarmingsystems.com.au/wp-content/uploads/2018/07/MFS-newsletter-June-18.pdf	73 farm businesses	Richard Simpson and Rebecca Haling, CSIRO

2018	Aug-18	farmgroup newsletter	ASHEEP newsletter article published August 2018. "ASHEEP projects update Phosphorus efficient pastures project" newsletter #50	~160 recipients (92 farm businesses)	ASHEEP
2018		farmgroup newsletter	ASHEEP published updates in its quarterly newsletter that is distributed to members, highlighting experiment progress.	~160 recipents (92 farm businesses)	ASHEEP
2018		farmgroup newsletter	Richard Hayes contributed a section to the MFS Newsletter which described the relative performance of varieties in the evaluation network, and highlighted the promising regeneration characteristics of cultivars such as Yellotas.	~73 farm businesses	Richard Hayes, Suzanne Boschma, NSW DPI
2018		farmgroup newsletter	an article appeared in the GSSA newsletter relating to the presentation Lisa Warn gave at the MLA Pasture Updates run by the Central Ranges branch of the Grasslands Society. The presentation included information on improving phosphorus use efficiency and the potential role of more P-efficient species like Serradellas.	circulation 500 people	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2019	May-19	farmgroup newsletter	An MFS newsletter article was distributed to members containing "P -efficient pasture experiments" updates.	distributed to the MFS membership (73 farm businesses)	ASHEEP
2019	Nov-19	farmgroup newsletter	ASHEEP newsletter article published November 2019 "ASHEEP P efficient pastures project update" by Inaya Stone	~160 recipients (92 farm businesses)	ASHEEP Inaya Stone
2019		farmgroup newsletter	A experiment update was included in the Southern Dirt spring field day booklet	circulation ~900	Melissa Jardine, Southern dirt
2020	Jan-20	farmgroup newsletter	Lisa Warn wrote an article about the field day and presentations at SIDN STH site for the GSSA's February 2020 newsletter. "GETTING PRODUCTIVE PASTURE IN CENTRAL VICTORIA"	circulation ~500	Lisa Warn, Ag consulting P/L and Central Ranges branch of Grassland Soc.
2020	Feb-20	farmgroup newsletter	ASHEEP newsletter article published February 202 "serradellas and the development on more P-efficient pasture systems" by Richard Simpson	~160 recipents (92 farm businesses)	ASHEEP Sarah Brown
Industry consultation					
2017		industry consultation/ influence	Bob Freebairn conducted property visits and took phone calls throughout the year in the northern NSW farm group areas where the research was discussed.	approx. 100	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2018	26-Mar-18	industry consultation/ influence	Fertiliser management advice provided to Holmes Sackett Pty Ltd, Wagga Wagga by Richard Simpson.		Richard Simpson and Rebecca Haling, CSIRO
2018	Sep-18	industry consultation/ influence	September – December 2018: Richard Simpson reviewed, and provided commentary and data analysis ideas for a paddock farmer-instigated soil fertility mapping study conducted by Tim Prance (SA).		Richard Simpson and Rebecca Haling, CSIRO
2018	early spring	CSIRO research station visit	David Hawkey, Tom Dickson and Ross Palmer (Heritage Seeds) visited the Ginninderra Experiment Station. The visit included a walk and briefing at the serradella flowering time experiment.		Richard Simpson and Rebecca Haling, CSIRO

2019	24-Jan-19	industry consultation/ influence	Richard Simpson (CSIRO) assisted Mr Lee Menhenett (INCITEC PIVOT) to prepare an article entitled: “Time for a new approach to pasture soil testing” in which the fertiliser/soil testing company adopted and promoted "Five Easy Steps" and project work on monitoring soil P levels with more frequent soil testing to capture P efficiencies on farms.		Richard Simpson and Rebecca Haling, CSIRO
2019	Feb-19	industry consultation/ influence	Meeting held with Tasglobal Seeds, Tas. (Robert Dent and Eric Hall) concerning results of several serradellas experiments and negotiating access to a new cultivar presenting in development and showing promise in the P efficient pasture research.		Richard Simpson and Rebecca Haling, CSIRO
2019	5-Jun-19	industry consultation/ influence	Richard Simpson and Rebecca Haling provided protocols to Gavin Peck (QDPI) for P-response pot experiments that mimic field pasture conditions and return reliable indications of P efficiency among pasture varieties.		Richard Simpson and Rebecca Haling, CSIRO
2019		industry consultation/ influence	Normal property consultations and enquiries cover P4P issues.	several hundred	R. Freebairn, Boggabri grazing group
2019	17-Aug-19	Visit to AgKI, PIRSA and local farm	A farm visit on Kangaroo Island occurred to discuss fertiliser use and soil test interpretation. Further discussions with PIRSA advisor on the potential for use of serradella in pastures on the island.		Richard Simpson and Rebecca Haling, CSIRO
2020	1-Feb-20	industry consultation/ influence	Follow up meeting held with Tasglobal Seeds and local seed production company in Launceston area, Tas. (Robert Dent, Eric Hall, Dr Rowan Smiith (UTAS), Laura Goward (PhD scholar), Rebecca Haling, Richard Simpson) to discuss most recent results of serradellas experiments, negotiate further access to new serradella that has shown promise in the P efficient pasture research.		Richard Simpson and Rebecca Haling, CSIRO
Information disseminated to external farmer groups and rural industry					
2017	6-Apr-17	information dissemination to farmer groups	Lisa Warn presented at the MLA Pasture Updates run by the Albury-Wodonga branch of the Grasslands Society. The presentation included information on improving phosphorus use efficiency and the potential role of more P-efficient species like Serradellas.	60 attendees	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2017	1-Aug-17	information dissemination to farmer groups	Monthly email updates on the progress and highlighhts of the experiment were sent to Southern Dirt's members		Emma Russell, Southern Dirt
2018	20-Oct-17	information dissemination to farmer groups	Lisa Warn presented at the MLA Pasture Updates run by the Central Ranges branch of the Grasslands Society, The presentation included information on improving phosphorus use efficiency and the potential role of more P-efficient species like Serradellas. Other speakers on the day spoke on the decision criteria and economics of applying lime (Jim Shovelton, Meridian) and	approx. 40 attendees	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland

			potential for variable fertiliser rate technology and the associated paddock pH and nutrient mapping tools (Brendan Torpy Precision Ag). Precsion Ag mapped two paddocks of Gerard Ryan’s (branch president and P4P project collaborator) and presented the results at the Pasture Update.		
2018	12-Apr-18	information dissemination to farmer groups	Lisa Warn presented a project update at the Central Ranges branch meeting of the Grasslands Society.	approx. 25 attendees	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2018	5-Dec-18	information dissemination to farmer groups	Following the presentations made at the joint Tablelands Farming Systems/Bookham Ag Bureau field day held at the Gunning field site, field day handouts were provided to both farmers groups for distribution to members that were unable to attend the actual event.	circulation ~200 via TFS/BAB networks	Richard Hayes, Suzanne Boschma, NSW DPI
2018		information dissemination to farmer groups	Monthly email updates on the progress and highlights of the experiment were sent to Southern Dirt's members		Melissa Jardine, Southern dirt
2019	9-Apr-19	information dissemination to farmer groups	Webinar for NSW DPI on pasture management including P efficient pastures.	est. 200 agronomists and farmers	R. Freebairn, Boggabri grazing group
2019	Jul-19	information dissemination to farmer groups	Lisa Warn prepared a summary of the branch’s work on phosphorus efficiency and alternative legumes which was printed in the GSSA 60th annual conference proceedings.	~500 members	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2019	Jul-19	information dissemination to farmer groups	Results from the experiments and an overall rundown of the project was highlighted in talks given by the Jim Virgona at a Grasslands Society of Southern Australia meeting	250 Attendees/members	Jim Virgona Graminus Consulting Pty Ltd.
2019	Jul-19	information dissemination to farmer groups	Results from the experiments and an overall rundown of the project was highlighted in talks given by the Jim Virgona at a Graham Centre Livestock Forum meeting	100 Attendees	Jim Virgona Graminus Consulting Pty Ltd.
2019		information dissemination to farmer groups	Richard Simpson and Cameron Gourley: NSW Grasslands Society Newsletter Vol.34, No2: Phosphorus soil test benchmarks for productive legume-based pastures.	circulation 300 members	Richard Simpson and Cameron Gourley
2019		information dissemination	Richard Simpson and Phil Graham: NSW Grasslands Society Newsletter Vol.34, No3: Practical application of soil test P benchmarks for phosphorus.	circulation 300 members	Richard Simpson and Phil Graham

		to farmer groups			
2019		information dissemination to farmer groups	Richard Simpson, Richard Hayes and Rebecca Haling: NSW Grasslands Society Newsletter Vol.34, No4: Progress in research to develop a more P-efficient pasture system.	circulation 300 members	Richard Simpson, Richard Hayes and Rebecca Haling
Newspaper and popular press aricles					
2016	2-Jun-16	newspaper/ popular press	Following the visit and presentation by Richard Simpson (CSIRO) at the MLA/GSSA Pasture update feld day, articles from the field day were featured in the Weekly Times newspaper.	circulation 330,000; major rural paper.	Lisa Warn, Ag consulting P/L and central ranges branch of Grassland
2018	26-Apr-18	newspaper/ popular press	Articles have been published in the “Down to Earth” column in *The Land* newspaper covering aspects of P4P research, highlighting the progress of the research project	82,470 weekly readers in NSW; major rural weekly newspaper.	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2018	17-May-18	newspaper/ popular press	Articles have been published in the “Down to Earth” column in *The Land* newspaper covering aspects of P4P research, specifically in relation to progressive experiment results	82,470 weekly readers in NSW; major rural weekly newspaper.	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2018	13-Dec-18	newspaper/ popular press	Bob Freebairn featured the Canberra site of the national serradella flowering time experiment in an article for ‘The Land’ newspaper (Down to Earth column). Title: Refine phosphorus use	82,470 weekly readers in NSW; major rural weekly newspaper.	Richard Simpson and Rebecca Haling, CSIRO
2019	3-Jan-19	newspaper/ popular press	Articles have been published in the “Down to Earth” column in The Land newspaper relating to soil test data from experiments gives value to pasture decisions	82,470 weekly readers in NSW; major rural weekly newspaper.	R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2019	Jun-19	newspaper/ popular press	Richard Simpson prepared short article for “Fuzzy” column of the Canberra Times newspaper on ‘phosphorus and global food security’.	82,470 weekly readers in NSW; major rural weekly newspaper.	Richard Simpson and Rebecca Haling, CSIRO
2019	25-Jul-19	newspaper/ popular press	Articles have been published in the “Down to Earth” column in The Land newspaper, highlighting Research into serradella roots.	82,470 weekly readers in NSW; major rural weekly newspaper.	R. Freebairn, Boggabri grazing group
2019	15-Aug-19	newspaper/ popular press	Articles have been published in the “Down to Earth” column in The Land newspaper, "More P efficient pastures”	82,470 weekly readers in NSW; major rural weekly newspaper.	R. Freebairn, Boggabri grazing group
2019	26-Jun-19	newspaper/ popular press	Richard Simpson (26 June 2019) "Phosphorus; a finite resource essential for life, critical for agriculture and food security". Published in ECOS, issue 256		Richard Simpson
Scientific presentations					
2017	6-Sep-17	scientific presentation	Managing the phosphorus cycle in clover-based pasture for more effective use of P fertiliser inputs, by Simpson R, Haling R, Virgona J, Ferguson N (2017), has been submitted as an invited presentation to the Grassland Society of		Richard Simpson and Rebecca Haling, CSIRO

			Southern Australia Annual Conference, Nagambie, Victoria (6-7 September, 2017)		
2018	26-Feb-18	scientific presentation	A poster summarising the project outcomes and future directions was on display at the UWA booth for the duration of the GRBA Research updates conference in Perth		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2018	10-13 Sept 2018	scientific presentation	Invited presentation by Richard Simpson at the Phosphorus in Soils and Plants (PSP6) Conference, Leuven, Belgium: "Progress in development of a more P-efficient grassland system for southern Australia", by Simpson RJ, Hayes RC, Sandral GA, Haling RA, Stefanski A, Boschma SP, Newell MT, Ryan MH, Kidd DR, Nutt BJ.	International science meeting	Richard Simpson
2018	10-13 Sept 2018	scientific presentation	Presentation by Rebecca Haling at the Phosphorus in Soils and Plants (PSP6) Conference, Leuven, Belgium: "Foraging for better root traits: phosphorus acquisition efficiency in a critical pasture species" by Haling RE, Becquer A, Warren A, Stefanski A, McLachlan JW, Kidd DR, Ryan MH, Sandral GA, Hayes RC, Flavel RJ, Guppy CN, Simpson RJ.	International science meeting	Rebecca Haling
2018	29-Aug-19	scientific presentation	At the Dowerin field days, Daniel Kidd presented a research poster "Phosphorus use efficiency in dryland pastures" and had some serradella and subterranean clover plants on display.	>25,000 visitors to field day	Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2018		scientific presentation	McLachlan J, Haling R, Simpson R, Flavel R, Guppy C (2018) Variation in P-acquisition efficiency among *Trifolium subterraneum* genotypes and the role of root morphology traits. *Poster presentation to the International Society for Root Research Conference (ISRR-10), Israel*.		Richard Simpson and Rebecca Haling, CSIRO
2018		scientific presentation	McLachlan J, Haling R, Simpson R, Flavel R, Guppy C (2018) Variation in P-acquisition efficiency among *Trifolium subterraneum* genotypes and the role of root morphology traits. Abstract prepared for a presentation at the *10th Symposium for the International Society of Root Research*, Israel 8-12 July.		Richard Simpson and Rebecca Haling, CSIRO
2019	5-Mar-18	scientific presentation	Richard Hayes presented a talk entitled "The Pasture:Soil interface" at an MLA Pasture Update located in Crookwell, NSW.	80 attendees	Richard Hayes, Suzanne Boschma, NSW DPI
2019	Feb-19	scientific presentation	Richard Simpson presented invited paper to the Australian Grasslands Association meeting, Launceston, on behalf of the team (RJ Simpson, RC Hayes, GA Sandral, RE Haling, A Stefanski, SP Boschma, MT Newell, MH Ryan, DR Kidd, BJ Nutt, JG Howieson, JW McLachlan, CN Guppy, RJ Flavel, JM Virgona and NJ Ferguson), entitled: "Improving the P- efficiency of grassland production in southern Australia"		Richard Simpson and Rebecca Haling, CSIRO

2019	12-Feb-19	scientific presentation	Australian Grasslands Association symposium, Launceston, Tasmania. The symposium was attended by Daniel Kidd (UWA), Richard Simpson (CSIRO) and Richard Hayes (NSW DPI) and the project outcomes were communicated to pasture researchers and representatives from MLA. Daniel Kidd submitted an abstract entitled "Physiological tolerance or physical avoidance? How some Australian forage legumes tolerate aluminium" describing the outcomes of the aluminium tolerance studies among serradella. Daniel also gave a PowerPoint presentation at the symposium on this topic.		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019	12-Mar-19	scientific presentation	The project was highlighted during a scientific presentation held by the Western Australian Livestock Research Council (WALRC) at Donnybrook. Daniel Kidd presented a brief overview of the project to numerous farmers, researchers and members of industry. An informative Q&A discussion session was set up by a Western Australian livestock research institution that enabled producers to feed into the MLA process the key issues that need to be researched and areas where attention and extension could be beneficial.	approx. 85 attendees	Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019	5-Jul-19	scientific presentation	Rhizosphere Conference, Saskatoon Canada, Funded by an AW Howard travel scholarship, Daniel presented a poster at the Rhizosphere conference on his liming field study with serradella. He reported on the changes in root exudation from forage and crop species in response to low pH and nutrient deficiencies and what impact liming has on root exudates. This work is primarily part of Daniels PhD but does highlight a potentially important mechanism for the tolerance of serradella to aluminium toxic soils types.		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019	14-Jul-19	scientific presentation	Following on from the Rhizosphere conference in Saskatoon, Daniel Kidd (UWA) also accepted an invitation to speak to researchers at the University of New Hampshire to discuss P-efficient legumes. Associate Professor Richard Smith was very interested in our extensive pasture legume options developed in Australia as they rely primarily on red and white clover in their organic forage systems. Both of these are relatively high fertility species, something we had discovered in this phosphorus efficiency project. As nutrient inputs are often low in an organic system it was obvious that they were not always able to meet the nutritional demands of these species. They were therefore interested in exploring more nutrient-efficient species that maintain yield with fewer inputs. In our system, this option would be serradella, but species such as birdsfoot trefoil and other clover types may be well suited to their environment. Daniel presented to Richard’s research group on the outcomes of the P efficiency project and visited their field experiments, which helped in the communication of the important findings of our work to an international audience.		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019	Aug-19	scientific presentation	Richard Simpson presented paper to the Australian Agronomy Conference, Wagga Wagga, on behalf of the team (Suzanne Boschma, Daniel Kidd,		Richard Simpson and Rebecca Haling, CSIRO

			Matthew Newell, Adam Stefanski, Rebecca Haling, Richard Hayes, Megan Ryan, Richard Simpson), entitled: “Flowering time responses of serradella cultivars”		
2019	Aug-19	scientific presentation	Richard Simpson presented on Kangaroo Island (SA) at the AgKI Conference on the topic of “Three things that ensure effective use of phosphorus fertiliser for pasture production”.	150+ farmers and consultants	Richard Simpson and Rebecca Haling, CSIRO
2019	25-Aug-19	scientific presentation	Australian Agronomy conference, Wagga Wagga, NSW. Daniel Kidd presented a conference paper “Liming changes more than the pH – A field study on wheat and pasture species” and presented at the conference. This work is part of Daniel’s PhD. However it has some excellent synergies with the controlled environment work he completed as part of the RRD4P project. Daniel Kidd and Megan Ryan also contributed to another project related conference paper on the serradella phenology experiment "Flowering time responses of serradella cultivars"	300+	Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
Scientific publications					
2017	Dec-17	scientific publication	Norton RM, Simpson RJ (2017) A five step approach to P use on pastures in Australia, *Better Crops with Plant Food*, publication on the web in December 2017; BC is an on-line fertiliser industry publication (International Plant Nutrition Institute) with a global circulation.	circulation 17, 000	Richard Simpson and Rebecca Haling, CSIRO
2017	24-Sep-17	scientific publication	Becquer, A., Haling, R., Stefanski, A., Richardson, A. and Simpson, R. (2017). Complementary phosphorus aquisition strategies of interplanted subterranean clover and white lupin increase sward yield in a low phosphorus soil. In: Doing More with Less. GJ O'Leary, RD Armstrong and L Hafner Eds. Proceedings of the 18th Australian Agronomy Conference, 24-28 September 2017, Ballarat, Vic, Australia (http://www.agronomyaustraliaproceedings.org/).		Richard Simpson and Rebecca Haling, CSIRO
2017		scientific publication	Haling RE, Brown LK, Stefanski A, Kidd DR, Ryan MH, Sandral GA, Nichols PGH, George TS, Lambers H, Simpson RJ (2017). Prospects for more phosphorus efficient subterranean clover. In: Doing More with Less. GJ O'Leary, RD Armstrong and L Hafner Eds. Proceedings of the 18th Australian Agronomy Conference, 24-28 September 2017, Ballarat, Vic, Australia (http://www.agronomyaustraliaproceedings.org/).		Richard Simpson, CSIRO
2017		scientific publication	Improving productivity of phosphorus deficient soils: nutrient foraging in pasture legumes *by* Warren A (2017) *with* Haling RE, Becquer A, Simpson RJ (supervisors) was completed as an internal report for the CSIRO Summer Studentship Program. This report covered details of the assessment of root traits that can deliver more P-efficient cultivars of subterranean clover.		Richard Simpson, CSIRO

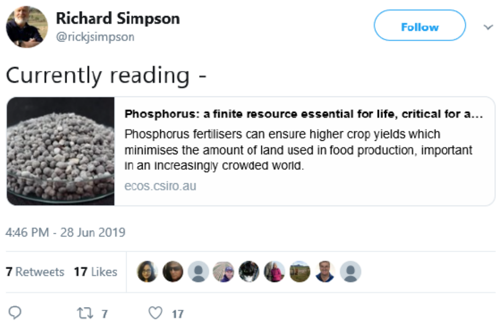
2019	Dec-19	scientific publication	D. R. Kidd, C. E. Di Bella, L. Kotula, T. D. Colmer, M. H. Ryan and G. G Striker (2019) Defining the waterlogging tolerance of *Ornithopus* spp. for the temperate pasture zone of southern Australia. *Crop and Pasture Science* doi.org/10.1071/CP19491		Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2019		scientific publication	McLachlan JW, Haling RE, Simpson RJ, Li, X., Flavel RJ, Guppy CN (2019). Variation in root morphology and P acquisition efficiency among *Trifolium subterraneum* genotypes. *Crop And Pasture Science*, *70*, 1015-1032.		Richard Simpson and Rebecca Haling, CSIRO
2019		scientific publication	Kidd DR, Ryan MH, Colmer TD, Simpson RJ (2019) The response of *Ornithopus* cultivars to aluminium and manganese in solution culture. Originally prepared for Crop and Pasture Science (now in revision pending further experiments in acid soil).		Richard Simpson and Rebecca Haling, CSIRO
2019		scientific publication	Boschma S, Kidd D, Newell M, Stefanski A, Haling R, Hayes R, Ryan M, Simpson R (2019) Flowering time responses of serradella cultivars. Proceedings of the 2019 Agronomy Australia Conference (http://agronomyaustraliaproceedings.org/images/sampledata/2019/2019ASA_Simpson _Richard_169.pdf).		Richard Simpson and Rebecca Haling, CSIRO
2019		scientific publication	Gourley CJP,Weaver DM, Simpson RJ, Aarons SR, Hannah MM, Peverill KI (2019) The development and application of pasture yield responses to phosphorus, potassium and sulphur fertiliser in Australia using meta-data analysis and derived soil-test calibration relationships. *Crop and Pasture Science* 70, 1065-1079.		Richard Simpson and Rebecca Haling, CSIRO
2019		scientific publication	Sandral, G., Price, A., Hildebrand, S., Fuller, C., Haling, R., & Stefanski, A. *et al.* (2019). Field benchmarking of the critical external phosphorus requirements of pasture legumes for southern Australia. *Crop And Pasture Science*, *70*, 1080-1096.		Richard Simpson and Rebecca Haling, CSIRO
2018		scientific publication	Haling RE, Brown LK, Stefanski A, Kidd DR, Ryan MH, Sandral GA, George TS, Lambers H, Simpson RJ. (2018) Differences in nutrient foraging among *Trifolium subterraneum* cultivars deliver improved P-acquisition efficiency. *Plant and Soil* 424, 539–554.		Richard Simpson and Rebecca Haling, CSIRO
2019		scientific publication	Brunton O (2019) Competitive interactions between serradella (Ornithopus spp) and subterranean clover (Trifolium subterraneum) in mixed pasture swards. Charles Sturt University (Hons Thesis)		Olivia Brunton
2020		scientific publication	McLachlan JW (2020) Root trait importance for phosphorus acquisition efficiency in Trifolium subterraneum. University of New England (PhD dissertation - under examination)		Jonathan McLachlan
2020		scientific publication	McLachlan JW, Haling RE, Simpson RJ, Flavel RJ, Guppy CN (2020) Root proliferation in response to phosphorus stress and space: implications for the study of root acclimation to low phosphorus supply and phosphorus acquisition efficiency. *Plant and Soil* (*in press*) doi: 10.1007/s11104-020-04535-y		Jonathan McLachlan *et al.*

2020		scientific publication	McLachlan JW, Flavel RJ, Guppy CN, Haling RE, Simpson RJ, (2020) Root proliferation and phosphorus acquisition in response to stratification of soil phosphorus by two contrasting Trifolium subterraneum cultivars. *Plant and Soil* (*in press*) doi: 10.1007/s11104-020-04558-5		Jonathan McLachlan *et al.*
2020		scientific publication	McLachlan JW, Becquer A, Haling RE, Simpson RJ, Flavel RJ, Guppy CN (2020) Intrinsic root morphology determines the phosphorus acquisition efficiency of five annual pasture legumes irrespective of mycorrhizal colonisation. *Functional Plant Biology* (under review following revision)		Jonathan McLachlan *et al.*
2020		scientific publication	Simpson RJ, Haling RE, Graham P (2020) Delivering improved phosphorus acquisition by root systems in pasture and arable crops. In. "Understanding and improving crop root function" (ed. Emeritus Prof Peter Gregory), Burleigh Dodds Science Publishing, London. (*in press*)		Richard Simpson and Rebecca Haling, CSIRO
Science networking					
2017	1-Mar-17	Researchgate	Scientific reports related to, underpinning, and arising from the project were promoted to a global scientific audience via Researchgate. The project profile was initiated in February 2017: https://www.researchgate.net/project/Phosphorus-efficient-pastures . By March 2017, it had 375 reads and a following of 92 scientists.	worldwide	Richard Simpson, CSIRO
2017	1-Aug-17	Researchgate	The P efficient pastures project had 643 reads and a following of 132 scientists	worldwide	Richard Simpson and Rebecca Haling, CSIRO
2018	1-Feb-18	Researchgate	The P efficient pastures project had had 1,027 reads and a following of 160 scientists	worldwide	Richard Simpson and Rebecca Haling, CSIRO
2018	1-Aug-18	Researchgate	The P efficient pastures project had 1,271 reads and a following of 179 scientists	worldwide	Richard Simpson and Rebecca Haling, CSIRO
2019	1-Feb-19	Researchgate	The P efficient pastures project had 1,558 reads and a following of 200 scientists	worldwide	Richard Simpson and Rebecca Haling, CSIRO
2019	1-Aug-19	Researchgate	The P efficient pastures project had 1,915 reads and a following of 221 scientists	worldwide	Richard Simpson and Rebecca Haling, CSIRO
2020	24-Apr-20	Researchgate	As of the 28th April 2020, the P efficient pastures project had 2,101 reads and a following of 233 scientists	worldwide	Richard Simpson and Rebecca Haling, CSIRO
Social media					

2018		social media	The experiment has been promoted via social media including various farmer groups websites, facebooks and twitter accounts throughout the course of the project. Twitter handle #P4Pastures		Melissa Jardine, Southern dirt
2017	7-Dec-17	social media	The project was launched on Twitter using the hashtag #P4Pastures during the training workshop for participatory research site leaders held in Canberra (7 December 2017). Information distributed by Twitter increased slowly as results from the projects become available. It was agreed among the participants that Twitter communications related to the project were only to be used for non-trivial" announcements. The initial objective was to promote communications nationally among the project's research and farm-group teams, but also to steadily attract a following from farmers outside of the project team.		Richard Simpson, CSIRO
2017		social media	Farmer groups in Northern NSW were involved tweeting progress updates and results in conjunction with other groups e.g. growth responses due to P treatments were tweeted in July of 2017 and pictures of deep soil coring at the Purlewaugh site on the 10th of November 2017.		R. Freebairn, S. P. Boschma, Boggabri grazing group, NSW DPI
2017		social media	ASHEEP and Jim Virgona were involved in tweeting project updates. In 2017, ASHEEP tweeted the experiment purpose and structure, and progress of the site was regularly tweeted e.g. the regeneration from the 2017-sown sites and the establishment of the new site (406 engagement to this tweet were recorded)	~160 recipents (92 farm businesses)	Jim Virgona and Graminus consulting P/L
2017		social media	Communication with external stakeholders is steadily increasing with several NSW DPI staff regularly active on Twitter and promoting the #P4Pastures 'brand' at appropriate opportunities. NSW DPI staff steadily built their social media networks to increase the impact of tweets later in the project as more results came to hand.		Richard Hayes, Suzanne Boschma, NSW DPI
2017		social media	Southern Dirt posted updates and photos of the project to both their Facebook and Twitter pages throughout the year e.g. a snapshot report of progress in the project was advised widely to increase awareness via twitter, and other site visits and activities by Southern Dirt were posted to both Facebook and Twitter regularly.		Melissa Jardine, Southern dirt
2017		social media	Twitter activity at this stage of the project was predominantly announcments reporting establishment and early pasture growth at participatory research sites (February 2017 to February 2018).		Richard Simpson and Rebecca Haling, CSIRO
2017		social media	Information on the experiment (experiment purpose and structure) was uploaded to ASHEEP website (https://www.ASHEEP.org.au/phosphorus-efficient-pastures) . Updates were posted regularly throughout the experiment period	~160 recipents (92 farm businesses)	(ASHEEP) Jim Virgona, Sarah Brown and Luke Marquis

2018	Mar-18	social media	Following the Redcliff field day (20 December 2017), a project page was published on the MFS website in March 2018, including reports and a project overview.	73 farm businesses	Doug Alcock and Graz Prophet consulting
2018		social media	Twitter posts were made consistenly by D. Kidd throughout the course of the project highlighting general info about the experiment, progress, results and presentations. Engagement and views of these tweets generally increased over time.	viewers approx. 718-6,063 at different times.	Daniel Kidd, Megan Ryan, and Parwinder Kaur, the university of WA
2018		social media	Twitter posts for this milestone period has included farmwalk announcements, insights into research activites in the project. (August 2017 to February 2018)		Richard Simpson and Rebecca Haling, CSIRO
2018		social media	Twitter activity since the last Milestone Report has included an announcement of the flowering time experiment with clear early vigour by a number of serradella cultivars (particularly French serradellas). (February 2018 to August 2018).		Richard Simpson and Rebecca Haling, CSIRO
2019		social media	Twitter continues to be used to announce new experiments, experimental results and communication of results. (February 2019 to August 2019).		Richard Simpson and Rebecca Haling, CSIRO
2019		social media	Activity since the last Milestone Report has included announcement of the experiment concerning acid soil tolerance by serradellas, field days and third-party reporting of seminars reporting results of P-efficiency experiments by Richard Simpson and Rebecca Haling. (August 2018 to February 2019).		Richard Simpson and Rebecca Haling, CSIRO
2020		social media	Central Ranges experiment site updates/photos and P4P information/photos were posted on Twitter on a regular basis.		Lisa Warn, Ag consulting P/L and central ranges branch of Grassland

Location key: ASHEEP (WA), Southern Dirt (WA), Boggabri Grazing Group (Northern NSW), Purlewaugh NSW Farmers (Northern NSW), Tablelands Farming Systems (Southern Tablelands NSW), Bookham Agricultural Bureau (Southern Tablelands NSW), Monaro Farming Systems (Monaro, NSW) and Central Ranges Grasslands Branch (Central Victoria)



Richard Simpson
@rickjsimpson
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Currently reading -

Phosphorus: a finite resource essential for life, critical for a…
Phosphorus fertilisers can ensure higher crop yields which minimises the amount of land used in food production, important in an increasingly crowded world.
ecos.csiro.au

4:46 PM - 28 Jun 2019

7 Retweets 17 Likes

7 17

SouthernDIRT @DirtSouthern · Mar 12
@DirtSouthern project officer Emma Russell gave a snapshot of local trials - Synergy & Profits using farm by products in mix farming @meatlivestock Phosphrous Efficient Pastures P4 #p4pastures & Increasing profits with dual purpose crops @meatlivestock

1 4

The Land @thelandnews · Sep 8
Set a soil fertility target and improve productivity

Set a soil fertility target and improve productivity
Research results to assist farmers with fertiliser decisions.
theland.com.au

3 5

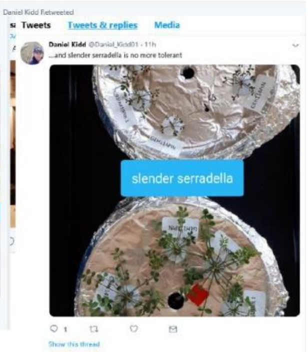
Rowan Smith @DrRWSmith · Jan 23
Some pastures knowledge amongst this group! Checking out some pod set in #serradella learnt so much this week - benefits of collaboration TIA/UTAS CSIRO @TasInAg @rickjsimpson @Laura_Goward @bethpenrose

1 13

Daniel Kidd Retweeted
Dr. Beth Penrose @bethpenrose · Mar 7

Daniel Kidd @Daniel_Kidd01 · 11h
What 2 weeks of waterlogging does to French and yellow serradella.
#P4Pastures #serradella #PhD @IOA_UWA

2 1 5

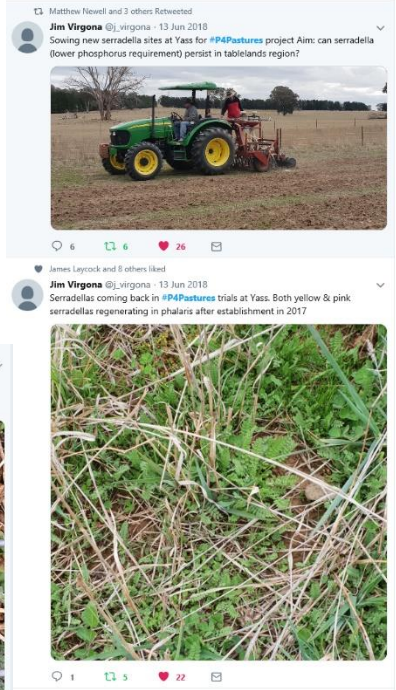


Daniel Kidd Retweeted
Tweets Tweets & replies Media

Daniel Kidd @Daniel_Kidd01 · 11h
...and slender serradella is no more tolerant

1

Show this thread



Matthew Newell and 3 others Retweeted
Jim Virgona @j_virgona · 13 Jun 2018
Sowing new serradella sites at Yass for #P4Pastures project Aim: can serradella (lower phosphorus requirement) persist in tablelands region?

6 6 26

James Laycock and 8 others liked
Jim Virgona @j_virgona · 13 Jun 2018
Serradellas coming back in #P4Pastures trials at Yass. Both yellow & pink serradellas regenerating in phalaris after establishment in 2017

1 5 22

Bradley John Nutt and 5 others Retweeted
Suzanne Boschma @SuzBoschma · 17 Jul 2017
Good response to different P rates in #P4Pastures producer group site @ Boggabri. Low=10kgP/ha

1 8 10

Lucinda Corrigan and 7 others liked
Jim Virgona @j_virgona · 22 Oct 2018
Serradellas hanging in at #P4Pastures site at Bigga Station - here is Avila sown 2017 in a stand with Holdfast GT phalaris

4 6 29

Neil Lewis Ballard and 2 others liked
Lisa Warn @LisaWarn2 · 1 Jun 2017
New #P4Pastures trial sown at Pastoria, Central Ranges Vic, evaluating serradellas vs subclover.

1 2 8

NSW DPI Agronomy and 4 others Retweeted
Richard Hayes @rickchayes · 30 Mar 2017
Lime applied to the Gunning #P4Pastures site aiming to test serradella rooting depth on acid soils

4 7 33

Nathan Ferguson Retweeted
Suzanne Boschma @SuzBoschma · 10 Nov 2017
Bob Freebairn & @NSWDPI boys soil coring #P4Pastures Participatory R&D site @ Purlewaugh to see where P located in leaky low PBI soil (19.3, 0-10 cm)

1 4 13

Rowan Smith and 6 others liked
Richard Hayes @rickchayes · 21 Jun 2018
Yellowtas yellow serradella showing promising year 2 regeneration across the network of #P4Pastures trials in southern NSW. A strong 2nd emergence of younger seedlings after recent ☔ thickens an already dense stand of seedlings from Feb rain - sites now open to grazing.

1 6 13

ASHEEP @ASHEEP_Inc · 6 Dec 2016

Stuart Kemp and 4 others liked

Lisa Warn @LisaWarn2 · 30 Apr 2019

#P4Pastures Sydney review meeting - more effective use of Phosphorus fertiliser in Aust pasture systems. #AWI #MLA

Daniel Kidd @Daniel_Kidd01 · 24 Sep 2018

Phenology experiment in W.A. comparing effect of sowing date on flowering time in most commercially available serradella cultivars with replicated experiments in Canberra, Cowra and Tamworth. #P4Pastures

Suzanne Boschma @SuzBoschma · 13 Aug 2017

Good P response @ Spring Ridge. Init soil Colwell P=10 mg/kg. Rates applied=0, 30, 60, 90kg/ha. Aim: calc rundown rate over 3 yr #P4Pastures

Lindsay Bell and 5 others liked

Richard Hayes @rickchayes · 4 Nov 2018

A challenging year for establishment of pasture legumes. Interestingly, the #P4Pastures serradella plots at this Yass site are looking ok compared to the clovers which are all-but absent. A promising result on this hostile granite soil.

Agrivet Business Consulting and 1 other Retweeted

Doug @Monaro_Sheepo · 24 Nov 2019

Avila Yellow Serradella is still battling on at the Bombala #P4Pastures trial in the face of one of the worst springs of the past 50 years. We need rain very soon!

Grassroots Agronomy and 4 others liked

Nathan Ferguson @nathgraminus · 4 Dec 2017

#P4Pastures field day at Bigga Serradella competing with Phalaris in the establishment year.

Nathan Ferguson @nathgraminus · 4 Dec 2018

#P4Pastures Serradella kicking goals with late spring rain sub clovers done for the year.

Daniel Kidd Retweeted

Nathan Ferguson @nathgraminus · 4 Dec 2018

#P4Pastures Field day at Merrel between Gunning & Crookwell. Comparing legume species, soil phosphorus and soil pH. Serradella kicking on in a tough spring.