Australian Grassland Association Inc.

SEEDS OF CHANGE
Hahndorf, South Australia
8-10th July 2025

Seeds of Change
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Program

Time	Author/Speaker	Title
Day 1 – Tuesday 8th July		
8:15-9:00	**REGISTRATION**	
	Session 1. Welcome and Setting the scene. Chair: Beth Penrose	
8:50-9:00	Opening Address – AGA President Rowan Smith	
9:00-9:20	Alan Humphries	Pasture breeding in South Australia
9:20-10:00	Various presenters	Pasture research in South Australia • Matthew Denton – University of Adelaide • James Webb – SARDI • David Peck – SARDI
10:00-10:30	**MORNING TEA**	
	Session 2. Beeding, cultivar development and evaluation. Chair: Jonathon McLachlan	
10:30-11.00	John Caradus	Benefits and risks associated with using genetic modification and gene editing to deliver traits of value in forages for Australian pastoral agriculture
11.00-11.30	Laura Goward	The potential for wider use of serradella in the pastures of southern Australia.
11.30-11.45	Rebecca Haling	Adaptation of novel germplasm and cultivars of serradella (*Ornithopus* spp.) to cold-climates in south-eastern Australia: flowering date and flowering date stability.
11:45-12:00	Richard Hayes	Failures in the Australian pasture seed market after 30 years of Plant Breeder's Rights.
12:00-12:30	**Student Competition Finalists. Chair: Jonathon McLachlan** • Haoran (Tony) Fan (University of Melbourne) • Angus Heslop (Lincoln University) • Neil Munday (NSW DPIRD)	
12:30-13:15	**LUNCH**	
13:15-13:55	**AgriFutures pasture research update** (10 minute presentations) • Meredith Mitchell – Overview of AgriFutures Pasture research • Daniele Giblot-Ducray - Monitoring Lucerne seed and parasitoid wasps to assess associated seed-loss and assist management • Scott Hutchings - Lucerne variety trial: assessment of optimum irrigation stress for seed production • Lilia Jenkins - Insecticide-resistant bluegreen aphids in southern legume crops and options for parasitoid biocontrol	
	Session 3. Pasture seed production, seed physiology and ecology. Chair: Cameron Allan	
13:55-14:10	Ruby Wiese	Swathing subterranean clover does not suck: a promising alternative to vacuum harvesting.
14:10-14:25	David Peck	Harvest and use of medic pods on-farm.
14:25-14:55	Phillip Nichols	Hard seed softening of diverse annual pasture legume cultivars in three contrasting environments of southern Australia.
14:55-15:10	Neil Munday	An initial investigation into the seed production and hard seed breakdown pattern of Trophy white clover.

	Focus on Pasture seed commercialisation. Chair: Janine Croser	
15:10-15:25	Richard Prusa	Challenges in pasture seed commercialisation
15:25-15:50	**AFTERNOON TEA**	
15:50-17:00	Industry Panel Facilitator: Janine Croser	Challenges and opportunities for pasture seed industry • John Caradus • Richard Prusa • David Peck • Meredith Mitchell • Mark Kester
17:00-17:05	**Housekeeping: Dinner venue, time etc**	
18:00-23:00	**Symposium Dinner - Adelaide Hills Convention Centre** **Guest Speakers – Richard Simpson and Cameron Allan**	

Day 2 – Wednesday 9th July			
Time	**Speaker**	**Driving time**	**Title**
7:45		20	Meet at bus at Adelaide Hills Convention Centre and depart for Waite
8:15 and 8:50	Bettina Berger, Yi Zhou		Australian Plant Phenomics Network (APPN). Robotic greenhouses, imaging of plants
8:15 and 8:50 (split group)	Alan Humphries, Nicholas Koch, Janine Croser		Tour of SARDI Plant Research Centre including: Australian Pastures Genebank, Speed breeding greenhouses & Seed Services
9:30		1.15	Morning tea on bus and depart Waite Institute for Marananga
11:00	Brett Nietschke, James Webb		Long Term Trials mixtures evaluation
12:00		15	Depart for lunch
12:15	Lunch		Chateau Yaldara
13:20		40	Depart for Eden Valley
14:00	James Webb		Eden Valley lucerne breeding field experiment
14:30		60	Depart for Palmer (inc Toilet stop at Palmer oval)
15:30	David Peck		Palmer low rainfall pastures trial
16:10	Alan Humphries, Tim Prance	60	Return to Hahndorf via Saltbush evaluation at Monarto and Amos Howard Memorial.
17:10			Arrival at Grunthal brewery.
18:00			Dinner at Grunthal brewery. **https://grunthal.com.au/**

Time	Author/Speaker	Title
Day 3 – Thursday 10th July		
8:45	**Opening and housekeeping**	
	Session 3. Pasture seed production, seed physiology and ecology (cont) Chair: Cameron Allan	
9:00-9:30	Rowan Smith	Improving seed germination performance of arrowleaf clover (*Trifolium vesiculosum* Savi.).
9:30-9:45	Ruby Wiese	Changes in harvest seed retention characteristics during maturation of subterranean clover (*Trifolium subterraneum* L.)
	Session 4. Pasture agronomy, nutrition and management. Chair: Phil Nichols	
9:45-10:15	Richard Hayes	Changes in pasture and soil properties with liming and superphosphate application over 12 years, on a range of soils in the Central Tablelands of New South Wales.
10:15-10:30	Tory Clarke	Rapid assessment of nutritional value and performance of pasture species in the field using chlorophyll fluorescence.
10:30-11:00	**MORNING TEA**	
11:00-11:30	Jonathan McLachlan	Root morphology and phosphorus requirements of tropical grasses and legumes
11:30-11:45	Clinton Revell	To Seed or to Plant? - Native Pasture Restoration in the Kimberley Rangelands, Western Australia.
11:45-12:00	Richard Hayes	Problems with the symbiotic competence of sainfoin (*Onobrychis viciifolia*) in Australia.
	Session 5. Current and future challenges. Chair: Clinton Revell	
12:00-12:30	Guangdi Li	Formulating pasture mixes with low CH_4 emission potentials – Grass/herb-annual legume mixes.
12:30-13:15	**LUNCH**	
13:15-13:45	Isabelle Kite	Evaluating the methane production and nutritional quality of pasture forages under current and future climate conditions.
13:45-14:15	Marja Simpson	Modelled potential distribution of C4 pasture species in current and future climates for Australia, with focus on southern Australia.
14:15-14:30	Beth Penrose	Elevated atmospheric carbon dioxide levels and seasonal rainfall change the mineral composition of temperate pasture grasses.
14:30-14:45	**Symposium wrap-up and close**	

Contents

Welcome Address

Hello and welcome everyone to the 7th Symposium of the Australian Grassland Association (AGA) here in Hahndorf, South Australia. This year's symposium is the first in South Australia and will focus on seed. South Australia is a key seed production state, with a strong focus on the breeding and production of lucerne and annual legumes. Now 13 years since the first in the series in Melbourne in 2012, previous symposiums have focussed on pasture legumes, perennial grasses, livestock productivity, soil constraints, and pasture resilience.

The AGA was established to facilitate the ongoing improvement and development of the pasture industry. The symposium series brings researchers, agronomists, advisors, and producers together to hear the latest in pasture research from leading pasture and forage researchers. Here we meet as equal, each bringing different perspectives on improving pastures and grazing industries.
To those advisors, agronomists and producers who are here, thank you for coming. You are at the front line of the challenges pastoral industries face but have the ability to influence and implement practice change when the value proposition and motivation collide. I ask you to challenge our scientists by asking questions of the research, exploring the implications, identifying barriers for adoption, and contributing solutions.

This symposium presents the opportunity to not only learn and expand our knowledge of grasslands, but also meet new contemporaries, build new networks, and reacquaint with old friends. You will hear from a number of impressive young scientists competing in the student competition. Each is a winner simply by being here, please ensure you take the time to introduce yourself and welcome them.
We are fortunate to welcome Richard Simpson (CSIRO) and Cameron Allan (ex MLA) who are in transition to retirement as dinner speakers. Richard Simpson has made a significant contribution in plant nutrition and pasture production, including studies across a range of pasture legumes. Cameron Allan influenced the agenda for pasture R,D&A across the feedbase program at MLA for many years. We thank them for their efforts, congratulate them on their achievements and look forward to hearing some of their career highlights.

I believe one of the great strengths of the symposium is that it encourages full paper submissions that are peer reviewed and published in Crop and Pasture Science. This ensures a high standard of presentations, and that valuable science is communicated across multiple mediums. I would like to congratulate guest editor Brendan Cullen and his team of reviewers on the publication of those papers.

It would be remiss not to mention the dry conditions experienced in many parts of southern Australia since the last symposium. While it's common for extended periods of moisture deficits in some regions, it was highly unusual for other regions and caused significant stress on some grazing businesses and families. A shout out to those who have been doing it tough.

I would like to thank our sponsors AgriFutures (Major sponsor), Meat and Livestock Australia and DLF Seeds (Session and Trade stall sponsors), and the AW Howard Trust (Student Travel Bursaries). These contributions are essential in keeping registration costs low and ensuring we have an enjoyable, yet professional event.

Finally, I would like to thank the committee. Treasurer Stuart Kemp has done a power of work behind the scenes on sponsorship and finances. Field tours have been organised by locals Alan Humphries and Richard Prusa, Carol Harris has again handled registration, Jono McLachlan the student competitions, Beth Penrose with auditing, while Cameron Allan, Clinton Revell, Kevin Foster, and Phil Nichols have also contributed as committee members.

We trust you will all have an enjoyable symposium.

Rowan Smith
President of the Australian Grassland Association

The Development and Commercialization of Pasture legumes in South Australia: Historical Perspectives and Genetic Resource Contributions

A. W. Humphries[A], C. Grant[B], N. R. Koch[A] and D. M. Peck[A]

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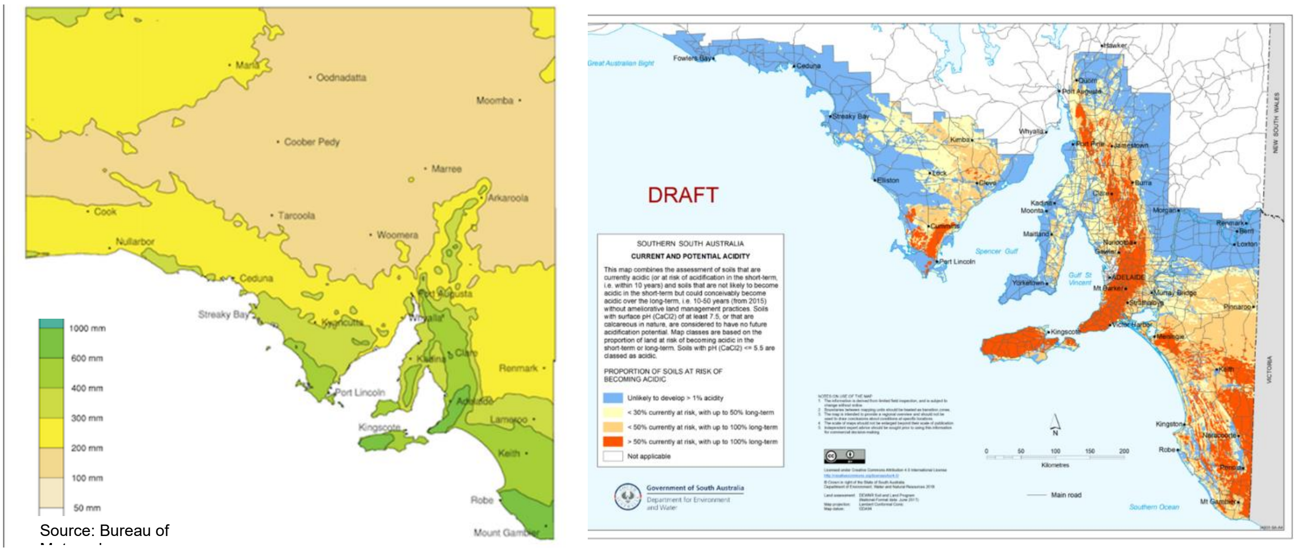
[B]Barenbrug Seeds Pty Ltd

Introduction

The pasture improvement and seed production industry in South Australia has underpinned sustainable agricultural development domestically and internationally for 125 years. The introduction and evaluation of forage species such as lucerne, annual medics, and subterranean clover have enabled sustainable livestock production, benefits to crop rotations and soil management in diverse climatic zones and transformed farming systems. This paper summarises the South Australian environment and key historical developments that have led to the modern-day activities of pasture breeding, commercialisation, seed production and exports.

The South Australian environment

Pasture production in South Australia is shaped by a diverse range of climatic and soil conditions. The southern agricultural region experiences a Mediterranean climate with annual average rainfall (AAR) between 450–850 mm. In contrast, northern and inland areas are semi-arid to arid, with rainfall below 300 mm are managed rangeland environments with limited sown agriculture (Figure 1). Soils vary from acidic sands and loams (pH 4.5–6.0) in higher rainfall zones to alkaline calcareous soils (pH 7.5–9.0) in low-rainfall regions. These conditions influence the selection of pasture species, with annual legumes such as medics (*Medicago* spp.) and vetch (*Vicia* spp.) dominating in low-medium rainfall environments rotated with cereal crops and a broader range of plants including subterranean clover, (*Trifolium subterraneum*), lucerne (*Medicago sativa*) and grasses such as ryegrass, (*Lolium spp.*), Phalaris (*Phalaris aquatica*) and cocksfoot (*Dactylis glomerata*) widely used in the medium to higher rainfall zones (above 500 mm AAR).



Source: Bureau of

Figure 1. **Average annual rainfall (mm) and soil pH of South Australia.**

History of pasture development and conservation in South Australia

Early introductions and discovery

Early introductions of exotic pasture legumes in South Australia were characterised by both deliberate actions and accidental naturalisation events. Lucerne was probably the first deliberately introduced legume, with reports in ‘The South Australian Newspaper’ in 1840 that the plant had “succeeded admirably” on early farms, especially in the dairying regions such as the Para Valley. The first reported discovery of a naturalised exotic legume occurred in 1889 when Amos William Howard recognised the potential value of subterranean clover growing along a creek near Mount Barker. Howard’s recognition of subterranean clover’s agronomic advantages, including its nitrogen-fixing capability, ability to regenerate without intervention and high feed value spurred his interest to promote the species. Howard overcame early technical challenges in harvesting the seed and soon began commercial sales, reaching up to one tonne annually by 1909. The acquisition of a clover huller from the United States in 1923 by Howard's son dramatically increased seed production capacity. By 1930, the subterranean clover industry contributed significantly to the Mount Barker economy, generating an estimated £50,000 annually (Gilbert, 1983). Amos Howards first subterranean clover cv. Mount Barker (stored in the Australian Pastures Genebank as accession APG 321) has been sown and subsequently naturalised across many areas of southern Australia over the last 100 years and is still present in today's pastures.

In 1941 history repeated itself when another farmer, Mr J.E Butler of Clare, requested C. A . Neal-Smith of the Waite Research Institute to formally identify a vigorous clover, which had been present on his farm for more than 20 years. The variety Clare was derived from this discovery and registered in South Australia in 1950-51, and was the first ssp. *brachycalycinum* cultivar. Clare was recommended for alkaline soils usually more suited to annual species of *Medicago* (Oram 1971).

Annual medics appear to have been introduced both accidentally and deliberately. Baron von Mueller introduced snail medic (*Medicago scutellata*) into Australia before turn of the 19th century, and it (eventually named cv. Robinson) was the original annual legume sown at Minnipa Research Centre from 1916-1921 (Oram 1971). Barrel medic (*Medicago truncatula*) is reported to have been naturalised in areas in South Australia, Victoria, and New South Wales before 1920. Following evaluation at the Waite Institute in 1937, Mr. Alf. Hannaford and Dr. H.C. Trumble selected an area on the property of Mr J Robinson of Noarlunga, which had a shorter spined barrel medic, for deliberate seed harvest and commercialisation of the variety "South Australian barrel medic", later renamed 'Hannaford'. Thus, there is an interesting connection between Mr J. Robinson of Noarlunga (now a seaside suburb of Adelaide) and the commercialisation of the first two medics in South Australia.

Pasture Genetic Resources in South Australia

Eric J. Crawford's career with the South Australian Department of Agriculture (1949–1989) was instrumental in advancing pasture cultivar development, particularly in the evaluation and introduction of annual legumes. In 1956, in collaboration with CSIRO scientists Dr. O. Frankel and Dr. A.R. Callaghan, Crawford initiated systematic pasture species evaluations at the Parafield Research Centre. His appointment as the first Plant Introduction Officer formalised his mandate to identify, introduce, and develop exotic pasture species for commercial use. The first report on Genetic Resources was published in 1964, marking the beginning of conservation of pasture genetic resources in South Australia.

Crawford conducted field evaluations of annual medics across South Australia, including at the Wanbi and Minnipa Research Centres. These evaluations led to the registration of several new medic varieties for South Australia, including Jemalong (collected and first registered in NSW), Harbinger and Paragosa (Oram 1971). Whilst these initial cultivar releases resulted from local collections of naturalised exotic legumes, Crawford and his colleagues soon recognised that further gains in productivity required targeted international collections to broaden the available diversity. International collections led to direct cultivar releases as well as important parents in controlled breeding programs.

International seed collection missions between 1967 and the early 1980s expanded South Australia's genetic resource base. Eric Crawford and and Murray Mathison led expeditions to Mediterranean and Middle Eastern countries — Israel, Cyprus, Turkey, Spain, Portugal, Iraq, Jordan, and Syria — acquiring diverse annual medics, clovers, and grasses (Smith *et al.* 2021). These collections were pivotal in establishing the Medicago Genetic Resource Centre, which by 1989 housed over 27,000 accessions and served both local breeding programs and global genetic conservation efforts. Cooperation with international organizations such as FAO, IBPGR, and ICARDA facilitated seed exchanges and fostered global evaluation programs, particularly for Mediterranean-type environments.

The Australian Medicago Genetic Resources Centre continued to grow under the curatorship of Geoff Auricht (~1985-1992) and Steve Hughes (1992-2017). Auricht and Hughes were passionate advocators of plant genetic resources and key proponents of the formation of an Australian Pastures Genebank, which eventually formed from the amalgamation of state-based collections in 2014 (Smith *et al.* 2021).

Today the Australian Pastures Genebank, held at SARDI in South Australia, is the custodian of the worlds most diverse and significant temperate and tropical pasture and forage collection. The APG currently holds over 85,000 accessions representing 2461 species, collected from 178 countries. Its ongoing maintenance is a commitment by the Australian Government under the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA). The APG conserves the diversity of Australia's pasture and forage species for use nationally and internationally as the basis for sustained and enhanced agricultural productivity and environmental preservation. In the last five years the APG has made its passport and trait observation data online at its GRIN-Global powered website and is partnering projects that are developing genomic data resources for *Medicago, Trifolium* and native grasses (Humphries 2024).

South Australian contributions to pasture legume cultivar development (1890 – present)
There have been at least 77 (non-exhaustive list) cultivars of annual medics, lucerne, clovers, vetch and other species commercialised from the South Australian public and private sector in the last 125 years (Figure 2). Pasture breeding activities initiated in the State Department of Agriculture (now PIRSA SARDI) in earlier years accelerated in response to the invasions of bluegreen (*Acyrthosiphon kondoi*) and spotted alfalfa (*Therioaphis trifolii*) in the late 1970s, which were devastating for both lucerne and annual medic production. In the last three decades plant breeding has continued to focus on production and adaptation, but other traits such as disease resistance, grazing tolerance, herbicide tolerance, flowering time and hardseededness have been integrated as a result of having a farming systems focus.

SARDI and Barenbrug have collaborated for 33 years to develop improved lucerne varieties for the Australian market (1992-2025) in what is the longest public-private partnership in Australia. South Australia has been early adopters of using molecular markers and speed breeding to develop cultivars more efficiently (Peck *et al.* 2015; Peck and Damin 2022).

Following the development of plant breeders' rights, two commercial companies, Pristine Forage Technologies and Springbrook Nominees, have also bred a range of pasture varieties for Australian and international markets.

The South Australian seed production industry and international exports
The Southeast region of South Australia represents a major centre for pasture seed production in Australia. Favourable climatic conditions, including moderate rainfall and temperate temperatures, combined with well-drained soils, and available irrigation water support the cultivation of a diverse range of pasture species. The region accounts for 83% of the country's lucerne seed production (Lucerne Australia n.d.). Annual medics and vetch are produced in the mid-north. South Australia contributes substantially to the national pasture seed supply, which totals approximately 30,000 metric tons annually, valued at AUD $210 million. Seed processing technologies employed in the region enhance seed quality and yield, supporting both domestic livestock industries and export markets.

The area of land sown for lucerne and other pasture certified seeds production has declined over the last 20 years (Figure 3, data source: Australian Seeds Authority Ltd).

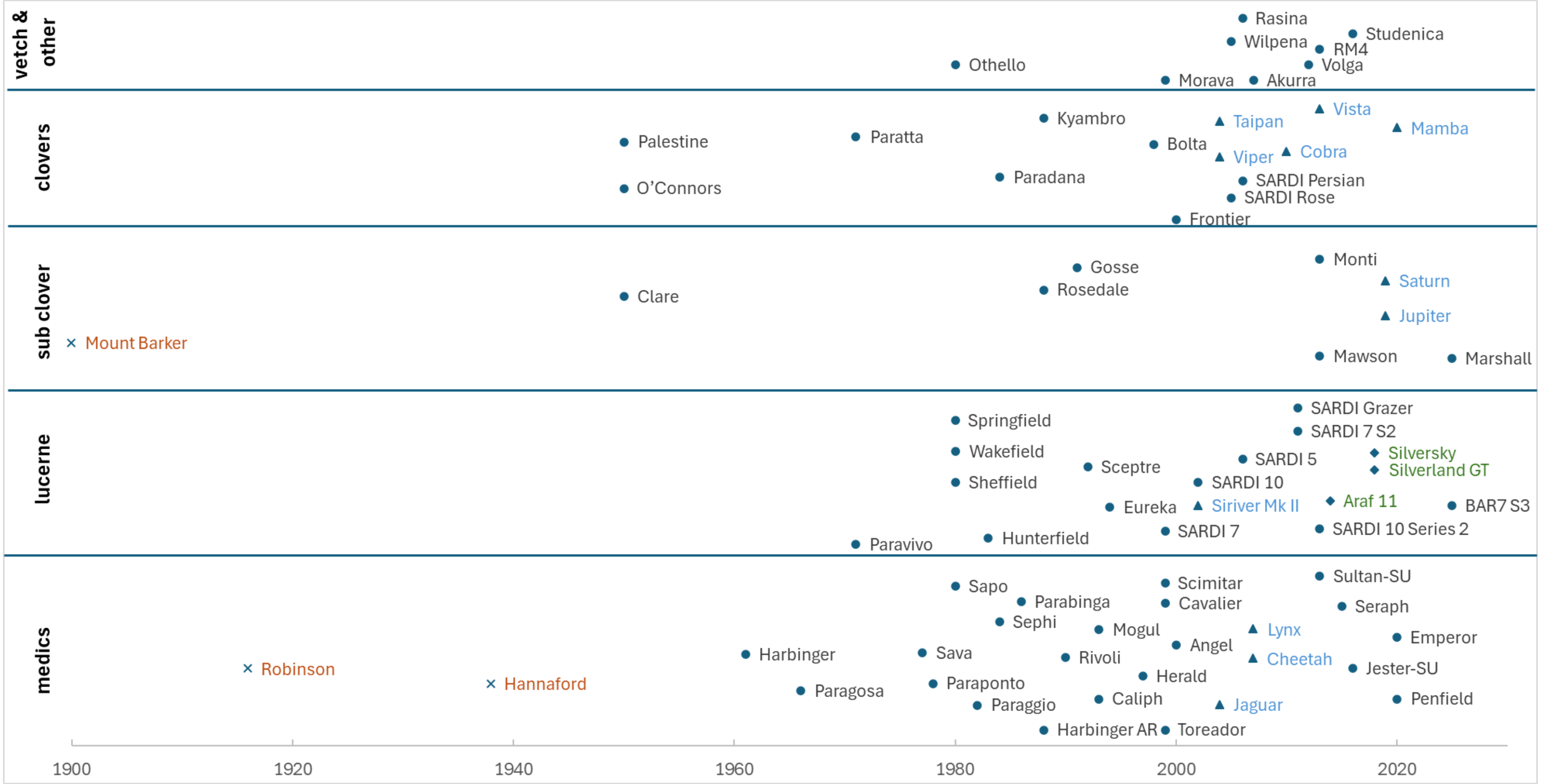


Figure 2. Timeline of South Australian pasture cultivar releases by species and organisation. Early private (×), SA Government (● now PIRSA SARDI), Pristine Forage Technologies Pty Ltd (◆) and Springbrook Nominees Pty Ltd (▲).

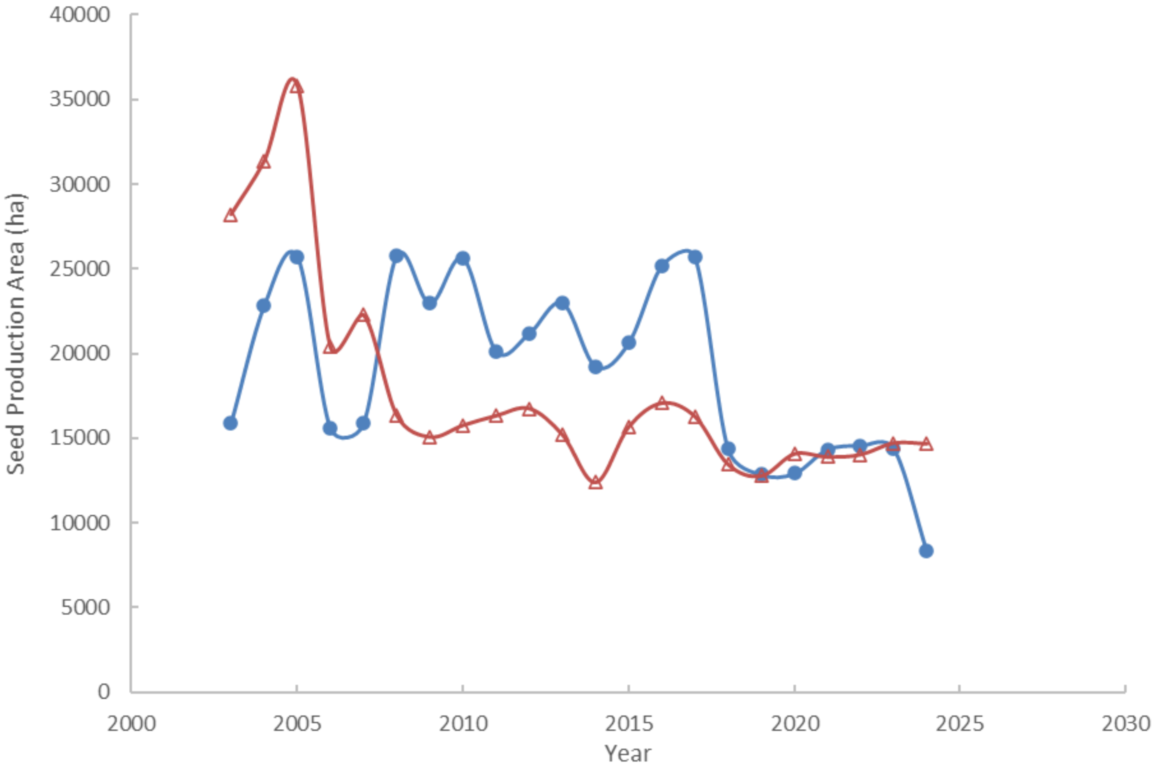


Figure 3. Pasture certified seeds production Area, 2003-2024 for lucerne (△) and all other pasture species (●). Certified statics compiled by Australian Seeds Authority Ltd from data provided by Seed Services Australia, AsureQuality Australia Ltd, AGWEST Plant Laboratories.

The instability of seed production area for lucerne has been associated with global market prices, the 2018 collapse in Middle East export market, and the worst drought on record over the last 24 months. Despite the relative collapse in the Middle East market (down from around 5,000 mt in 2017), this region remains one of Australia's three greatest seed production export markets, together with the USA and Argentina (Figure 4).

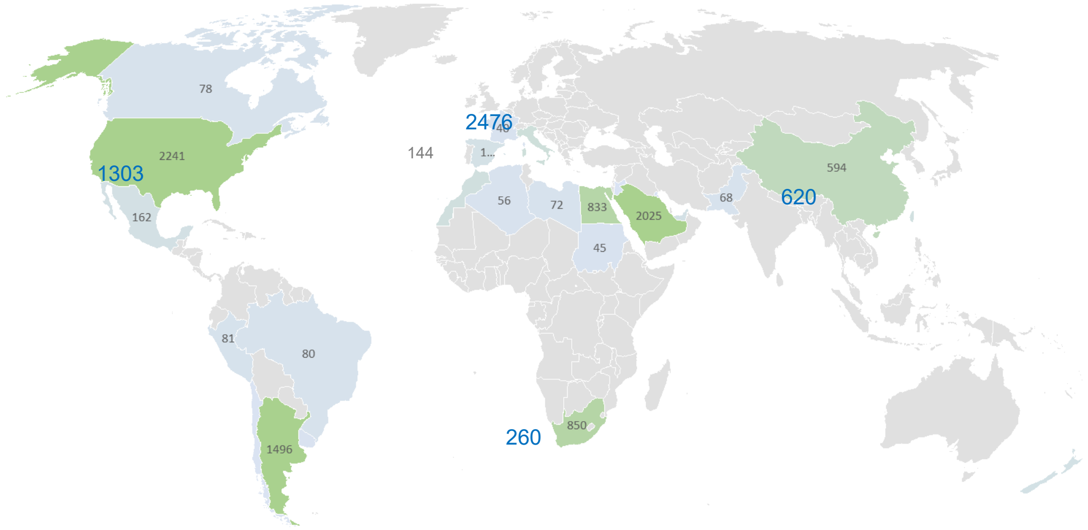


Figure 4. Australian lucerne (black font, 2023/2024) and clover (blue font 5-year total, 2019-2024). seed exports by country (source Colin Grant, Barenbrug, data downloaded from the Australian Bureau of Statistics).

Lucerne seed is exported from Australia to around 20 countries (with Chile, Greece and Kenya not shown in Figure 4) around the world, with adaptation to rainfed environments and tolerance to grazing widely recognised. In contrast, clover seed exports are dominated by only four environments, France, USA, South Africa and China.

Conclusion

The 125-year anniversary of South Australia's pasture improvement and seed production industry reflects a blend of pioneering innovation, applied research, and genetic stewardship. From early lucerne plantings and the introduction and discovery of subterranean clover and annual medics, agricultural scientists and farmers have collaborated to build resilient, productive farming systems.

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Notes:

Benefits, risks and challenges associated with using genetic modification and gene editing to deliver traits of value in forages for Australian pastoral agriculture

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Abstract: *Plant breeding is about expanding and exploiting the genetic potential of plants. In many crop species genetic modification (GM) has been a valuable option for delivering improved economic and environmental outcomes. However, while genetic modification has been extensively used for 30 years to advance trait expression in crops, including those used for animal feed such as maize and soybean, it has rarely been used in the development of commercialised cultivars of grazed grassland species. While genetically modified plants will not solve all challenges confronting managed grasslands systems, they have the potential to alleviate some, including those associated with environmental perturbations. The aim here is to review why the uptake of GM technologies as another means of providing solutions for the pastoral sector has been slow. Identification of traits that might have application and benefit in grassland systems for which genetic modification or gene editing will be required for their delivery, and regulatory concerns that need to be considered when adopting GM forage and pasture plants will also be discussed.*

Keywords: gene editing, pasture, plant breeding; transgenic

Introduction

Humankind has domesticated a range of wild plant species through plant breeding over the past 10,000 years (Hallauer 2011). Through simply using phenotypic selection and the creation of populations with desired plant traits some important crop plants today exhibit little resemblance to their original wild relatives. However, understanding the principles of inheritance (Stenseth *et al.* 2022) and the impacts of natural selection (Ospovat 1995) has only had short history in comparison. Modern plant breeding methods have included interspecific hybridisation, development of polyploids and double haploids, use of heterosis through crossing genetically diverse and often inbred populations, creation of mutations through use of chemicals and radiation, use of genetic and molecular markers for selection of desired traits, genomic selection, genotyping by sequencing, and then within the last 30 years genetic engineering/ modification and now targeted gene editing (Caradus 2024). Over time these methods have ensured a more efficient and accurate development of traits of value. However, it is these more recent methods involving genetic modification and gene editing that have been regulated to varying degrees in many jurisdictions to ensure that the resulting products have a demonstrated low level of risk to human health and the environment (Caradus 2023).

Genetic engineering or modification is defined here as the manipulation of an organism's genes by introducing, eliminating or rearranging specific genes using the methods of molecular biology particularly those techniques referred to as recombinant DNA techniques (USDA 2022). Gene editing differs from previous techniques in manipulating the plant genome by being more efficient, precise or targeted and more versatile (Songstad *et al.* 2017). The aim here is to address the benefits and risks of developing new forage cultivars using modern genetic modification and gene editing methods.

Genetically modified forage cultivars

To date only two genetically modified forage cultivars have been made available commercially. Both are cultivars of lucerne or alfalfa (*Medicago sativa*) which "holds a major place in cultivated grasslands worldwide …..for ruminant feeding, given its high yield and nutritional properties" (Ghaleb *et al.* 2021). The two cultivars are Roundup Ready™ alfalfa to aid weed management (Putman *et al.* 2013) and HarvExtra™ to provide low lignin feed to improve digestibility (Barros *et al.* 2019).

Reasons for the relatively low level of investment into genetically modified forage and pasture cultivars is possibly the result of:

1. **Cost and time associated with development.** Timeframes for development of genetically modified and gene edited cultivars can still be long and as a result costs can be considerable. Some of this is driven by the need to undertake tests to ensure safety of the resulting cultivar and identify any likely risks of the trait or the processes used to develop it. Genetic modification and gene editing has been most frequently used with in row crops (e.g. maize, soybean, canola, cotton (ISAAA 2022)) which have large market sizes that ensure a good return on the investment. Forage and pasture plants in comparison are generally of lower value and have comparatively small market sizes.
2. **Regulatory hurdles**. In most jurisdictions plants derived from genetic modification are regulated to ensure any risks associated with them are identified and quantified. For gene edited plants the extent of regulation can vary depending on jurisdiction (Caradus 2023). Costs of de-regulation in the U.S. associated with genetically modified plants can reach US$70m for a forage in the U.S. (Stephen Temple

pers. comm.), and from US$100m to US$150m per event for row crops like maize and soybean (Mark McCaslin pers. comm). Between 2008 and 2012 the estimated cost of discovery, development and authorisation of a new plant biotechnology trait was $136m, of which 23% was the cost of discovery, and 26% the cost of science for regulation and registration, and time from discovery to commercial launch was about 13 years (McDougall 2011). A study undertaken in the period from 2017 to 2022 estimated that the cost of discovery, development and authorisation of a new plant biotechnology-derived genetic trait commercialised was US$115.0m (AgbioInvestor 2022). These amounts include the cost of work to provide information on risk associated with the genetically modified event as required by the USDA (US Department of Agriculture), FDA (Food and Drug Administration) and EPA (Environmental Protection Agency) as well as tracking, reporting and stewardship of each and every planting of the de-regulated crop in the U.S. In New Zealand applications to the Environmental Protection Authority for genetically modified organisms cost NZ$27,500 (EPA 2025). Currently in Australia there is no cost for applying to the gene regulator for the intentional release of a genetically modified organism into the environment (OGTR 2023). A recent a survey of an international panel of plant biotechnology experts established that the cost of the development and commercial release of genome edited crops will depend (not unexpectedly) on whether the gene edited crop is regulated as a genetically modified crop or not (Lassoued *et al.* 2019). Comparing North America and Europe they estimated the of cost development through to commercial release if not regulated as genetically modified to be US$15m and US$5m, respectively, but of regulated as genetically modified to be US$33m and US$14m, respectively.

3. **Consumer concerns** have been related to safety of food and feeds derived from genetically modified plants, their impact on non-target organisms, development of resistant insect populations or increased weediness, negative impacts on biodiversity, transgene escape into wild or non-GM populations, negative impacts on rhizosphere microorganism, and/ or gene transfer to consumers from GM food and feed. However, after 25 years of growing and consuming genetically modified crops these risks associated with the use of GM crops have proven to be low to non-existent (Vega Rodríguez *et al.* 2022). Additionally, since the first genetically modified crops entered the food chain there have been no proven recorded cases of health-related issues (Panchin and Tuzhikov 2017; Abdul Aziz *et al.* 2022).
4. **Real or perceived co-existence challenges**. Coexistence of genetically modified crops with other crops is a significant area of contention, and particularly for perennial outcrossing plants. While co-existence has been and is possible it is reliant on agronomic strategies such as planting times, crop placement, separation distances and physical containment to stop pollen dispersal and seed movement, which could be assisted by using biological/molecular containment through genetic manipulation to disrupt the pollination and fertilisation process (Caradus 2025).
5. **Lifecycle effects.** Forage and pasture plant species tend often to be perennial and outcrossing while many row crops are annuals and some are inbred or have low outcrossing tendencies. Some important traits for forages and pasture plants such as persistence only manifest after several years and so timeframes for monitoring can be extensive resulting in delayed development of the final product.

Benefits associated with using genetic modification and gene editing

The benefits of new cultivars no matter how they are developed will be related to the challenges and opportunities facing the primary sector segment that those plants will be entering. For Australia the primary challenges facing the farming sector include, economic conditions and costs, weather, staffing, government policy, financial viability, climate change, natural disasters and biosecurity (Statista 2024). Plant breeding may provide solutions for situations caused by climate change and biosecurity breaches. Managing climate change will require both technologies that mitigate the effects of climate change and/or improve plant adaptation to climate change. Principal biosecurity issues will likely result in increased pest and pathogen pressure.

Examples of plant traits that might alleviate some of these primary sector challenges and could be delivered preferentially through genetic modification and gene editing are listed in Table 1. These examples indicate that many plant traits of value that will contribute to providing benefit to pastoral agriculture in Australia could be delivered through using either genetic modification or gene editing. Their ability to be used will depend on the regulatory regime imposed and level of risk determined to be associated with each of these traits and the genes involved.

Table 1. Examples of plant traits of value for Australian pasture and forage plants that might be delivered preferentially through genetic modification and gene editing.

Plant Trait	Benefit and species	Genes involved	Reference
		Genetic modification	
Condensed tannin expression	Reduced methane and nitrous oxide emissions – white clover	Insertion of transcription factor TaMYB14-1	Caradus *et al.* 2022; Roldan *et al.* 2022
High metabolisable energy	Reduced methane and nitrous oxide emissions – perennial ryegrass	Insertion of two genes - DGAT an oil synthesising enzyme, and CysOle which encapsulates and protects lipid droplets. DGAT - Diacylglycerol O-acyltransferase CysOle - Cysteine oleosin	Winichayakul *et al.* 2020; Beechey-Gradwell *et al.* 2022
Drought tolerance	Improved water use efficiency - maize	Expresses the cold shock protein B (CSPB) from *Bacillus subtilis* to maintain cellular functions under stress conditions	Chang *et al.* 2014; ISAAA 2016
Disease resistance	Alfalfa mosaic virus (AMV) resistance – white clover	Expressing the viral coat protein gene encoded by the sub-genomic RNA4 of AMV	Panter *et al.* 2012
	White clover mosaic virus (WClMV) resistance – white clover	Expression of a mutated form of the coat protein gene of WClMV	Voisey *et al.* 2001
Pest resistance	Control of porina (*Wiseana* spp.)	Cry1B gene from Bt*	Voisey *et al.* 1994
	30 pests in a range of crops	Bt* genes	Blanco *et al.* 2016
	Control of European maize borer - maize	Bt* gene	Hutchison *et al.* 2010
	Control of maize rootworms (*Diabrotica* spp.) - maize	Bt* gene	Pellegrino *et al.* 2018
		Gene editing	
Increased lipid content	Increased seed oil content and fatty acid compositional changes in oilseed species – *Camelina sativa* [high lipid linked to methane emission reduction - Grainger and Beauchemin 2011]	Knockout of gene FAD2 – SDN1	Jiang *et al.* 2017; Subedi *et al.* 2020
Dought tolerence	Improved ability to withstand water-deficit - lucerne	Small insertions/ deletions (indels) with gene MsSPL8– SDN1	Singer *et al.* 2022
	Drought stress tolerance - maize	Novel allelic variation through insertion of a native maize promoter GOS2 – SDN3	Shi *et al.* 2017
Disease resistance	Powdery mildew resistance (*Blumeria graminis* f. sp. *tritici*) - Wheat	Gene deletion – TaEDR1- enhanced disease resistance1 - SDN1	Zhang *et al.* 2017
	Bacterial blight resistance *Xanthomonas oryzae* pv. *oryzae* - rice	Gene insertion – OsSWEET13 sucrose transporter gene - SDN1	Zhou *et al.* 2015
Pest resistance	Tryptamine converted to serotonin resulting in reduced growth in plant hoppers and stem borers - rice	Small insertion (indel) – CYP71A1 gene encoding tryptamine 5- hydroxylase - SDN1?	Lu *et al.* 2018

* Bt - *Bacillus thuringiensis*

Concluding comment

While genetic modification and gene editing have been rarely commercialised in forage and pasture species research studies do indicate that, if there is willingness by regulators to approve resulting technologies, there are significant potential opportunities to deliver traits of value using these techniques. However, for this to be effectively achieved the following will be required:

1. Costs of development and regulation need to be proportionate to the value of the resulting pasture or forage crop.
2. Concerns about co-existence of traits developed through genetic modification and gene editing need to be effectively managed at all stages of the plant's life cycle and any resulting food or feed products.
3. Benefits need to be quantified, and any perceived or real risks need to be understood and managed to allay societal concerns.
4. Regulatory processes need to be trusted and clearly shown to deliver evidence-based risk assessments. Costs associated with obtaining regulatory approval need to be affordable to both small startup companies, public institutions, as well as multinationals.

Genetically modified crops have provided considerable benefits to farmers, consumers, and the environment, and have the potential to solve current challenges and as a result improve not simply economic outcomes but also the environment. Like any new technology genetic modification can bring risks, but these can be monitored and quantified and allow decision to be made about commercial, societal and environmental benefits versus real risks (Caradus 2022).

While genetically modified plants will not solve all challenges confronting managed grasslands systems, they do have the potential to alleviate some, including those associated with environmental challenges. Regulatory oversight of genetically modified technologies should be based on the benefit/ risk of the product and not simply on the process/technology used to deliver the product (Gould *et al.* 2022). The science community also has a responsibility to work with government and industry decision-makers to counter misinformation and provide evidence-based information that outlines the social, environmental, and economic benefits of genetic modification through both listening and engaging in a constructive and empathetic manner.

Conflict of interest

The author is employed by Grasslanz Technology Ltd. which has a R&D investment portfolio that includes both genetic modification and gene editing of forages and microbes to provide mitigating solutions to current environmental and animal welfare issues facing both New Zealand and other pastoral economies.

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Notes:

The potential for wider use of serradella in the pastures of southern Australia

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Abstract: *This review examines the potential for the wider use of serradella in southern Australian pastures that have historically been dependent on subterranean clover (Trifolium subterraneum L.). Serradellas (Ornithopus spp.) are annual legumes native to the Mediterranean region and adoption of serradella use in Australia has largely occurred in comparable climates. The most widely adopted aerial-seeded legume species for use in grazed farming systems in southern Australia have been yellow serradella (O. compressus L.) and French serradella (O. sativus Brot.). These species have been particularly successful on deep, sandy, acidic soils where they have had superior production to subterranean clover. Recent research supports the use of serradellas on a greater range of soil types provided suitable agronomic management. For instance, selection of cultivars with attributes that: optimise flowering time for a given environment, favour timely hardseed breakdown, and tolerate selected herbicides to enable cost-effective weed control. Wider use of serradella species in south-eastern Australia (e.g., permanent pasture systems, ~ 6 million ha) provides an opportunity to reduce the reliance on phosphorus fertiliser, reduce the exposure of a pasture to species specific disease or insect outbreaks and provide a cheaper option for seed production. Barriers to adoption in new temperate environments largely relate to the capacity of currently available cultivars for competitive production and long-term persistence in temperate environments where winters are longer and colder. Factors that may constrain the use of serradella are identified, with the aim of addressing how to overcome potential limits to adoption, and areas of future research are proposed.*

Notes:

Adaptation of novel germplasm and cultivars of serradella (*Ornithopus* spp.) to cold-climates in south-eastern Australia: flowering date and flowering date stability

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Abstract:

Context: There is scope to use serradellas (Ornithopus spp.) as a self-regenerating annual legume in permanent, high rainfall pasture systems of south-eastern Australia. Currently, only a limited number of cultivars have been identified that have both hardseed and mid- to late-maturity.

Aims: Flowering date and flowering date stability (i.e., ability to flower at about the same date irrespective of germination date) of these serradellas were assessed to determine their suitability for use in target environments.

Methods: Experiments were conducted at four cold-climate locations (Glen Innes, Bombala, NSW; Canberra, ACT; Launceston, Tasmania) in 2021 and 2022. Replicated strips of up to ten cultivars and breeding lines of French serradella (O. sativus), two cultivars of yellow serradella (O. compressus), along with subterranean clover (Trifolium subterraneum) controls were sown in March and May. Plants were monitored for time to first flower. The subterranean clover cultivars had known maturity rankings (i.e. flowering times; early, mid or late maturity) and were used to benchmark the maturity of the serradellas.

Results: Across all locations, flowering date instability was observed amongst the serradellas, with cultivars/genotypes flowering up to two weeks earlier when sown early. Despite this, median flowering date (i.e., 50%-flowering) of the serradellas was correlated across the four sites.

Conclusions: Two cultivars of yellow serradella (Avila and Yellotas) and one cultivar of French serradella (Rosa) consistently ranked as having mid-late maturity, while some novel breeding lines of French serradella had late- to very late maturity.

Implications: Several hardseeded serradellas are potentially adapted to permanent, high-rainfall pastures in south-eastern Australia. It is recommended that these serradellas are subjected to broader testing to confirm long-term persistence and productivity.

Notes:

Failures in the Australian pasture seed market after 30 years of Plant Breeder's Rights.

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Abstract:

Context: Plant Breeder's Rights (PBR) were introduced in Australia in 1987-94 but consequences of the new legislation have only recently become apparent.

Objective: To assess the impacts of PBR legislation on Australian pasture cultivar development.

Methods: This desk-top study includes three components: 1. A contrast of PBR with the scheme it replaced; 2. An analysis of registered pasture cultivars, including those marketed by six major seed companies and listed examples of poor seed supply of agronomically important cultivars; and 3. Three case studies demonstrating deficient aspects of PBR registration.

Results and Conclusions: As of April 2024, 807 cultivars were registered in Australia from 127 pasture species, most of which (~100) could be defined as niche species. There was poor support for PBR by the companies sampled, with only 31 % of marketed cultivars having PBR protection, and 48 % being unregistered. There is limited evidence of genetic gain in pasture cultivars post-2010 in part attributable to the lack of requirement in new cultivars for 'characters of merit', coupled with poor cultivar descriptions that obscure such determinations. Market changes due to PBR are clouded by the withdrawal of the public sector from cultivar development and evaluation, which commenced at about the same time. Nevertheless, recommendations are offered for improvements to PBR as they relate to pasture species.

Significance: Ongoing genetic gain in most Australian pasture species seems unrealistic. Preserving previous gains by maintaining availability of older cultivars of merit may be a more realistic objective.

Notes:

Monitoring Lucerne seed and parasitoid wasps to assess associated seed-loss and assist management

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Abstract: *This paper reports on the use of DNA tests to monitor seed losses associated with lucerne seed wasp (LSW) and two parasitoid wasp infestations in each load of harvested lucerne seed delivered to the cleaning sheds. Test results were reported to growers to enable them compare losses between crops and seasons. Data collected between 2018 and 2023 indicates that the value of seed loss across the industry ranged from $1.5M in 2020 to as high as $13M in 2018. Seed losses were greater in crops harvested late; individual crops typically mature between January and April. The relationship between LSW and parasitoid wasps also appears to contribute to seasonal variation in seed losses. Other factors may be important and requires further investigation. The ultimate aim is to develop a model to predict epidemics soon enough for growers to implement management practices to reduce losses.*

Key words: parasitoid wasps, seed losses, industry wide testing

Introduction

Lucerne seed wasp (LSW), *Bruchophagus roddi*, is a serious pest in lucerne crops capable of causing large production losses, >70% in some crops. The magnitude of the losses varies greatly between seasons and with crop commencement dates, which determines the harvest date (De Barro 2001). LSW reproduces on lucerne and to a lesser extent annual medic and clover seed (Seago 2022). One wasp can lay individual eggs in multiple seeds and the species can complete several generations per season when different crops in the district flower over an extended period. There are two main parasitoid wasps of LSW, *Idiomacromerus perplexus* and *Pteromalus sequester*; these lay an egg in seeds already infested by LSW. When the parasitoid larvae hatch, they feed on the LSW larvae. At harvest, infested seeds contain either a single LSW, or parasitoid wasp. Each wasp represents a lost seed. The infested seeds are removed by the seed cleaning sheds along with other impurities.

To reduce seed losses, producers can commence the seed crop phase in early November to facilitate flowering earlier in the season, when LSW populations are relatively low. Growers who want extra grazing or to cut hay will commence the seed crop in December. However, these crops are at higher risk of infestation as they flower later, when LSW populations have increased. Prior to this project, growers were only aware of LSW when populations were very high and contributed to high cleanout rates and gummed up seed cleaning equipment, as occurred in 2013. This paper reports on the use of new DNA tests for LSW and the two parasitoid wasps, calibrated to report results as wasps per gram of sample, to support the industry monitor seed losses and ultimately develop a model to predict epidemics in time for growers to implement appropriate management decisions.

Methods

This study investigated LSW and parasitoid wasp associated seed losses from lucerne seed crops in South Australia in samples collected from the 2018-2023 harvests. Each load of lucerne seed delivered to the cleaning sheds are routinely sampled and tested for the declared weed golden dodder (*Cuscuta campestris*) using a DNA test. The tests for LSW and the parasitoid wasps were added to the testing protocol.
To measure the LSW and parasitoid wasp levels, molecular tests were developed using the TaqMan MGB format, the standard used by SARDI Molecular Diagnostic Centre (https://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_research). The three tests were calibrated initially using 200g samples spiked with known numbers of wasps and then used in a number of field studies. This allowed converting qPCR results to equivalent wasp number per gram of seed delivery sample.

Wasp DNA levels were analysed statistically against various parameters including harvest year and time.

Results

A total of 4,097 delivery samples from the six harvests between 2018 and 2023 were tested. With industry assistance, 2,015 samples were linked to specific paddocks and locations, and data was provided on irrigation method, day of delivery to the seed cleaning sheds, and 1,875 of these samples had data on weight of uncleaned seed delivered to the sheds. Of the samples linked to specific locations, 1,947 were from the southeast region and 68 from the mid-north region of South Australia.

Cost of LSW

The clean seed production and average losses for the industry were extrapolated from the samples linked to paddocks that had data on amount delivered and cleanout rates. Each wasp measured was considered a lost seed. The value of the losses was based on the farmgate clean seed prices for each year, which were $5.50 per kg in 2018, $4.30 in 2019, $4.50 in 2020, $5.85 in 2021, $7.00 in 2022, and $7.50 in 2023.

The annual losses based on the concentrations of LSW, and parasitoid wasps in the harvested seed delivered to the cleaning sheds was $13 million in 2018, $2 million in 2019, $1.5 million in 2020, $5.7 million in 2021, $7.4 million in 2022 and $2.7 million in 2023 (combined cost $32.4 million). Note: these results do not include seed losses associated with wasps not captured by the harvester.

Variation in total wasp populations between years

Most samples came from the southeast region centred around Keith (Figure 1). Maps of average concentrations of total wasps (LSW plus parasitoid wasps) were produced using samples with location information, assigned to a 15km grid (Figure 1). The results show total concentrations varied across the southeast and mid-north regions both within districts and between harvest years.

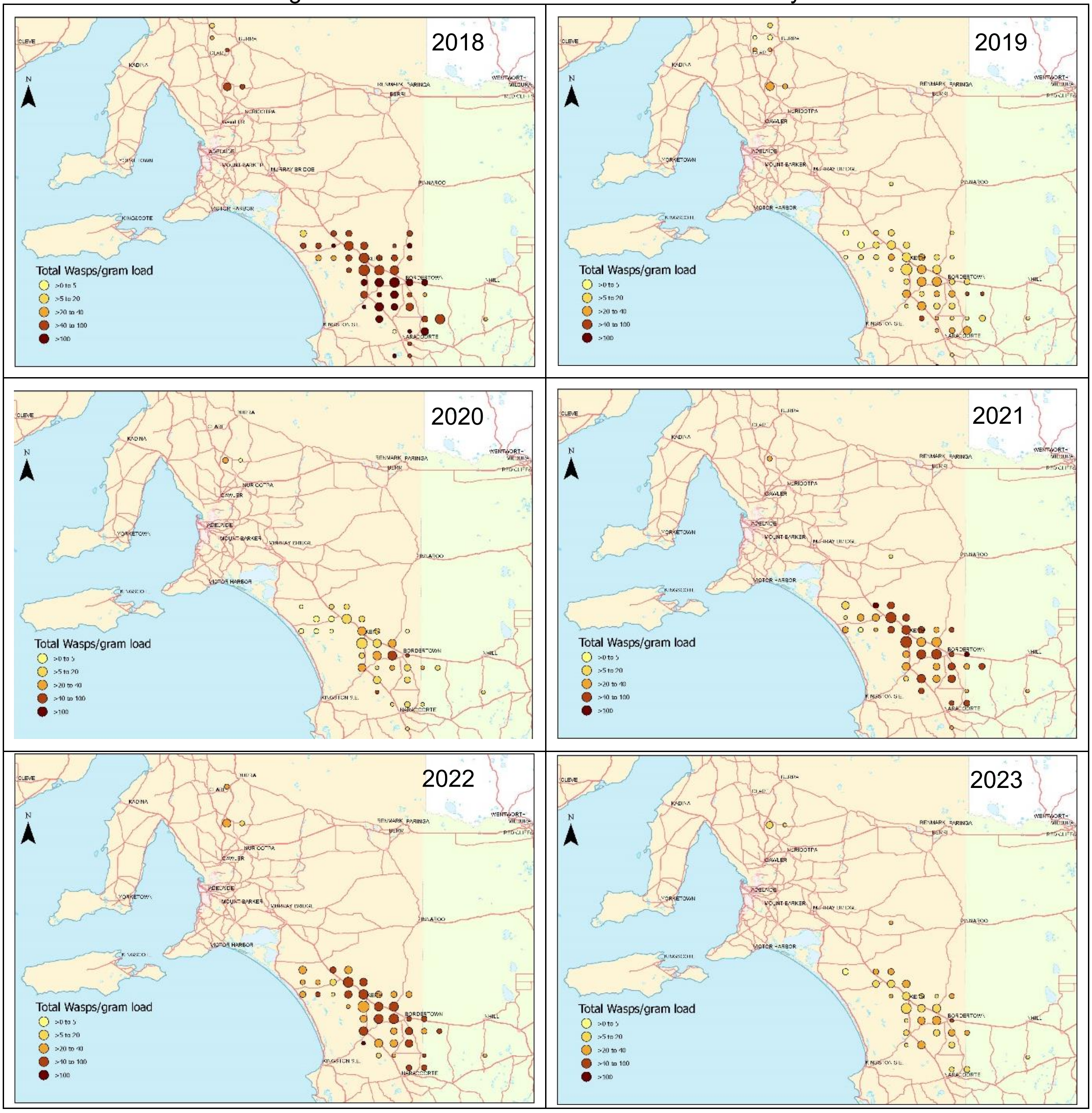

Figure 1. Average total (LSW plus parasitoid) wasps per gram of seed delivery sample from farms mapped to 15km grid. Results are displayed using a colour scale grading from yellow < 5 to dark brown >100 total wasps per gram.

Changes in LSW and parasitoid wasp population densities between years

The relationship between LSW and parasitoid wasps changed within and between seasons (Figure 2). In 2018, average parasitoid populations were large and outnumbered LSW more than two-fold. In 2019, both LSW and parasitoids populations crashed, with parasitoid levels still outnumbering LSW, but by a greatly reduced margin. In 2020, the relationship began to shift with LSW populations increasing while parasitoid levels declined. In 2021 and 2022, both LSW and parasitoid populations increased, but parasitoid levels were greater. The 2022 harvest finished a month earlier than normal, this may have contributed to the lower LSW and parasitoid densities in 2023.

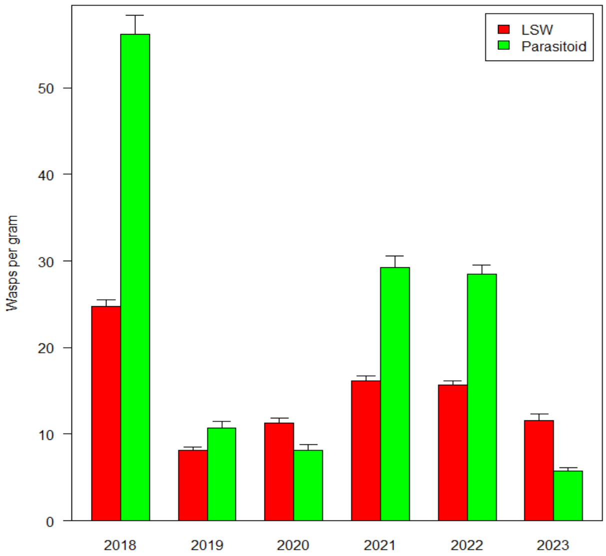

Figure 2. Average LSW and parasitoid wasps per gram of seed delivery sample by year.

Correlations with day of delivery to seed cleaning sheds

Lucerne crops usually mature between January and April. For each harvest from 2018 to 2023, the average total wasp infestation increased significantly with later deliveries; while the rate of increase varied between seasons it was always highly significant ($p < 0.0001$) (Table 1). There were also large variations in levels of infestation between deliveries on the same day, and this increased with day of delivery. Some of this variation may have been due to producers storing some seed on farm prior to delivery to the sheds.

Table 1. Annual regressions for total wasps (LSW plus parasitoids) per gram delivery sample against delivery day of year (proxy for harvest date). Slope is the change in wasps per gram delivery sample per day.

Year	Slope	SE	t	p value
2018	0.88	0.15	6.02	<0.0001
2019	0.33	0.06	5.30	0.0000
2020	0.52	0.06	9.28	0.0000
2021	0.74	0.08	9.36	0.0000
2022	0.78	0.10	7.98	0.0000
2023	0.49	0.08	6.02	0.0000

LSW as a percent of total wasps declined significantly in later deliveries (**Error! Not a valid bookmark self-reference.**). This decline was due to parasitoid wasp numbers increasing at a faster rate than LSW. However, average LSW levels still managed to increase significantly with day of delivery in each year from 2018 to 2023, the greatest increases occurred in 2020 and 2023.

By contrast, LSW as a percent of total wasps declined significantly with delivery date for each harvest except in 2023. The latter result was probably due the early finish to harvest in 2022 (**Error! Not a valid bookmark self-reference.**).

Discussion

The data collected between 2018 and 2023 showed that LSW was present every year and in all seed crops. The average loss during this period was $5.4 million per year, however these varied greatly ranging from $13 million in the 2018 harvest to $1.5 million in 2019 harvest. Differences in growing season conditions and relative population densities of LSW and the parasitoids likely contributed to the variation and require further investigation to develop a predictive model.

Table 2. Annual regressions for LSW as a percentage of total wasps per gram delivery sample against delivery day of year (proxy for harvest date). Slope is the change in percent LSW per gram delivery sample per day.

Year	Slope	SE	t	P value
2018	-0.33	0.03	-10.18	0.0000
2019	-0.33	0.05	-6.20	0.0000
2020	-0.24	0.05	-4.34	0.0000
2021	-0.57	0.04	-15.53	0.0000
2022	-0.21	0.04	-4.77	0.0000
2023	-0.01	0.07	-0.19	0.8500

LSW and parasitoid wasp-related seed losses vary greatly between crops and, across the years about 35% of the losses occurred in 10% of paddocks. Identifying and changing management practices in these crops should achieve large benefits for the industry.

Some very strong trends were observed in this study. In each season as delivery day was delayed from January to April, total wasps (LSW plus parasitoid wasps) per gram delivery sample increased significantly. As seed losses are directly correlated with total wasps, later maturing crops are therefore at greater risk. Interestingly, these crops are potentially useful as a source of parasitoid wasps, as LSW as a percentage of total wasps is relatively low. High carryover of parasitoids may suppress multiplication of LSW populations in the following season.

While the tests are useful to determine what happened in the previous season, there is not sufficient understanding to predict seasons at high risk of LSW early enough for growers to manage the risk. The factors driving changes in LSW populations appear to be complex and require further investigation. Some crops of particular interest are the late harvested crops that have low levels of infestation. This may be due to use of management practices not currently recognised as important in moderating risk of LSW infestation. Other explanations include the crop may be isolated, locally high parasitoid wasp populations in previous years may have suppressed LSW populations, or incorrect harvest date was considered (e.g. the crops may have been harvested early and stored on farm before delivery).

Conclusions

Testing the harvest delivery samples has provided the lucerne seed industry with a practical way to monitor the cost of LSW.

More work is required to develop a model to predict losses in future crops, however the results obtained so far indicate crop closure dates, growing season conditions and changes in the ratio of LSW and parasitoid wasp populations are likely to be important factors. There may also be effects of other management practices that have yet to be identified.

There is a need for more management practices to reduce risk of seed loss, one worth exploring is using screenings from late harvested crop with high parasitoid concentrations to inoculate subsequent early maturing crops to reduce early LSW multiplication rates.

Acknowledgments

AgriFutures Australia for funding this project. Lucerne Australia, in particular seed producer Guy Cunningham for providing industry input, communicating project outcomes to seed producers, and establishing a technical committee to oversee testing and management of LSW.
Lucerne seed cleaners, Greg Excell Limestone Coast Seeds and Andrew Fulton Tatiara Seeds for assisting with reporting results and assigning samples to grower crops. The SARDI Molecular Diagnostics team whose commitment underpinned the achievement of project outcomes.

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Notes:

Lucerne variety trial: assessment of optimum irrigation stress for seed production

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Abstract: *This study evaluated the seed yield performance of 31 lucerne (Medicago sativa) varieties over five years under three irrigation stress regimes: standard (farmer practice), moderate, and high stress. The trial was conducted in Keith, South Australia, over five years to reflect the productive life of a commercial lucerne stand and align with industry practices. Results demonstrate that delayed irrigation can significantly improve seed yields, particularly under high-stress conditions. Significant yield differences were also observed between varieties, with varieties SW18NPK91, SARDI 10 Series II, Heritage 10 and SARDI 7 Series II consistently higher yielding across each strategy across the years. The high stress irrigation practice yield ranged from 0.6t/ha – 0.7t/ha. While moderate stress irrigation practices yielded between 0.62t/ha and 0.38t/ha. Standard watering practices yielded between 0.44t/ha and 0.61t/ha. Yield variability across seasons suggests that optimal irrigation depends on environmental conditions and variety characteristics. This paper presents a gross margin analysis comparing irrigation treatments to identify the most economically advantageous varieties and watering strategy.*

Key words: Medicago sativa, variety trial, gross margin, water management

Introduction

Lucerne is a cornerstone of Australia's pasture seed industry, contributing approximately $95 million to the national economy (Copping *et al.* 2024). With over 80% of production concentrated in South East South Australia, reliable data on varietal performance under local conditions is essential (Hudson 2017). Growers frequently seek evidence-based recommendations to optimise seed yield over the typical five to seven-year productive stand life. Lucerne seed growers in the South East are part of a prescribed wells water allocation plan and are under increasing pressure to optimise water use efficiency within crops. With this background industry wanted to compare varieties and seed yield under modified irrigation management systems and evaluate the effect varying irrigation management had on gross margin (Copping *et al.* 2024). In response to concerns over inconsistent performance of imported varieties and limited yield data availability (Humphries 2018), a five-year trial was conducted to benchmark varieties and evaluate the impact of irrigation management on seed yield and profitability.

Methods

The seed yield trial was established on 22 June 2018 on a sandy loam soil at Warrawee Park, near Keith, SA (36°11'49.8"S 140°20'39.6"E). The area is part of a Mediterranean climate, characterised by warm, dry summers and mild, wet winters. Keith experiences average temperatures of around 30°C in January (summer) and 15°C in July (winter). Rainfall is concentrated in the winter months, with a growing season rainfall of 460mm. The trial site had a soil moisture probe, and weather station installed which was used to plan irrigation scheduling.

Thirty-one proprietary and benchmark lucerne varieties (Table 1) were evaluated under three flood irrigation regimes: standard, moderate stress, and high stress. Treatments were applied over three replicated plots using a randomised complete block design. Standard irrigation followed farmer practice, while stress treatments delayed irrigation based on plant stress indicators. Moderate stress was at the point where plants began to wilt, and high stress was at the point where plants displayed early signs of leaf drop prior to watering. Seed yield was measured annually at harvest, and analysed through factorial AOV (see Copping *et al.* 2024). Hay yield was assessed in spring through cuts. A gross margin analysis was conducted using average yield data, standard market prices, and estimated irrigation costs.

The herbage trial was sown on 22 June 2018 by direct drill with a plot planter at a seeding rate of 3 kg/ha into a moist seed bed (five rows × 0.5 cm depth × 0.25 m row spacing), and managed under a standard watering strategy (farmer practice). There were two replicates of the 31 varieties in a randomised complete block design, with the individual plot size 10 m × 1.25 m. Forage cuts were made about every six weeks across the growing season to evaluate and compare dry matter (DM) weight (t/ha) and DM percentage across the 31 varieties. The first cuts from the seedling stand were made in August 2018, with the final cut taken from the herbage trial in May 2021.

Table 1. Lucerne varieties included in the trial.

Company	Name	Dormancy
Seed Force	SF 914QL	activity 9, commercial since 2016
	SFR27-030	activity 7/8 to be launched in 2019
	SFR27-032	activity 6, not yet commercial
	SFR27-033	activity 7/8, not commercial
Pasture Genetics	L71	7
	L92	9
	GTL60	6
Alforex Seeds/	Magna 995 (was 901)	9
Naracoorte Seeds	Magna 868	8
Upper Murray Seeds	Silverland	5
	Silverosa GT	7
	Silverado	9
	Silversky	11
PGG	Siriver MK II	9
	Titan 9	9
	AR245	
	AR323	
SGI	SW18NPK90	8/9
	SW18NPK91	9
	SW18NPK92	10
Heritage Seeds/FGI	Sardi 10 Series II	10
	Heritage 10	
	Heritage ST	
	Sardi 7 Series II	7
Other	SC01	8
	SC02	8
	SC03	9
	SC04	9
	SC05	9
Public	Siriver	6
	Aurora	6

Table 2. Irrigation dates for each watering strategy from 2018-2019 to 2022-23.

Strategy	First	Second	Third	Fourth
		2018-2029		
Standard	16/11/2018	23/12/2018	10/1/2019	29/1/2019
Moderate	16/11/2018	24/12/2018	12/1/2019	2/2/2019
High	16/11/2018	24/12/2018	15/1/2019	9/2/2019
		2019-2020		
Standard	4/12/2019	22/12/2019	12/1/2020	18/2/2020
Moderate	4/12/2019	22/12/2019	14/1/2020	
High	4/12/2019	22/12/2019	21/1/2020	
		2020-2021		
Standard	25/11/2020	19/12/2020	12/1/2021	4/2/2021
Moderate	25/11/2020	24/12/2020	21/1/2021	
High	25/11/2020	6/1/2021		
		2021-2022		
Standard	9/12/2021	28/12/2021	15/1/2022	6/2/2022
Moderate	9/12/2021	28/12/2021	20/1/2022	
High	9/12/2021	28/12/2021	28/1/2022	
		2022-2023		
Standard	10/12/2022	28/12/2022	16/1/2023	6/2/2023
Moderate	10/12/2022	29/12/2022	20/1/2023	
High	10/12/2022	29/12/2022	28/1/2023	

Results and Discussion

Multiyear seed yield summary

After the first year, the high-stress irrigation consistently produced higher seed yields than standard watering (Fig. 1), except in 2021-22 when seasonal rainfall disrupted the irrigation schedule resulting in insignificant differences. There were significant yield differences observed between varieties, with SW18NPK91, SARDI 10 Series II, Heritage 10 and SARDI 7 Series II consistently yielding higher across each strategy across the years (Fig. 2).

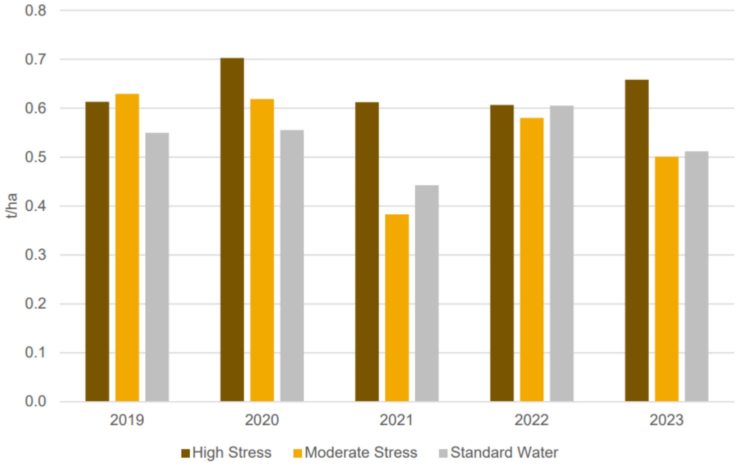

Figure 1. Total average seed yield (t/ha) for each watering strategy per year (2019–2023).

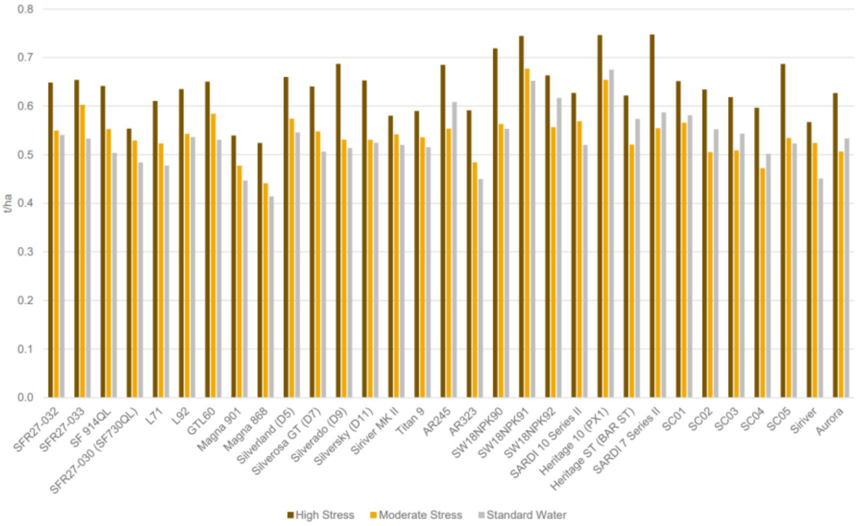

Figure 2. Total average seed yield (t/ha) across years by variety for each watering strategy (2019–2023).

Herbage trial results

Figure 3 shows the average dry matter yield (t/ha) for each variety across each watering strategy at biomass cut timing 1 (September) and 2 (November).

Gross margin analysis

Gross margin analysis of the three watering strategies in the seed trial was undertaken for years 2 to 5 (Table 3). These calculations accounted for all variable costs (including the cost of watering) and income from a spring hay cut (using data from the biomass assessment made each spring) and seed yield only. More information can be found in Copping et al (2024). The gross margins do not comprehensively reflect the cost of production, although individual growers could calculate this using Lucerne Australia's Cost of Production template (available to all members of Lucerne Australia, Carter and Heywood, 2008).

The gross margin analysis showed some variation, but implementation of the high-stress strategy consistently delivered a higher gross margin in every year evaluated compared to both the standard and moderate-stress strategies. These differences can be attributed to a combination of higher seed yield, lower watering costs and, in the case of year 5, historically high lucerne seed prices.

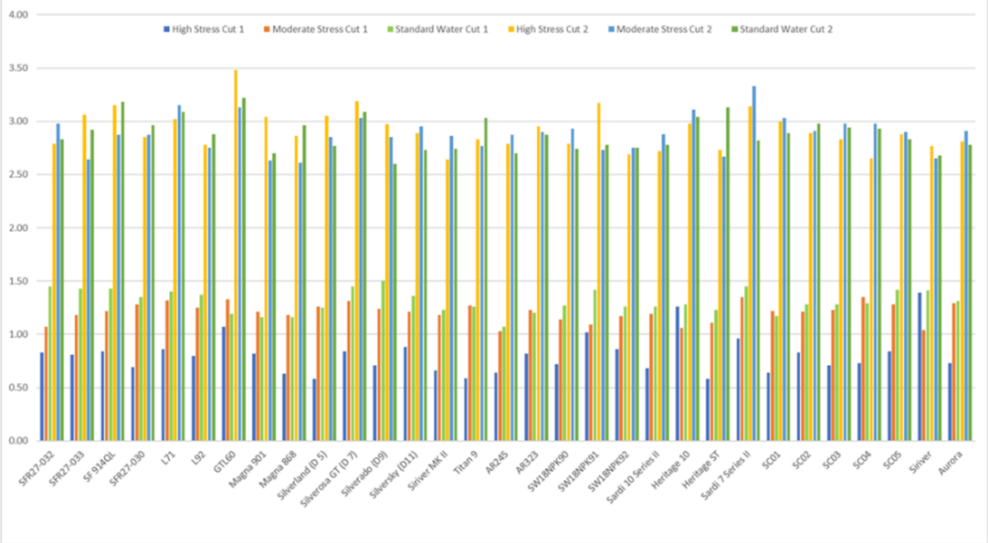

Figure 3. Total average dry matter yield (t DM/ha) per variety for each watering strategy at Biomass Cut 1 (September) and 2 (November).

Table 3. Gross margin for the three irrigation strategies.

Irrigation strategy	Gross Margin ($/ha)			
	2020	2021	2022	2023
Standard	$2,882.77	$2,070.87	$3,074.31	$2,913.17
Moderate	$3,483.27	$1,930.37	$2,976.14	$3,034.15
High	$3,633.07	$3,063.77	$3,166.67	$4,209.89

Conclusions

The results from this five-year lucerne variety trial represent valuable information for growers in the main lucerne seed-production area of Australia. The Australian lucerne seed industry has traditionally been protective of its production data, resulting in limited analysis of seed performance being available, especially in consistent growing conditions. This trial data has demonstrated which varieties produce high seed yields and how varieties perform under a higher rate of moisture stress, giving growers guidance on water use in the future. There is strong evidence that delaying irrigation timing in a controlled, strategic manner leads to higher seed yields across any variety. The degree of irrigation delay and the specific timing is likely to vary between soil types and irrigation methods, but this data supports anecdotal evidence.

Under the high-stress watering strategy, the seed yield responses to delayed irrigation timing were generally consistent for the 31 varieties evaluated across the five years. However, seed yield responses to the moderate-stress strategy were not consistent compared to the standard watering strategy. Further, there were consistent differences in seed yield between the varieties evaluated across the years, with significant yield differences observed with varieties, SW18NPK91, SARDI 10 Series II, Heritage 10 and SARDI 7 Series II (Copping et al 2024). The extension of the seed trial component for an additional two years (2022 – 2024) has allowed further validation of varietal performance across seasons under modified irrigation.

Acknowledgments

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Notes:

Insecticide-resistant bluegreen aphids in southern legume crops and options for parasitoid biocontrol

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Abstract: *The emergence of insecticide-resistant Bluegreen aphids (BGA; Acyrthosiphon kondoi) poses a new management challenge to Australian pastures and pulse crops. Our study aimed to address uncertainties for the management of insecticide-resistant BGA and preventing future resistance evolution through three avenues. First, we conducted an insecticide surveillance program to assess the distribution of resistant BGA. We identified insecticide-resistant BGA at 21 new locations across Southern Australia, raising concerns about the effectiveness of current insecticide control strategies in multiple regions. Second, we conducted baseline sensitivity assays to evaluate the potential of three alternative insecticide modes of action for future management of BGA. Third, we gathered baseline data on a key natural enemy: parasitoid wasps, to evaluate biocontrol options for BGA. We found unexpectedly low diversity among parasitoids, with Aphidius ervi being the sole species parasitising BGA in multiple states and crop types. While A. ervi shows promise as a biocontrol agent, most current insecticides registered for BGA control will have off-target effects that damage natural A. ervi populations. In turn, selective insecticides alongside a broader suite of integrated pest management methods are needed to mitigate the future spread of insecticide-resistant BGA.*

Introduction

Bluegreen aphids (BGA; *Acyrthosiphon kondoi*) are common pests of lucerne, medic, clover, and pulses in Australia and several nations abroad (Bailey, 2007). BGA harms crop growth and yield by feeding on upper leaves, stems, and buds while secreting bioactive compounds into plants (Edwards *et al.* 2008). BGA also transmit plant viruses between crops, including cucumber and alfalfa mosaic virus (van Leur *et al.* 2021). BGA outbreaks can escalate rapidly due to their short (~10-day) generation time and rapid dispersal via winged morphs. Thus, growers must actively manage this pest to mitigate the risk of crop damage.

Insecticides have long been a reliable and cost-effective method to control BGA. However, a reliance on a narrow group of broad-spectrum insects placed BGA populations under strong selection pressure. In 2021-22, research found that three BGA populations in lucerne paddocks in South Australia and New South Wales had evolved resistance to organophosphates, carbamates, and synthetic pyrethroids (Chirgwin *et al.* 2024). This was the first recorded case of insecticide resistance in this species globally, raising concerns about new risks to Australia’s pastures and pulse industries.

Our study aimed to address uncertainties about insecticide-resistant BGA and mitigate the risk of further resistance evolution through three avenues. First, we investigated an insecticide resistance surveillance program from 2022 to 2025, collecting BGA populations across Southern Australia and conducting bioassays to test their insecticide resistance. Second, we ran baseline sensitivity assays to assess the potential of three alternative insecticides to manage BGA in the future. Third, we gathered baseline data on a key natural enemy: parasitoid wasps, to assess how to enhance biocontrol options.

Methods

Insecticide resistance surveillance

During the life of the project, BGA field populations were collected from pasture, pasture seed, and pulse crops as part of a resistance surveillance program. Sampling occurred across Vic, Southern NSW and SA, with a focus on regions with reported chemical control failures (e.g., SA–VIC border). Populations were collected directly by the project team or submitted by growers and agronomists. We used toxicology-bioassays to assess the presence and magnitude of resistance in BGA to organophosphates, carbamates, and synthetic pyrethroids. Toxicology-bioassays were used since the resistance mechanism(s) for these chemicals and species had not previously been identified. Leaf dip methods previously established for BGA were used (Chirgwin *et al.* 2024) for all bioassays. All field-collected aphid populations were tested alongside a known insecticide-susceptible population.

Baseline insecticide assays

We examined two BGA populations known to be resistant to organophosphates, carbamates, and pyrethroids compared to a susceptible BGA population. We tested Flupyradifurone, Sulfoxaflor, and Flonicamid at six to eight concentrations from 0.0001-100× of the field rate for BGA or other aphid species. Mortality was recorded 72 h post exposure for flupyradifurone, 144 h for flonicamid, and 48 h for sulfoxaflor. Dose–response curves were fitted using binomial logistic regression to estimate LC_{50} values. Resistance ratios were then calculated by dividing field population LC50s by the susceptible population LC50.

Parasitoid surveys

We screened parasitoid wasps in all BGA populations collected during the resistance surveillance. After bringing a BGA population to the lab, we isolated aphids into 55mm Petri dishes with 1 % (w/v) agar and lucerne leaves, keeping them at 20°C for three weeks. Under these conditions, parasitoids typically develop within 2 weeks inside an aphid before emerging as adults. Each emerged adult parasitoid was placed in 100% ethanol and stored at -20°C until later morphological identification.

Results & Discussion

The surveillance program identified insecticide-resistant BGA in new locations and crop types (Fig. 1). A total of 21 new populations resistant to organophosphates, carbamates, and/or synthetic pyrethroids were found in southern Australia, a far wider distribution than originally reported by Chirgwin *et al.* (2024). The distribution has expanded west (to the Eyre Peninsula), north in New South Wales (near Tamworth), and southeast to multiple locations in Victoria. This wider distribution of resistant BGA clones likely results from selection pressures due to the use of these insecticides, promoting the evolution of resistant strains. Reducing this selection pressure is a challenge for pasture industries, as these insecticides control various pests (e.g., red-legged earth mites and other aphids). Insecticide-resistant BGA were more prevalent in lucerne than in other crops. Most populations gathered from lucerne in South Australia showed insecticide resistance. In contrast, insecticide-resistant BGA populations were rarer in other crop types, although they were found in lentil crops in South Australia (Yorke Peninsula) and Victoria (Wimmera and Mallee).

Figure 1. Map of southern, mainland Australia showing BGA populations that were susceptible or resistant to organophosphates, carbamates, and/or synthetic pyrethroids.

BGA showed lower resistance to carbamates than to organophosphates and pyrethroids. Most populations resistant to chlorpyrifos also showed resistance to alpha-cypermethrin, but only about half showed clear resistance to pirimicarb. Furthermore, the resistance ratios for pirimicarb were typically lower than those for chlorpyrifos and alpha-cypermethrin. Recent studies are starting to reveal the mechanisms behind pirimicarb resistance in BGA (Thia *et al.* 2024), but it is unclear whether epigenetic factors influence resistance levels. In some aphid species, epigenetic mechanisms confer greater resistance to pirimicarb in response to certain environmental cues. Nonetheless, according to our findings and discussions with agronomists, pirimicarb seems effective in managing BGA when used under optimum temperature conditions. Pirimicarb operates through three mechanisms: contact, translaminar (systemic) action, and fumigant effects (Turner, 1995). The fumigant effect is most effective at cooler temperatures (20 °C to 30 °C). However, its effectiveness can diminish in cooler conditions, commonly found in winter and spring. Therefore, some instances of pirimicarb control failures for BGA are likely due to a combination of low-level resistance and suboptimal application temperatures.

No evidence of field-evolved resistance was found in our baseline tests of the three modern insecticides (Fig. 2). As such, our results show no cross-resistance among flupyradifurone, sulfoxaflor, or flonicamid with the resistance mechanisms related to organophosphates, carbamates, and synthetic pyrethroids in BGA. This baseline sensitivity data serves as a benchmark for future insecticide resistance monitoring. However, chemical registrations differ from state to state. Sulfoxaflor was recently registered for use on BGA in lucerne, but not on other crops. Flonicamid is listed on a limited emergency use permit for BGA in lucerne seed crops. Flupyradifurone is used in other countries (e.g., USA) for BGA but is not registered in Australia. Sulfoxaflor and Flonicamid may also aid integrated pest management (IPM) methods, as these chemical modes of actions are less toxic to some beneficial invertebrates (e.g., ladybirds, hoverflies) than organophosphates, carbamates, or synthetic pyrethroids (McDougall *et al.* 2022).

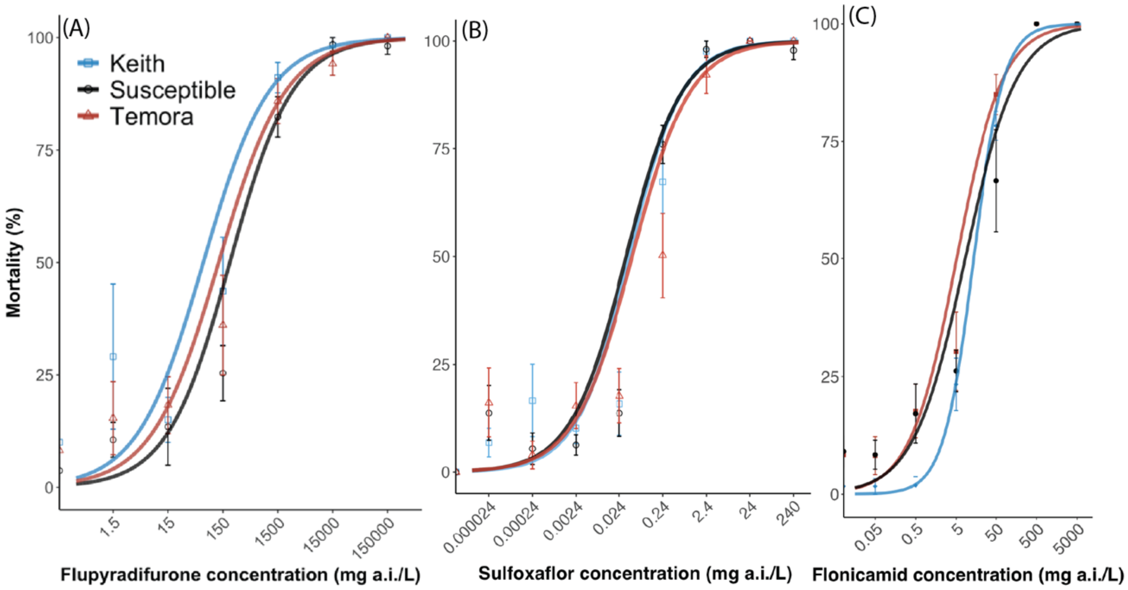

Figure 2. Dose-response curves for two field-collected BGA populations and a known susceptible BGA population in response to (A) flupyradifurone, (B) sulfoxaflor, and (C) flonicamid.

We identified 132 parasitoids from 25 locations in South Australia, New South Wales, and Victoria, including four crop types (clover, lucerne, vetch, and lentils; Fig. 3). The diversity of parasitoids was surprisingly low across regions and crops, with all parasitoids being the same species: *Aphidius ervi.* Previous research has indicated that aphid pests in Australia are attacked by multiple parasitoid species. For instance, in the study by Ward *et al.* (2021) the parasitoids of several aphid species common to Australian grain crops were investigated, and on average, each aphid species was attacked by four parasitoid species. Given this lack of parasitoid diversity for BGA, management methods, especially chemical use, need to be carefully designed to support *A. ervi* populations. Unfortunately, insecticides currently registered for controlling BGA in pastures and seed crops (including sulfoxaflor) appear toxic to *A. ervi* (Overton *et al.* 2021).

Aphidius ervi have several desirable traits for an effective biocontrol agent. *A. ervi* is a generalist parasitoid of multiple aphid species in legume crops (Milne, 1999). *A. ervi* is also known to use olfactory cues to seek out legume crops, and each female can parasitise over 300 aphids in their brief (2-3 week) lifecycle (Hagvar and Hofsvang, 1991). *A. ervi* is mass-produced by commercial Australian breeders, which may provide growers the option to boost natural *A. ervi* populations for future BGA management. This process, known as augmentation, includes mass-rearing a natural enemy species via commercial breeders and subsequently releasing these individuals into the fields to artificially increase natural enemy populations. Augmentation reduces the time lag between pest populations hitting economically damaging levels and natural enemies responding effectively, whether through migration or breeding, to control the pest density (Eilenberg *et al.* 2001). Still, augmented parasitoids have primarily been used for pests within small or protected cropping areas. The effectiveness of augmented *A. ervi* in a large area or pasture crops remains to be tested.

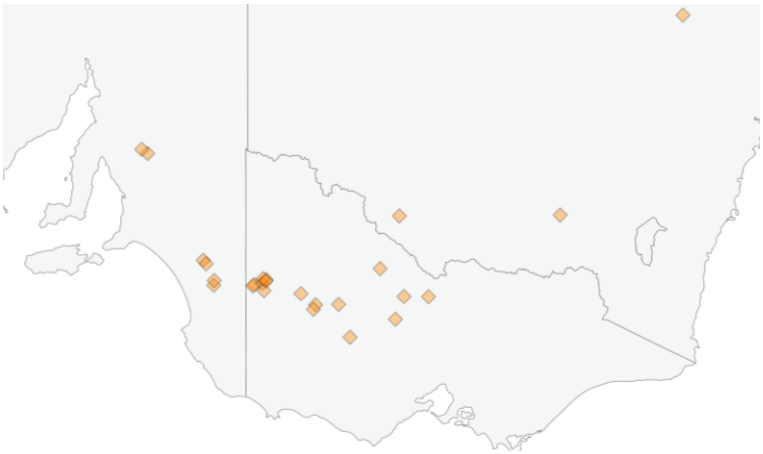

Figure 3. Locations of the BGA populations where parasitoid wasps were collected and identified in south eastern Australia.

Conclusions

The emergence of insecticide-resistant BGA necessitates a shift in managing this pest. Here, we present alternative chemical and biocontrol methods to help facilitate this transition. However, further steps are required to sustainably manage BGA alongside many other pests that co-occur with BGA in pastures. For example, red-legged earth mites, cowpea aphids, and mirids are managed using the same limited range of insecticides needed to control BGA. Consequently, BGA populations are likely subjected to ongoing selection pressures favouring resistant clones, even when they are not the main target of the insecticide applications. Additional initiatives, such as developing multi-pes Insecticide Resistance Management Strategies (IRMS), improving monitoring methods for natural enemies of BGA, and investigating aphid-tolerant cultivars, warrant further exploration in the future.

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Notes:

Swathing subterranean clover does not suck: a promising alternative to vacuum harvesting

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Abstract: *The buried seeds of subterranean clover (Trifolium subterraneum L.; subclover) are commonly harvested with a suction-based Horwood-Bagshaw (HB) clover harvester in late summer, following multiple tillage passes. However, this process is inefficient, causes soil disturbance and erosion and relies on outdated technology. Swathing is a promising alternative, whereby roots are cut and the green sward lifted and windrowed, ready for burr pickup and threshing by a combine harvester. A trial on a commercial seed crop in Capel, Western Australia, compared swathing by a modified peanut digger on November 25 (Day 1) and December 1 (Day 2), 2022 to a single HB pass on February 13-14, 2023 following tillage. Each treatment was applied to four 75 m-long plots with samples from five quadrats per plot taken to measure seed yield, plant biomass, soil moisture, seed weight and germinability and seed collection efficacy. Canopy greenness was also assessed. Swathing showed high seed collection efficacy with 100% of available seed remaining attached to swathed plants on Day 1 and 75% on Day 2, while 54% of seed was collected by the HB harvester. Mean seed weight was 12-18% lower for swathed seeds than for HB-harvested seeds. Germinability was 83% for seeds swathed on Day 1 and almost 100% on Day 2, similar to that of HB-harvested seeds. As germinability was lower and highly variable for Day 1, we investigated its relationship to canopy greenness. We conclude that swathing is a promising approach for harvesting subclover, but timing relative to sward senescence is critical.*

Corresponding Crop & Pasture Science paper:
Wiese RC, Ryan MH, Nichols PGH, Moss WM, Wintle B, Zago YA, Hunt L, Hamblin A, Guzzomi AL (2025) Swathing subterranean clover (*Trifolium subterraneum*) does not suck: a promising alternative to vacuum seed harvesting. *Crop & Pasture Science* **76**, CP24357. https://doi.org/10.1071/CP24357

Notes:

Harvest and use of medic pods on farm

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Abstract: *Annual medics are widely grown as ley legume pastures on alkaline soils. However many pastures are rundown and low levels of renovations are practiced. The aim of this work was to determine if medic pods can be harvested with a grain harvester and if storing pods for 2-3 summers allows for sowing rates of < 100 kg pods/ha. Barrel medic cv Sultan-SU and strand medic cv Seraph were grown in medium and low rainfall areas over two years, desiccated and pods harvested with a grain harvester. A seed softening study was conducted to determine what sowing rate is required to achieve 200 plants/m^2. Seed softening studies were also done on seven medic cultivars stored for two years, and several medic cultivars stored for 4-15 years. With desiccation, 870-2000 kg pods/ha were harvested at the mid-rainfall site and 370-700 kg pods/ha at a low rainfall site in a wetter year. Minimum sowing rate for plot harvested pods stored for three years was 140 kg pods /ha. For medic cultivars stored for two years, Scimitar had a sowing rate of 33 kg/ha but other cultivars required > 100 kg/ha. Storage of medic pods for 4-15 years allowed sowing rates of < 100 kg pods/ha for cultivars Cavalier, Herald, Paraggio, and Scimitar but not for Caliph, Jester, Sultan-SU, Toreador and Tornafield. For most cultivars, the need to store pods for four or more years will make this method unviable. Scimitar can be trialled on small areas to see if it is suited for wider adoption.*

Key words: hardseed, clover harvester

Introduction

Annual medics *(Medicago* spp.) are widely grown as ley pasture legumes in alkaline soils in southern Australia (Nichols *et al.* 2012). Annual medics provide high quality and high protein feed to livestock, and fix nitrogen for the benefit of following grain crops. The cost of seed and low growth of pastures in the establishment year is regularly reported as a constraint to pasture legume adoption. French serradella (*Ornithopus sativus*) pod segments can be harvested with a grain harvester (Nutt *et al.* 2020), and in preliminary work Peck *et al.* (2021) found that early desiccation of medic pastures allows pods to be harvested with a grain harvester. Hardseeded pod segments of French serradella can be sown in late summer and early Autumn, soften during Autumn and establish with the break (Nutt *et al.* 2020). This provides multiple benefits including cheap establishment costs, completing pasture sowing ahead of the peak crop sowing period, early livestock feed, and increased seed set in years with dry spring (Nutt et al. 2020). A key component of this method is that French serradella seeds soften quicker in the dark (i.e. sown) than in the light. By contrast annual medics at 2 cm soil depth soften at the same rate as pods on the surface (Taylor 2005). However, stored medic pods soften quicker than medic pods harvested in the last growing season (Harrison *et al.* 2020). The present study follows on from Peck *et al.* (2021) with the goal of developing a system which allows medic pods to be harvested on-farm and sown in late summer to early autumn to establish medic pastures.

Methods

Desiccation and harvest of pods

Medic pod harvesting trials were sown with 10 kg/ha of the strand medic (*M. littoralis*) cv Seraph and the barrel medic (*M. truncatula*) cv Sultan-SU at two South Australian sites [Palmer (low rainfall site; 299mm mean rainfall) and Kingsford (medium rainfall site; 468mm mean rainfall)] in 2022 and 2023. Trials were sown after knockdown herbicide controlled the initial germination of the growing season. Plots were 10 m x 1.5 m with six replicates in 2022 and four in 2023. At Palmer in 2022, a second trial was sown at 50 kg/ha to mimic a regenerating medic pasture, which was mowed until early flowering to simulate grazing. The beginning and end of peak flowering was observed. Gallardo *et al.* (2003) report that medic seeds are desiccant tolerant and have high vigour after 400 growing degree days (GDD) and pods dehisce after 900 GDD. On a weekly basis the observed daily temperature, forecast daily temperature, and climate temperature data was used to predict when seeds developed from late flowers are at 400 GDD and when pods from early flowers are at 900 GDD. Desiccation day was confirmed with field observation of plants senescing and pods beginning to fall, combined with a weather forecast of four fine days with light winds. Plots were desiccated with 2 l/ha of paraquat and harvested with a small plot grain harvester four days later.

Use of medic pods

Medic pods (8g) were placed inside pockets in strips made from flywire (Nichols *et al.* 2025) and placed on the soil surface at Adelaide in mid-February and mid-March 2024. Cohorts of pods placed in the field were: 1) machine harvested pods of Seraph and Sultan-SU stored for 1-3 summers (i.e. harvest studies); 2) current medic cultivars (spineless burr medic (*M. polymorpha*) cultivars Scimitar and Cavalier; barrel medic cultivars Penfield, Emperor, Sultan-SU and Jester-SU; strand medic cultivar Seraph; and French serradella cultivar Fran$_2$o) grown at Waite in 2021, naturally senesced, pods collected and stored inside a shed for 2 summers; 3) a range of medic cultivars stored in a shed for 4-15 years (mid-March only). Five replicates were used for cohort 1 and 2, and three replicates for cohort 3. Pods were collected 20th April (25th percentile time of autumn break at Lameroo, Flohr *et al.* (2021)), removed from flywire pockets and planted at a depth of 2cm in pots with potting mix in a shade house, well-watered for three weeks and plant establishment counted. Plant establishment counts were converted into weight of pods (kg/ha) required to achieve 200 plants/m^2.

Results

Harvest of pods with grain harvester

900 GDD after peak flowering started, coincided with medic pastures starting to senesce with some pods fallen on the ground and many pods having changed from green to grey. Four days after the desiccant was applied, many pods were still attached and were able to be picked up and threshed with small plot grain harvester (Fig. 1). At the dry site Palmer, 584±85 kg/ha of Seraph and 370±46 kg/ha of Sultan-SU pods were harvested in 2022 despite the preferred desiccation day being delayed one week by rainfall and wind. For the simulated regenerating strips, 703±101 kg/ha of Seraph pods and 520±82 kg/ha of Sultan-SU pods were harvested. In 2023 with a dry late winter and early spring, plant growth and pod set were insufficient to attempt pod harvest. At the medium rainfall site Kingsford, in 2022 (wet spring) a pod yield of 1740±349 kg/ha was achieved for Sultan-SU and 1965±164 kg/ha for Seraph. At Kingsford 2023 (dry spring), pod yield of 871±137 kg/ha was achieved for Sultan-SU and 1273±194 kg/ha for Seraph. It was estimated that the grain harvester harvested 40-60% of total pods. Bulk densities of pods are 412±13 kg/m^3 for Seraph (like oats) and 194±3 kg/m^3 for Sultan-SU.

Figure 1: Medic pods were attached to the plant four days after desiccant applied (left image) and vine and pods were able to be picked up and threshed with a small plot grain harvester (right image).

Use of medic pods

Number of plants established varied greatly (Fig. 2) and the required sowing rate to achieve 200 plants/m^2 varied with storage time and cultivars (Table 1 & 2, Fig. 3). Required sowing rate for pods harvested with a grain harvester are presented in Table 1. Pods from the dry site Palmer had lower sowing rates than from the medium rainfall site. Pods placed in the field in mid-March required higher sowing rates especially when stored for only 1 summer. For pods of cultivars stored for 2 summers (Fig. 3), only Scimitar burr medic and Fran$_2$o French serradella had sowing rate ≤ 50 kg/ha. Placement time had little effect on sowing rates except for Jester-SU and Seraph when required sowing rate greatly increased with a mid-March placement. For pods stored for ≥ 4 years (Table 2), Scimitar, Herald and Paraggio had required sowing rates ≤ 50kg/ha. Jester and Caliph required high sowing rates, though when pods were threshed plenty of seeds of a healthy colour were found and when scarified had 100% germination. This indicates that the high sowing rates in Jester and Caliph is a lack of seed softening rather than loss of seed during long storage.

Discussion

This project found that early desiccation allowed medic pods to be harvested with a grain harvester in both years at a medium rainfall site, but not in a dry year at the low rainfall site. This agrees with the results of Peck *et al.* (2021) who found pods could be harvested at a medium rainfall site (Adelaide) but not at a low rainfall site (Minnipa) in a low rainfall year. Earlier sowing increases plant growth (Nutt *et al.* 2020) and will increase the chance of harvesting pods in low rainfall areas. Farmers can have dedicated nursery paddocks with new cultivars or harvest regenerated pastures grazed up to early flowering. However the plant establishment work found that only the burr medic cv Scimitar (and French serradella Fran2o) had enough seed softening with two years storage for the required sowing rate to be ≤ 50 kg pods/ha. Scimitar provides an opportunity for farmers to trial the method at a small scale (e.g. 5 ha) to see if it works on a commercial farm. Medic species are matched to soil type and burr medics are less widely grown than barrel and strand medics (Nichols *et al.* 2012). The development of barrel and strand medic cultivars with seed softening traits like Scimitar (or Fran2o French serradella) would allow for this practice to be more widely recommended.

Table 1: Required sowing rate (kg/ha) for Seraph and Sultan-SU pods stored for 1-3 summers (# sum.) and harvested with grain harvester at different sites.

Cultivar	site	# sum.	Sowing rate kg/ha February	Sowing rate kg/ha March
Seraph	Kingsford	1	814 ± 61	1261 ± 416
Seraph	Kingsford	2	720 ± 92	960 ± 75
Seraph	Palmer	2	156 ± 14	191 ± 14
Seraph	Adelaide*	3	138 ± 5	161 ± 7
Sultan-SU	Kingsford	1	622 ± 49	1058 ± 217
Sultan-SU	Kingsford	2	792 ± 119	899 ± 155
Sultan-SU	Palmer	2	204 ± 7	260 ± 17
Sultan-SU	Adelaide*	3	176 ± 8	212 ± 14

* Pods harvested by Peck *et al.* (2021)

Table 2: Sowing rate (kg/ha) for medic pods stored for 4-15 years and placed in the field mid-March.

Cultivar (yr.)	Sowing rate (kg/ha)
Herald (15)	29 ± 2
Scimitar (12)	32 ± 3
Scimitar (4)	35 ± 0
Paraggio (6)	47 ± 3
Paraggio (15)	49 ± 5
Cavalier (12)	62 ± 5
Cavalier (4)	84 ± 7
Tornafield (15)	135 ± 17
Sultan-SU (4)	176 ± 23
Toreador (12)	715 ± 113
Jester (12)	1616 ± 504
Caliph (12)	> 9500

Figure 2: Plant establishment in pots (two pots per treatment) three weeks after sowing.

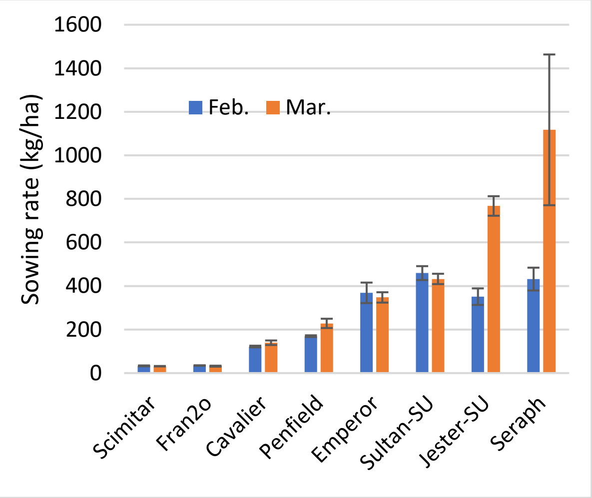

Figure 3: Required sowing rate (kg/ha) for pods stored for 2 summers and placed in the field in mid-February or mid-March.

The focus of this project was developing a cheaper source of medic seeds on-farm. Results may also be relevant to the production of commercial medic seed. Moss *et al.* (2021) report that subterraneum clover (*Trifolium subterraneum*) faces a serious risk from the current practice of using Horwood Bagshaw Clover Harvesters which has an expensive, slow and labour-intensive process combined with poor reliability and maintainability of harvesters that are now at least 30 years old. As the commercial medic seed industry uses the same harvesters, they are also at serious risk from the ageing equipment. Harvesting pods with a grain harvester and threshing pods with a stationery thresher could assist medic seed growers, thus ensuring commercial seed of an important pasture option for alkaline soils. For the commercial seed industry seed quality work is required (e.g. seed germination %, plant vigour).

The required sowing rate was influenced by cultivar, time of placement in the field, storage time, and growing season. Seed softening is a two-stage process with stage I (pre-conditioning) being the accumulation of heat and stage II (softening) requiring temperature fluctuation, with both stages being affected by cultivar (Taylor 2005). The cultivars with less softening with a March field placement may require higher temperature fluctuations (i.e. stage II) or more heat units (stage I). The dry site Palmer had greater softening than the medium site which agrees with Taylor (2005) reporting that seeds grown in hot dry areas soften more than seeds grown in cool wet areas. Apart from Scimitar, we are unable to offer advice on how long to store pods before sowing them. The best advice is to conduct small scale annual February sowing trials from three years storage onwards, and when enough softening occurs sow medic pods the following year. To conduct a softening trial use an accurate balance and sow X g of pods into soil without any medic pods and count emergence after the opening rains. The sowing rate for 200 plants / m^2 equals 2000X/number of plants emerged (e.g. if sow 3g and 100 plants emerge the sowing rate = 2000 x 3 / 100 = 60 kg pods/ha). The need to do this testing, along with the storage time, means that the sowing of medic pods other than Scimitar is unlikely to be a viable option for most farmers.

Conclusions

With early desiccation, useful amounts of medic pods were harvested in a medium rainfall site in all years and in a low rainfall area except in dry years. However only the burr medic cultivar Scimitar had enough seed softening after two summers of storage for the required sowing rates to be ≤ 50 kg/ha. Farmers growing Scimitar have a pathway to adopt this research, but pods of other cultivars are not suited to sowing on farm. The ability to harvest medic pods with a grain harvester may be of assistance to commercial medic seed production. Plant breeders could develop barrel and strand medic cultivars with softening patterns like Scimitar or French serradella Fran$_2$o.

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Notes:

Hard seed softening of diverse annual pasture legume cultivars in three contrasting environments of southern Australia

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Abstract: *Hardseededness (seed coat impermeability) is the most important form of seed dormancy in annual pasture legumes. Softening of hard seeds occurs over the summer-autumn months, allowing seeds to germinate, while residual hard seeds remain for germination in future years. This paper reports on the magnitude and timing of hard seed softening over summer-autumn among 42 annual pasture legumes in 15 species at three contrasting locations in southern Australian (Perth, Adelaide and Canberra). Twenty entries were common to each site. Freshly ripened seeds in nylon mesh pockets were either placed on the soil surface or buried at 2 cm depth and sampled for germination testing every 28 days until early winter. Initial hard seed levels were generally greater than 80%, apart from French serradella (Ornithopus sativa) cultivars Cadiz and Serratas with ≤ 6.5%. The rate and extent of seed softening over the summer-autumn period was greatest in Perth and least in Canberra, with Adelaide intermediate. Subterranean clovers (Trifolium subterraneum) had the most softening, while annual medics (Medicago spp.) and yellow serradellas (Ornnithopus compressus) had the least. Burial reduced the amount of seed softening in most species and was particularly pronounced for subterranean clover. However, pod burial increased the amount of seed softening in yellow serradella. These results provide a greater understanding of how the seed dormancy attributes of different pasture legumes can be better matched for adaptation to different environments and farming systems in southern Australia and other regions of the world with similar climates.*

Notes:

An initial investigation into the seed production and hard seed breakdown pattern of Trophy white clover.

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Abstract: *Little is known about the hard seed breakdown pattern in white clover (Trifolium repens L.) and how it affects soil seed reserves. Two separate experiments were conducted near Cowra, NSW to address this knowledge gap. The first was conducted in 2019 to track the hard seed breakdown pattern. The second was conducted in 2023 to quantify annual seed inputs. The softening of the hard seed of cv. Trophy was observed to occur in both autumn and spring. Forty six percent of the cv. Trophy seed had softened by mid–autumn and after 46 weeks most of the seeds had softened with less than 2% remaining hard. The number of inflorescences and seed production of cv. Trophy was measured under field conditions and found to be similar to cv. Haifa. in all measured flowering traits (P>0.05). It is proposed that the softening of hard seeds occurs in response to seasonal temperature fluctuations typical of both Mediterranean climates (mild winters and hot summers) and temperate climates (cool winters and warm summers). Typically, in southern Australia two distinct periods of softening will occur, limiting the ability to build a long-term seed bank for regeneration. Modelling of the seed production and hard seed breakdown of cv. Trophy indicates annual seed inputs need to be >30 000 seeds/m^2, or ~160 kg/ha, for soil seed reserves to be maintained above the minimum required to enable adequate seedling regeneration to occur until the following spring.*

Key words: Softening pattern, persistence, perennial legume

Introduction

White clover (*Trifolium repens* L.) is an important perennial legume in south-eastern Australia's high rainfall permanent pasture environments. However, it is highly susceptible to summer moisture stress, leading to population decline. Cultivar development in Australia has focused on improving the persistence of the established plant by selecting for an increased number of branching stolons, with regeneration from seed being a secondary consideration (Lane *et al*. 2000). The timing of seedling emergence is thought to be one of the factors contributing to poor seedling regeneration, with seedlings observed to emerge during late autumn/early winter when temperatures are suboptimal for white clover growth (Archer & Robinson, 1989). The failure of germinable seeds to reach the seedling stage is a common occurrence in white clover with large variability in establishment percentages (9-73%) previously observed (Brock & Kane, 2003). Post-drought seedling regeneration is dependent on soil seed reserves. Archer & Rochester (1982) observed that two years after abundant inflorescences were produced, soil seed reserves were adequate to re-establish the entire sward. Where abundant inflorescences had not been observed in the preceding two years, soil seed reserves were frequently at or below the minimum of 1000-2000 seeds/m^2 suggested by Archer & Rochester (1982) for seedling regeneration to occur. The longevity of soil seed reserves in this species is relatively unknown. In this study, two experiments were conducted using cv. Trophy to quantify annual seed inputs and hard seed breakdown patterns.

Methods

Experiment 1: Hard seed breakdown

The hard seed study was conducted at the Cowra Agricultural Research and Advisory Station, NSW; (S33°48.211' E148°42.236'; average annual rainfall 624 mm). A complete account of the hard seed study, excluding the white clover data, was previously published by Newell *et al.* (2023). Here we present the white clover data compared with 2 subterranean clover (*T. subterraneum* L.) cultivars, Coolamon and Goulburn, and 2 yellow serradella (*Ornithopus compressus* L.) cultivars, Avila and Yellotas. All seed was grown at the Cowra Agricultural Research and Advisory Station in 2018. Pouches containing 100 seeds (clover) or pod segments (serradella) of an individual cultivar were joined together to form strips based on sampling dates. The strips were laid on the soil surface on 18 January 2019. Pouches were harvested at 28, 55, 89, 138, 180 and 320 days post layout. Three replicates per cultivar per sampling date were used. At each harvest date, seed from each pouch was placed on filter paper in a 90mm diameter Petri dish with 3.5ml of distilled water, then incubated in the dark at 15°C for 14 days. After 14 days, any seed that had imbibed or had an emerged radicle was removed. The remaining seeds were considered hard and used to calculate the hard seed percentage.

Experiment 2: Seed production

Plots of white clover cvv. Haifa and Trophy were sown at the Cowra Agricultural Research and Advisory Station in a randomised complete block design on 11 May 2023 at a rate of 4 kg/ha. The seed had previously been scarified and was inoculated with group B rhizobia prior to sowing. The seed was dropped on the surface of a cultivated seed bed and lightly covered with trailing harrows. Single superphosphate was applied at 114 kg/ha at the time of sowing. The plot size was 1 m x 3 m and replicated 3 times. Establishment counts were taken on 20 July 2023 using a 1 m x 1 m quadrat divided into 100 (10 x 10 cm) squares. The number of plants in 10 of these squares on a diagonal transect was recorded. Each plot was counted in 2 different locations.

On 10 November 2023, 10 inflorescences with a senescent peduncle were selected at random from each plot. The inflorescences were dissected, and the number of florets per inflorescence counted. The seed was rubbed out by hand and cleaned using a seed air blower. The total amount of seed was weighed, and four sub-samples of 100 seeds were removed and weighed to calculate the 1000 seed weight. The cessation of flowering for both cultivars occurred by 17 November when the florets of all the inflorescences had senesced. The number of mature inflorescences in a 50 cm x 50 cm quadrant was counted and, along with the seed weight of the 10 inflorescences and the 1000 seed weight, used to calculate seed yield.

Data analysis

All data analysis was performed using the statistical software program R (Version 4.3.1), including the package ‘rstatix’. A two-sample t-test was performed using the ‘t_test’ function to compare floral traits (inflorescence/m^2, florets per inflorescence, seed yield, seeds/m^2 and 1000 seed weight) in cv. Trophy and cv. Haifa. In order to estimate the percentage of seeds softening throughout the year, a polynomial regression analysis was carried out on the softening pattern of cv. Trophy from Experiment 1. The independent variable was days since the start of the experiment and dependent variable was the hard seed percentage. The curve was fitted using the built in ‘lm’ function, The fit of the model was validated with a residual plot; with residuals randomly scattered around zero in no visible pattern. The seed production values for cv. Trophy from Experiment 2 and the polynomial regression equation was used to calculate the number of softened seeds available on a monthly basis through-out the year. The establishment percentage (number of established seedlings per m^2 /number of viable seeds sown per m^2) of cv. Trophy from Experiment 2 was used to estimate the number of germinable seeds expected to survive long enough to develop one trifoliate leaf.

Results

Experiment 1

All cultivars had high initial levels of hard seed ranging from 88–98% (Figure 1). After 55 days, 77% of the white clover cv. Trophy seeds remained hard. The softening rate then increased, with 54% of seed remaining hard at day 89. The seed continued to soften until day 138, when it paused, with 28% of hard seed remaining. The seed began to soften again after 180 days with less than 2% of seed remaining hard after 320 days. The softening pattern of cv. Trophy was most similar to that of cv. Yellotas with <3% difference at days 28 and 55. The rate of softening of cv. Trophy and cv. Yellotas were broadly parallel from day 89 to day 184, after which the rate of softening of cv. Trophy was more rapid than cv. Yellotas. After 320 days, cv. Trophy had the lowest residual hard seed of all the cultivars tested.

In the subterranean clover and the serradella, distinctly different patterns of softening were observed between the cultivars of the same species.

Experiment 2

Seedling density was similar in both cultivars, with cv. Trophy at 115 plants/m^2 (13.4% of seed sown) and cv. Haifa at 107 plants/m^2 (17.9% of seed sown). Flowering commenced for both cultivars at the end of September and had ceased by 17 November. The difference between the cultivars in inflorescence number, floret number, seed production and 1000 seed weight was not significant at *P*=0.05, but with all *P*-values <0.11 (Table 1).

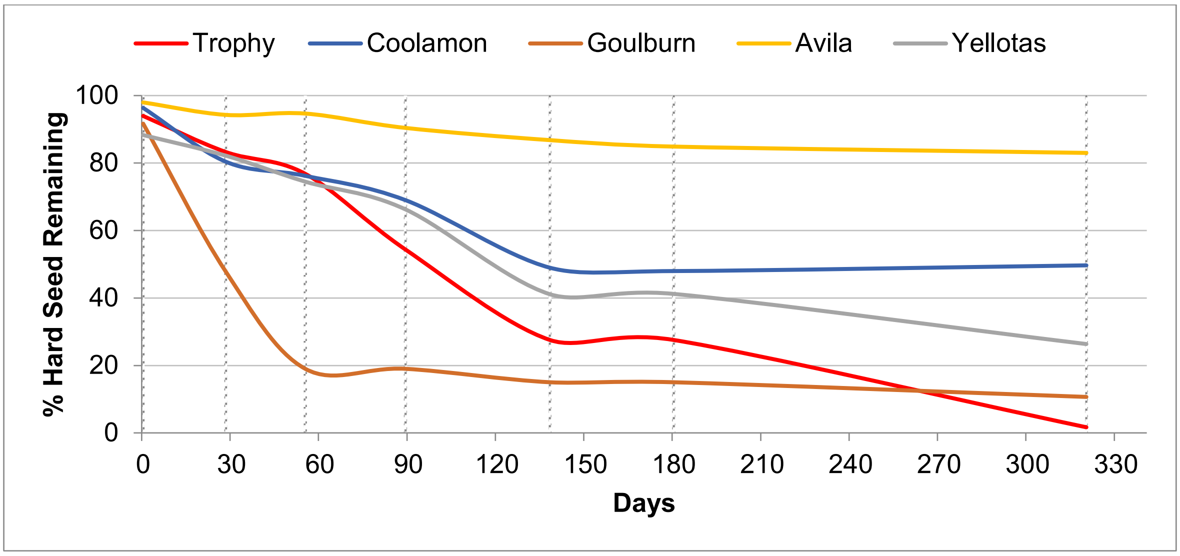

Figure 1. Seed softening patterns of white clover cv Trophy, subterranean clover cvv. Coolamon and Goulburn and yellow serradella cvv. Avila and Yellotas. Dashed vertical lines represent harvest dates.

Table 1. Flowering traits of white clover cvv. Haifa and Trophy, including inflorescence density, florets per inflorescence, seed yield, seed density, and 1000-seed weight.

	Inflorescence/m^2	Florets per inflorescence	Seeds yield (kg/ha)	Seeds/m^2	1000 seed weight (g)
Haifa	456	90	696	119774	0.58
Trophy	360	77	454	84 880	0.53
P-value	0.07	0.07	0.09	0.09	0.11

Modelling

The polynomial regression equation ($y = 0.0008x^2 - 0.545x + 97.239$, $r^2 = 0.94$) predicted that number of softened seeds available per month peaked at ~13 000 at day 30, declining to ~1000 at day 330. The number of softened seeds making it to seedling stage was predicted to be ~1800 at day 30, declining to 140 by day 330 (Figure 2).

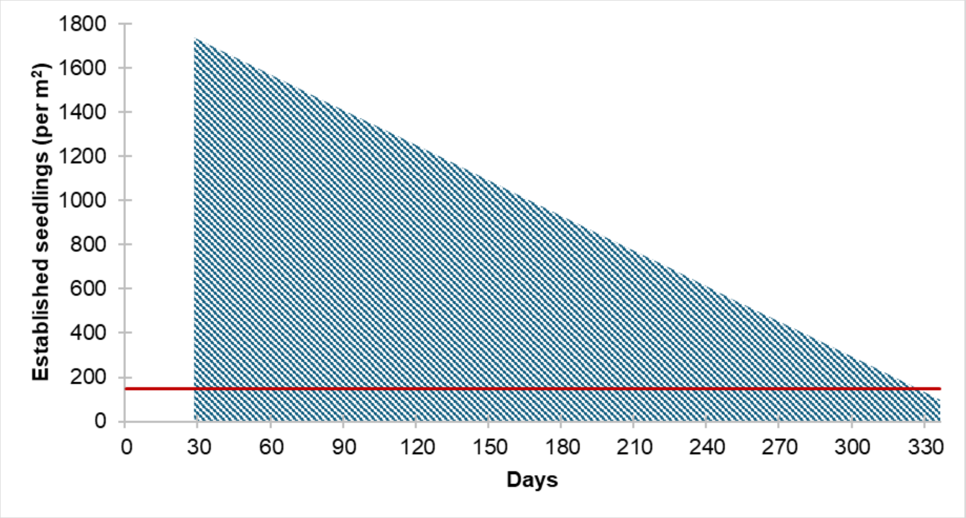

Figure 2. Estimated seedlings of cv. Trophy reaching one trifoliate leaf stage that emerge from hard seeds. Shaded area represents estimated seedling establishment. Solid red line represents the assumed minimum 150 plants/m^2 required to reestablish a sward. A polynomial regression curve was fitted to the seed softening pattern of cv. Trophy to estimate the percentage of seeds softening throughout the year. Seed production values from Experiment 2 and the polynomial regression curve were used to calculate the number of softened seeds available on a monthly basis. The expected number of softened seeds to result in a seedling was estimated using the establishment percentage from Experiment 2.

Discussion

The seed softening pattern of cv. Trophy showed a steady decline from the start of the experiment until day 55 (early autumn) after which the rate of softening increased. This is consistent with the findings of Kelman & Blumenthal (1992) that suggested white clover needs declining temperatures for seed softening to occur. The pause in softening between day 138 (the start of winter) and day 180 (mid-winter) would indicate that winter temperatures were unsuitable to facilitate the softening of the seed. This could be due to insufficient day/night temperature fluctuations at this time of year, as white clover requires fluctuating temperatures for softening (Robinson, 1960; Van Assche *et al.*, 2003).

Robinson (1960) and Van Assche *et al.* (2003) proposed that white clover requires a period of exposure to cold temperatures (<5°C) before day/night temperature fluctuations facilitates the softening of the seed. This was also observed in the present study with the resumption of softening past day 180. The results of the current study suggest that white clover responds to two different environmental conditions for seed softening to occur, switching between them as the seasons change. The first is the ‘Mediterranean type environment’ categorised by high summer temperatures and higher day/night temperature fluctuations (Quinlivan, 1961). The second is the ‘temperate type environment’ categorised by low winter temperatures followed by more minor temperature fluctuations (Van Assche et al., 2003). These two distinct periods of softening resulted in little hard seed remaining in less than 12 months. This could explain why white clover struggles to maintain sufficient soil seed reserves except after periods of prolific flowering (Archer & Rochester, 1982) and points to the requirement for regular flowering of white clover for long-term persistence (Hayes et al. 2019).

The previously described model was used to estimate the required annual seed inputs to maintain the seed bank at adequate levels. One hundred and fifty plants/m^2 was assumed as the minimum requirement to reestablish an entire white clover population (Praat *et al.*, 1996). Assuming the loss of the entire population of mature white clover plants and no existing seedbank, cv. Trophy would require 30 000 seeds/m^2 or ~160 kg/ha (~130 inflorescences/m^2) to set the previous year for seed reserves to be high enough to supply >1100 soft seeds/m^2 every month, for seedling regeneration to occur between late summer and mid-spring. Flowering would then be required again to replenish soil seed reserves, should seedling regeneration be required again in the following year. The seed production observed in this study easily exceeds the modelled requirements, however no grazing occurred during the flowering period. Chapman and Anderson (1987) observed that grazing may remove 89-97% of inflorescences. If assumed losses of 89-97% due to grazing are applied to the inflorescence production in this study, total seed input falls to 2500-9000. This is well below the modelled requirements.

Kelman & Blumenthal (1992) produced perhaps the only published softening pattern of cv. Haifa. It was conducted in a growth chamber under a fluctating temperature regime, with the temperatures changed periodically to represent seasonal changes. At the start of that experiment, 95% of of the cv. Haifa seed was hard but softened rapidly to only 18% after 5 months (Kelman & Blumenthal, 1992), compared to ~25% in cv. Trophy after the same duration in the present study. The pattern of softening of cv. Haifa as seen in Kelman & Blumenthal (1992) appears to be more rapid than that of cv. Trophy in this study, which may simply reflect the different methodologies used but supports the general conclusion that a low proportion of residual hard seed remains in white clover after 12 months, requiring on-going seed production to maintain soil seed reserves.

Further research should look to validate the responses in both flowering attributes and hard seed characteristics over a broader range of locations and seasonal conditions. Considering that Chapman and Anderson (1987) observed that 65% of white clover seed disappeared to unknown causes, the required annual seed inputs may be greater than is suggested here.

Conclusion

The softening pattern of hard seeds in Trophy white clover, where softening occurs in both autumn and spring, limits the cultivar’s ability to establish a persistent seed bank. To maintain adequate soil seed reserves for full regeneration to occur until mid-spring, annual seed inputs must exceed 30 000 seeds/m², or ~160 kg/ha. These findings highlight the need for consistent seed production to foster adequate regeneration.

Acknowledgements

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Notes:

Improving seed germination performance of arrowleaf clover (*Trifolium vesiculosum* Savi.)

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Abstract: *The germination performance of five arrowleaf clover cultivars; Cefalu, Seelu, Zulu1, Zulu2 and Arrotas were characterised. Physical dormancy preventing water uptake by the seed was found to be the major factor inhibiting germination. Mechanical scarification for 40 seconds proved the most successful treatment in improving germination percentages in all 5 cultivars. Maturity was found to strongly influence germination. The germination % of seeds harvested earlier in the season was greater than seeds harvested later untreated. Seeds harvested later had a higher level of hardseededness, and a higher level and rate of germination following scarification than seeds harvested early in the season. Seed colour was observed as an indicator of maturity. Seeds graded into orange, brown and red sub lots contained greater proportions of hard seed indicating advanced maturity. Yellow sub lot had a higher germination %, contained few hard seeds and took longer to germinate indicating pre-maturity. Seed density was also observed as an indicator or maturity. Germination % increased with increasing density for seed harvested earlier in the season but decreased with increasing density for seed harvested later in the season. The germination % of both early and late seed lots increased with increasing density once scarified for 40 seconds. A linkage between seed maturity and seed colour, and also seed maturity and seed density was found. Germination percentages of seeds in soil were less than recorded for the same seed lot tested under standard laboratory conditions. In conclusion, industry reports of poor establishment in the first season could be due to either hardseededness or inappropriate scarification or that the seed used may have been immature.*

Notes:

Changes in harvest seed retention characteristics during maturation of subterranean clover (*Trifolium subterraneum* L.)

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Abstract: *Subterranean clover (*Trifolium subterraneum *L.; subclover) remains the most widely sown annual pasture legume in southern Australia due largely to its distinct trait of seed burial which promotes high levels of persistence. However, seed burial makes seed harvesting inherently difficult. Current harvesting methods are effective yet inefficient and cause soil degradation. Swathing offers an alternative in which plant roots are cut, plants are lifted and windrowed, and the dried plants later harvested with a combine. As swathing occurs much earlier than conventional subclover seed harvesting, the key to success is timing: maximising seed retention while ensuring sufficient seed quality and yield. To investigate this trade-off, plants from six subclover cultivars were harvested on nine or ten dates during seed development. Seed retention was quantified through measurement of the attachment strength of seed-containing burrs to peduncles and harvest loss determined during sampling. Burr attachment strength decreased over time for all cultivars, while harvest loss was variable but generally increased over time, indicating that earlier swathing is desirable for seed retention. Burr attachment strength may be a valuable predictor of harvest or swathing loss, but only when plants are less mature.*

Key words: swathing efficiency, harvest efficiency, peduncle

Introduction

Subterranean clover (*Trifolium subterraneum* L.; subclover) remains the most widely sown annual pasture legume in southern Australia due, in part, to its shallow burial of seed-containing burrs (Nichols *et al*. 2012). This seed-burial trait promotes strong persistence yet makes the seed harvesting process inherently difficult and costly (Moss *et al*. 2021a). To overcome the inefficiencies and environmental degradation associated with the current subclover seed harvesting method, Moss *et al*. (2022) suggest a swathing approach using technology adapted from the peanut (*Arachis hypogaea* L.) industry: this involves cutting plant roots prior to full crop maturity, windrowing plants and then collecting dry plants with a combine harvester. This approach shows promise, but success is dependent on timing. Swathing must occur while stems and peduncles retain some sap to ensure seed-containing burrs remain attached to plants. However, swathing plants too early may have a detrimental impact on seed yield and quality as seeds are immature. Similar issues are seen for peanuts, where pod losses during swathing (or 'digging') increase over time and digging "too late" results in large yield losses (Colvin *et al*. 2018; Sorensen *et al*. 2015, 2017). Digging losses are often related to peg strength – the strength of the attachment between a peanut pod and the stalk or gynophore attached to it – with weakened pegs blamed, in part, for the losses (Sorensen *et al*. 2015; Thomas *et al*. 1983). While this relationship is well-established for peanuts, little research has examined the relationship between burr to peduncle attachment strength and swathing losses for subclover. As part of an overall goal to understand trends in seed quality and burr retention for prediction of ideal timing for swathing subclover, we examined changes in burr attachment strength and harvest loss during seed maturation and senescence for six subclover cultivars.

Methods

Six Australian subclover cultivars, representing two maturity types from each of the three subspecies (Table 1), were planted in four replicated field plots each containing four rows of 48 plants at the UWA Shenton Park Field Station (31.95º S, 115.80º E) from May 8, 2023. Plots were irrigated twice weekly until November 2 for the early flowering cultivars (Dalkeith, Monti and Mawson) or until November 9 for the late flowering cultivars (Carver, Napier and Antas). Sampling occurred weekly from October 23 to December 14 with an additional sample taken in late January or early February 2024 to represent the timing of conventional subclover seed harvesting. This resulted in the number of sampling dates being nine for the late flowering cultivars and ten for the early flowering cultivars.

Table 1. The six subclover cultivars studied. Maturity type describes flowering trends. Date of first flower is the date when at least 50% of plants had at least one open flower: the mean of four plots is presented. The corresponding number of days from seeding to first flowering (DFF) is also given.

Subspecies	Cultivar	Maturity type	First flower	DFF	Cultivar	Maturity type	First flower	DFF
subterraneum	Dalkeith	Early	20 August	104	Carver	Late	12 September	127
yanninicum	Monti	Early	30 August	114	Napier	Late	17 September	132
brachycalycinum	Mawson	Early	1 August	85	Antas	Late	22 September	135

Harvest loss

On each sampling date, a quadrat (20 cm x 10 cm) was placed in each plot. The perimeter of each quadrat was cut with a knife and plant material and attached burrs within the quadrat area were lifted and collected. Any burrs “left behind” were collected using a shovel and sieve and kept separately as a proxy for swathing losses. Burr samples collected with the plant material and those left behind were both threshed and cleaned, and the seeds weighed. Harvest loss, representing the weight of seeds left behind as a proportion of total seed yield, was then calculated.

Burr attachment strength

From each quadrat sample, five burrs with intact, connected peduncles were randomly selected. Peduncles still attached to the plant were carefully cut at the peduncle-stem junction. Within eight hours of collection, the tensile force required to separate each burr from its peduncle was measured according to methods of Moss *et al.* (2021b). Measurements of seed quality and plant development stage were also collected but are not reported here.

Statistical analysis

RStudio (RStudio, Boston, MA, USA, version 4.3.3) was used to create strip plots and analyse the effect of cultivar, plot, sampling date and their interactions on burr attachment strength (linear regression; date inverse-transformed to improve model fit) and harvest loss (beta regression). Beta regression tested the effect of cultivar, plot and burr attachment strength on harvest loss. In all three models, interactions were significant ($P<0.05$) so the effect of either date or burr attachment strength and their interaction with plot was analysed for each cultivar. For burr attachment strength, a non-transformed linear model was compared to an inverse-transformed model for each cultivar and the better model was used for analysis.

Results

Burr attachment strength for each cultivar decreased over time (Figure 1; Table 2). For Dalkeith, there was an interaction between plot and date but this was not the case for any other cultivar. R^2 values for these models ranged from 0.019 – 0.315.

Harvest loss increased over time for Monti, Mawson, Carver and Napier (Figure 1; Table 2). However, for Dalkeith and Antas, harvest loss did not change with time. For Monti, Carver and Napier there was an interaction between plot and date.

There was a significant relationship between burr attachment strength and harvest loss for Carver and Napier (Figure 2; Table 2), with an interaction between burr attachment strength and plot for Carver but not Napier. However, for the remaining cultivars there was no relationship between burr attachment strength and harvest loss.

Table 2: Effect of date on burr attachment strength (linear regression) and harvest loss (beta regression) and effect of burr attachment strength on harvest loss (beta regression), with plot interactions included. Results are for six subclover cultivars harvested on nine or ten dates from October 2023 to February 2024. Harvest loss was measured for one quadrat sample from four plots of each cultivar. Burr attachment strength was measured on five burr-peduncle samples from each quadrat. For linear regression models the model form and R^2 value is given. Significance: n.s. $P > 0.05$, * $0.01< P < 0.05$, ** $0.001< P < 0.01$ and *** $P < 0.001$.

Response variable		Burr attachment strength			Harvest loss		Harvest loss	
			P-values		Chisq-values		Chisq-values	
Cultivar	Model	R^2	Date	Date x plot	Date	Date x Plot	Burr attachment strength	Burr attachment strength x plot
Dalkeith	Inverse	0.22	***	**	n.s.	n.s.	n.s.	*
Carver	Inverse	0.32	***	n.s.	***	**	***	***
Monti	Linear	0.21	***	n.s.	***	*	n.s.	n.s.
Napier	Inverse	0.23	***	n.s.	***	*	***	n.s.
Mawson	Linear	0.02	**	n.s.	***	n.s.	n.s.	*
Antas	Inverse	0.08	***	n.s.	n.s.	*	n.s.	n.s.

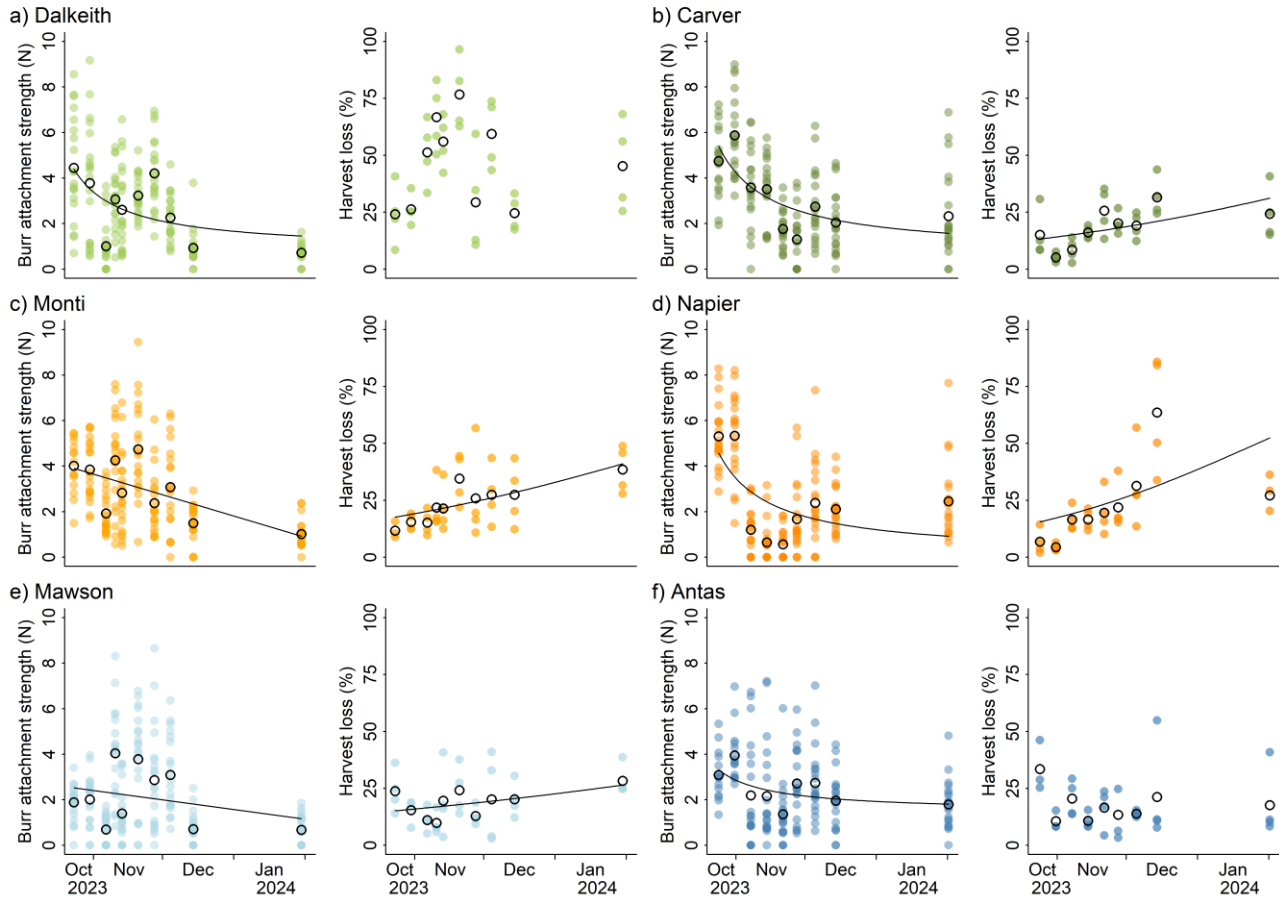

Figure 1: Changes in burr attachment strength and harvest loss over time for subclover cultivars (a) Dalkeith, (b) Carver, (c) Monti, (d) Napier, (e) Mawson, and (f) Antas. Data were collected on nine (b, d, f) or ten (a, c, e) dates from one quadrat sample (harvest loss; n=4) or five burr-peduncle samples (burr attachment strength; n=20) in each of four replicate plots for each cultivar. Each solid, coloured circle represents a sample while hollow circles represent the means. The relationship between burr attachment strength or harvest loss and date, if significant, is shown with a line. Statistical outcomes are shown in Table 2.

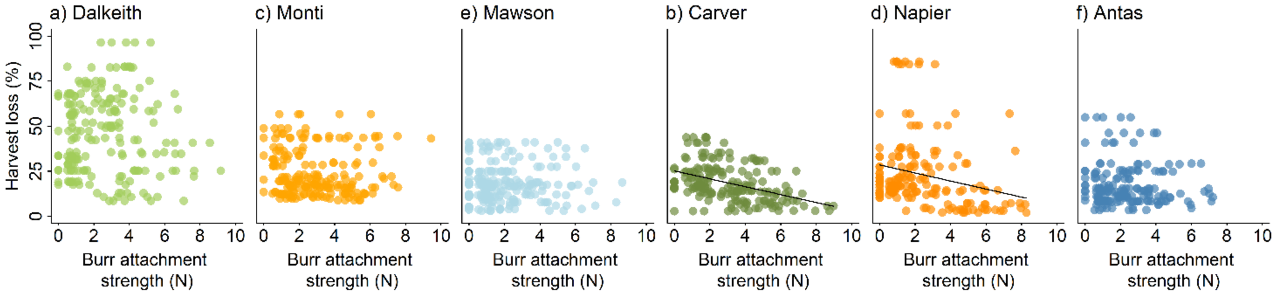

Figure 2: Relationship between burr attachment strength and harvest loss during seed maturity for subclover cultivars (a) Dalkeith, (b) Carver, (c) Monti, (d) Napier, (e) Mawson, and (f) Antas harvested on nine (b, d, f) or ten (a, c, e) dates. Harvest loss was measured for one quadrat sample in each of four replicate plots while burr attachment strength was measured for five burr-peduncle samples in each quadrat. Each solid, coloured circle represents a sample. The relationship between burr attachment strength and harvest loss, if significant, is shown with a line. Statistical outcomes are shown in Table 2.

Discussion

For peanuts, prior to digging, peg strength tends to decrease over time while pod loss tends to increase (Colvin *et al*. 2018; Sorensen *et al*. 2015, 2017). Similarly, burr attachment strength in subclover tended to decrease over time for subclover Dalkeith and Monti (following an increase at very early sampling dates) in trials by Moss *et al*. (2021b) and for Dalkeith, Carver, Monti, Napier, Mawson and Antas in our study. However, harvest or swathing losses, which have not yet been well-studied for subclover, appear less consistent. While harvest loss increases over time for Monti, Mawson, Carver and Napier, matching trends for peanut pod loss, for cultivars Dalkeith and Antas there are no changes. Although harvest loss as measured in this study is not equivalent to swathing seed loss, it is assumed that trends in harvest loss should be indicative of trends in swathing loss. Therefore, for four cultivars these results suggests that earlier swathing will increase seed retention and swathing efficiency. For Dalkeith and Antas, however, outcomes are less clear. Dalkeith showed high levels of harvest loss and strong variability, suggesting that this cultivar may be more susceptible to external, environmental factors than the other cultivars. Further investigation is warranted to support future implementation of a swathing technique. Antas belongs to ssp. *brachycalycinum* which tend not to have strong burr burial traits (Francis *et al*. 1972; Katznelson and Morley 1965). As cultivar Mawson, also ssp. *brachycalycinum*, shows a very gentle increase in sampling harvest loss over time, perhaps this subspecies is less prone to swathing losses.

Examination of the relationship between burr attachment strength and sampling harvest loss suggests for the early maturity type cultivars, in addition to Antas, burr attachment strength is not a good predictor of harvest loss. However, there is a relationship between the two variables for Carver and Napier. As later maturing varieties, Carver and Napier were generally at earlier stages of maturity compared to cultivars Dalkeith, Monti and Mawson on the same sampling dates and data for these cultivars were therefore captured from a greater range of plant development stages. Therefore, these results may suggest that at less advanced stages of maturity, burr attachment strength is a good predictor of harvest loss, while as maturity advances this relationship becomes less reliable. This is also supported by a decrease in variance with increasing burr attachment strength (corresponding to earlier dates) for Carver and Napier in Figure 2. Perhaps, as burr attachment weakens, the impact of factors such as soil texture and moisture, burr morphology and depth of burial on harvest loss increase. The indeterminate nature of subclover may also contribute to large variability in measurements.

In many cases, an interaction between plot and the predictor variable of interest indicates there was some spatial variability within the testing site which impacted relationships between date, burr attachment strength and harvest loss. As the site was uniform in terms of soil type, slope and preparation, it is likely that much of this variability was driven by inconsistencies in irrigation management. These results therefore imply the relationships presented may change significantly across locations differing in soil type or climate.

Conclusions

The attachment strength of seed-containing subclover burrs to peduncles decreased over time, while harvest loss tended to increase, suggesting that earlier swathing will promote greater seed retention and greater swathing efficiency. However, harvest loss for cultivars Dalkeith and Antas did not change over time. Results also suggest that burr attachment strength may be a useful indicator of harvest loss, but only for greener, less mature plants. Further research is warranted to investigate whether these relationships hold true during field-scale swathing operations. To identify the ideal time for swathing subclover, an understanding of changes in seed quality over time and its relationship with seed retention is crucial and will be a focus of future research.

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Notes:

Changes in pasture and soil properties with liming and superphosphate application over 12 years, on a range of soils in the Central Tablelands of New South Wales

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Abstract:
Context. The decision to apply lime to pastures requires an understanding of its enduring effects on pasture and soil properties across contrasting soil types.

Aims. This study examined historical data to quantify the enduring effect of lime, with contrasting rates of superphosphate, on soil chemistry and pasture productivity over 12 years.

Methods. Five experimental sites were established in 1978 on the Central Tablelands of New South Wales, Australia.

Key results. Averaged across the 5 experimental sites, where the cation exchange capacity was 2.34–5.16 cmol(+)/kg, initial lime application resulted in an increase of 0.3 pH units/t lime applied. After 12 years, an increase in pH equating to 0.1 pH units/t lime was still observed. In the absence of lime, soil pH declined by ~0.02 pH units/year. Averaged across all lime treatments at all sites, the annual pH decline was ~0.07 pH units/year. Lime generally increased exchangeable calcium and magnesium, and decreased aluminium throughout the 12-year study period: the longevity of response generally proportional to the quantum of lime applied. Where differences in exchangeable potassium and available soil P due to lime existed, both properties were generally lower where the heavier rates of lime were applied. Annual applications of superphosphate were associated with lower soil pH and exchangeable soil K, and higher Al saturation, but differences did not emerge until between 2 and 7 years after the experiment commenced. Lime generally increased pasture biomass, although differences between the highest and nil lime treatments declined progressively. There was an almost universal positive response in annual DM associated with superphosphate application. Lime had little effect on the composition of pastures dominated by D. glomerata, in contrast to superphosphate, which often increased the content of D. glomerata and/or T. subterraneum and reduced the percentage of weeds. Lime consistently increased the productivity of M. sativa, but this was a minor sward component and failed to persist at most sites. Pasture yields generally increased with %Ca but yield responses to %Al were more variable.

Implications. Lime had an enduring legacy on pasture productivity and soil properties, still evident after 12 years following application across contrasting soils.

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Notes:

Rapid assessment of nutritional value and performance of pasture species in the field using chlorophyll fluorescence

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Abstract: *To identify and address in-season reductions in pasture productivity and quality there is a growing need for rapid, accurate and non-destructive methods to assess plant performance in the field. Here, we tested the suitability of rapid in-field chlorophyll fluorescence measures for predicting pasture productivity and nutritional values in perennial ryegrass (Lolium perenne) and white clover (Trifolium repens), under differing nitrogen fertilisation rates at the Tasmanian Dairy Research Facility in Elliot, Tasmania.*
Effects of variable synthetic nitrogen (N) fertilisation application rates on pasture productivity and nutritional quality, alongside the accuracy of rapid chlorophyll fluorescence measures to capture these parameters, were evaluated twice in a replicated block design experiment across the growing season in collaboration with the Dairy High2 Research Program. White clover and perennial ryegrass (grown at an 80:20 species ratio) under either 300 kg N/ha or 150 kg N/ha were measured at the leaf-level by chlorophyll fluorescence alongside comprehensive leaf physiology measures including net CO_2 *assimilation for validation of plant performance, and elemental analysis of nutritional content of the forage. Additional data from the High2 trial including environmental conditions, soil conditions and milk production parameters were also integrated to further validate the predictive model. We demonstrate that rapid chlorophyll fluorescence measures can capture the impacts of varying nitrogen fertilisation levels, alongside other dynamic in-field stress conditions, on ryegrass and white clover performance and nutritional value. This tool will support improved management strategies towards mitigating environmental and resource stress impacts on pasture productivity and sustainability.*

Notes:

Root morphology and phosphorus requirements of tropical grasses and legumes

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Abstract:

Context: Tropical pasture species are often grown in nutrient-deficient soils that can limit plant productivity and persistence. However, little is known about the root traits and critical phosphorus (P) requirements of many tropical grasses and legumes. Characterisation of these traits would enable P-efficient species to be used when soil fertility is poor and agricultural inputs are limited, or for important root traits to be selected when breeding for P efficiency.

Aims: To assess the shoot yield, root morphology and critical P requirements of a range of commonly-grown tropical pasture species.

Methods: Five tropical grasses and seven tropical legumes were grown in pots to investigate shoot and root responses to applied P (0–120 mg P kg^{-1} *soil) in a low-P soil.*

Key Results: The shoot yield of each species increased in response to applied P, yet there were differences in maximum shoot yield (1.7–9.8 g DM pot^{-1}*) and critical external P requirements (12.8–38.0 mg P kg*$^{-1}$ *soil) among the species. The acquisition of P was primarily associated with the development of root length when plants were grown in soil that enabled near-maximum growth (e.g.* $R^2 = 0.38$*–0.40 in the 15–30 mg P kg*$^{-1}$ *soil treatments).*

Conclusions: There are differences in root morphology and P-acquisition efficiency among tropical pasture species that could influence species selection and nutrient management.

Implications: Phosphorus-efficient species should be grown in soils that are known to be P deficient, or where P fertilisation is not an option, and species could be paired based on their P requirements.

Notes:

Native Pasture Restoration in the Kimberley Rangelands, Western Australia - Vegetative propagation of Bundle bundle (*Dichanthium fecundum*) and Ribbon grass (*Chrysopogon pallidus*) from wild collected plants.

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Abstract: *The pastoral industry has a long association with the Kimberley region of Western Australia. As a result of past land management practices, there has been a loss of some of the more desirable pasture grasses from these rangelands. Direct seeding is understood to be an economical means to return native plants to degraded landscapes. However, with increasing demand combined with poor seed production it is becoming more difficult to source adequate seed for rangeland rehabilitation. In response, research was undertaken to evaluate the effectiveness of using vegetative propagation of two native grass species to produce tubestock for planting as an alternative to overcome the difficulty of sourcing seed. Bundle bundle (Dichanthium fecundum) was tested using one 18-month-old plant grown from seed under nursery conditions. Twenty-seven plants were propagated with 81% of plants surviving after 6 months growing in glasshouse conditions. Ribbon grass (Chrysopogon pallidus) was tested by harvesting tussocks of wild-grown plants collected in the Kimberley region and transported back to Perth for division. A total of eight wild harvested tussocks were divided into 72 plants with 96% surviving after six months growing in a glasshouse then nursery. Fifty of these plants were chosen for planting in a seed production area (SPA) in Perth. With the addition of fertiliser and water, 100% of plants survived. Twelve months after planting out in the SPA, C. pallidus plants began to flower and set seed. Seed quality (% seed fill) was 27-fold greater in seed collected from the SPA than wild collected seed.*

Key words: tubestock, vegetative propagation, rangelands,

Introduction

The pastoral industry in the Kimberley region of Western Australia is an important economic contributor to the state. However, as result of past land management practices and variable wet seasons, there has been a decline of the more desirable native pasture grasses resulting in a loss of feed-base productivity. To reverse this decline, research is being conducted to help restore important native pasture grasses in this region. Direct seeding is understood to be an economical means to return native plants to large landscapes, however, it is becoming more difficult to source adequate seed numbers for restoration and rehabilitation purposes due to increasing demand and poor seed production (Neville *et al.* 2016). Establishing a Seed Production Area (SPA) is considered a viable option for commercial seed production, however, for some species seed production may be naturally low or of poor quality (measured by seed fill).

Dicanthium fecundum (Bundle bundle, Curly bluegrass) is a desirable, long lived tussock grass generally found on plains and loam to clay soils in the Kimberley rangelands, often growing in association with other perennial grass species (Ryan et al. 2013). Wild seed collection of this species has generally been successful, but seed-fill (as a measure of seed viability) can be variable (Golos and Revell 2025). *Chrysopogon pallidus* (Ribbon grass) is also an important component of more productive pastures in the Kimberley rangelands, growing in clay soils on alluvial plains (Schoknecht and Payne 2010; Ryan *et al.* 2013). After three years of seed collecting, only a limited quantity of seed has been collected as flowering seems to be early in the wet season and limited to areas that have been burnt prior to the commencement of the wet season. Also, the seed quality has been very poor with seed fill ranging from 0% to 3%.

In response, research was proposed to evaluate the effectiveness of using vegetative propagation to produce tubestock for planting as an alternative to overcome the difficulty of sourcing seed. For *C. pallidus* in particular, vegetative propagation of tubestock from wild harvested tussocks may be an alternative source of plants for pasture restoration. As well as direct planting of tubestock in restoration sites, tubestock can be planted out in a SPA and, with suitable agronomic practices (irrigation and fertiliser), seed production and seed quality maybe improved (Golos and Revell 2025).

Methods

Locations

Wild seed was collected from *Chrysopogon pallidus* and *Dichanthium fecundum* plants growing on Napier Downs (17°19'41"S 124°49'48"E) and Mount House Stations (17°02'57"S 125°42'09"E) located in the West

Kimberley Region of Western Australia. The climate is tropical savanna (Koppen) with hot humid summers (BOM 2024). Both species were found growing in cracking clay soils on alluvial plains.

Due to logistical and accessibility issues in setting up an experimental Seed Production Area (SPA) in the Kimberley, a trial site was established at the University of Western Australia (UWA) research facility in Shenton Park, Perth (31°57'4.8"S 115°47'37.5"E) (Golos and Revell 2025). The soils are yellow-brown sands of the Spearwood dune system of the Swan Coastal Plain (McArthur and Bettenay 1960). The climate is subtropical/Mediterranean, with a warm dry summer and cold wet winter (BOM 2024).

Vegetative propagation of tubestock from nursery grown plant

The potential for using tussock division for propagating grasses for planting was initially trialed with *D. fecundum* in February 2023. A one-year-old plant propagated from seed and grown-on in a 4-litre pot in a nursery was selected for division. Culms/leaves were cut to 10 cm height and the root ball cut to 12 cm length. The tussock was then gently pulled apart to ensure minimal loss of roots for each tiller. Some divisions contained multiple tillers where attached tillers had not yet developed their own roots. All divisions were potted (plastic tube 5 cm square x 12 cm deep) into a proprietary potting mix (supplied by Richgro, Jandakot, Western Australia) and grown in nursery conditions.

Vegetative propagation of tubestock from wild collected plants

To trial vegetative propagation of *C. pallidus*, eight tussocks were dug up from two locations (five from Mulgowie paddock and three from Narradong paddock) on Napier Downs station on the 25th of August 2023. Tussocks were dug to a minimum of 25 cm depth to recover as much roots as possible, any plant material with no or badly damaged roots was discarded. Excess soil was carefully removed by breaking the tussock into smaller clumps to avoid damaging roots, and leaves cut back to approximately 10 cm. All plant material was wrapped in wet newspaper and placed in a plastic bag containing a small amount of water and sealed for transport to Perth. Plants were stored indoors at ambient temperature before further division and planting into pots on the 28th of August 2023.

Prior to further division, plant material was placed into buckets of water to soften the hard setting clay to aid division of clumps into individual tillers with intact roots. The method followed was the same as for *D. fecundum* (see above). Some small clumps containing multiple tillers were kept intact and planted into larger pots to ensure the survival of some plant material for all 8 eight tussocks sampled. A total of 72 potted plants ranging from 4 to 12 pots/tussock was produced (Table 2). All pots were put into a glasshouse with automated ventilation and irrigated 2-3 times a day.

Planting of tubestock in the seed production trial site

Seven months after propagation, fifty *C. pallidus* plants were chosen for planting in the SPA on 5-6 February 2024. To plant tubestock, fertiliser (Seamungus® by Neutrog Australia) was added (1 handful) to the planting hole and mixed with soil before planting. Plants were hand watered for two weeks after planting, plus received overhead irrigation (approximately 30 minutes, 2-3 times/week) that had previously commenced in the SPA on 27th November 2023. All plants were fertilised with NPK Blue (Baileys Fertilisers, Western Australia) on the 26th of February and 25th of November 2024. Seed was collected from *C. pallidus* weekly once flowering had commenced.

Seed harvesting

All seed harvesting, from the wild and SPA, was done by hand by lightly pulling at inflorescences to remove ripe florets and dropping into a bucket. Grass florets were x-rayed to determine seed fill using a Faxitron Specimen Radiography System (MX-20 Cabinet X-ray Unit) (Faxitron, Wheeling, Illinois, USA).

Results

The one-year-old *D. fecundum* plant propagated from seed and grown-on in a 4-litre pot in a nursery, successfully produced 22 out of a total 27 divisions (81.5% survival). Of the 72 potted plants of *C. pallidus*, only 3 plants died (95.8% survival), all being single tiller divisions in tubes representing 93.5% of tubes (Table 1). All fifty (100%) of the *C. pallidus* plants planted in the SPA have survived one year after planting. During the second summer (12 months after planting) 60% of plants began to flower (65% from Mulgowie, 52% from Narradong) from January to February. Ripe seed was collected from the SPA from 7th February to 11th March 2025. Mean seed fill was 36.2% (n=6) for seed collected from the SPA (Figure 1) compared to 1.3% (n=2) from wild collected seed.

Table 1. Number of plants propagated from eight *Chrysopogon pallidus* tussocks collected from paddocks on Napier Downs pastoral station and their survival. A selection of these plants was transplanted to a Seed Production Area (SPA) in Perth.

Plant Identification	Location	Divisions	survived	% survival	planted in SPA
PGOL NP2309-1	Mulgowie	6	6	100.0	5
PGOL NP2309-2	Mulgowie	12	11	91.7	7
PGOL NP2309-3	Mulgowie	4	4	100.0	3
PGOL NP2309-4	Mulgowie	10	10	100.0	7
PGOL NP2309-5	Mulgowie	9	9	100.0	7
PGOL NP2310-1	Narradong	6	6	100.0	5
PGOL NP2310-2	Narradong	11	10	90.9	8
PGOL NP2310-3	Narradong	14	13	92.9	8
Total		72	69	95.8	50

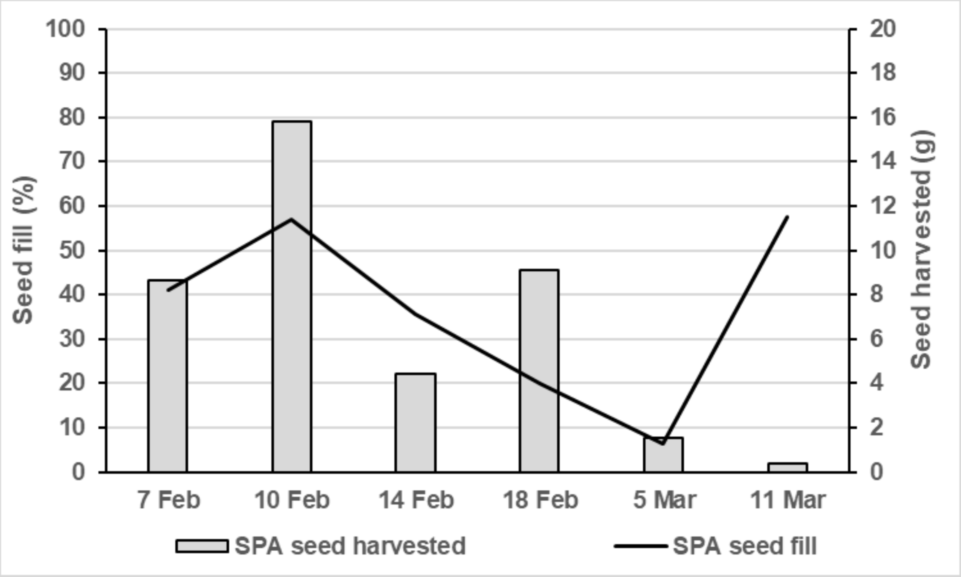

Figure 1. Seed harvested (g) and seed fill (%) of *Chrysopogon pallidus* seed collected from the Seed Production Area (SPA) in Perth, Western Australia during the summer of 2025. Note: seed fill of wild collected seed averaged 1.3%.

Discussion

This research has demonstrated that tubestock can be successfully produced vegetatively by division of both nursery produced and wild harvested grass tussocks with high rates of survival. Further, tubestock produced vegetatively can be successfully planted out under favourable agronomic conditions (irrigation and fertiliser) provided in a SPA. Another study found survival of grasses propagated from tillers in glasshouse conditions can be high, but highly variable between species (Matheus *et al.* 2025). Further research is required to trial planting tubestock under less favourable conditions in rangelands, although another study found high survival rates of grasses planted in post-mine sites that were propagated from tillers (Figueiredo *et al.* 2018).

The motivation to trial the vegetative propagation of *C. pallidus* was due to the very low fill of wild collected seed. Importantly, seed quality from plants grown under favourable agronomic conditions resulted in a large quantity of seed with 27-fold greater seed fill than wild collected seed. This suggests that SPA's could fulfill the seed requirements for restoring this species in degraded rangelands and possibly negate the need for planting of tubestock. Another interesting observation was that *C. pallidus* in the SPA was flowering 1 year after planting (during the 2nd summer). This contrasts with wild populations where no flowering was found in grasses unless they had been burnt before the onset of the summer wet season. It is unclear what the exact mechanism is that stimulates flowering of *C. pallidus,* but it seems that disturbance and dividing of mature tussocks may somehow partially replicate fire, as only 60% of plants in the SPA flowered compared to nearly 100% of wild plants burnt by fire. It is unclear if *C. pallidus* plants growing in the SPA will flower in following years or will require fire or perhaps cutting back to stimulate flowering and hence seed production.

D. fecundum generally establishes well from seed, but vegetative propagation from tussocks can also be used to expand a seed production area of this species. Other perennial grass species forming tussocks may also be suited to vegetative propagation through separation of tillers.

Conclusions

This research successfully demonstrated that wild harvested tussocks of *Chrysopogon pallidus* can be successfully divided to produce tubestock for planting. In addition, planting of tubestock in a SPA and grown with the addition of irrigation and fertiliser resulted in improved seed quality (seed fill) compared to wild harvested seed. Further research is required to test the feasibility of using nursery produced tubestock for planting in degraded sites and compared to seeding to determine the most cost-effective method of returning *C. pallidus* to degraded rangelands. Vegetative propagation of *Dichanthium fecundum* can also be used as a technique to cultivate plants for a seed production area.

Acknowledgments

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Notes:

Problems with the symbiotic competence of sainfoin (*Onobrychis viciifolia*) in Australia

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Abstract: *There has been sporadic interest in the perennial legume, sainfoin (Onobrychis viciifolia), since its introduction to Australia in the 1970's, however, the species has never achieved commercial success. Poor symbiotic competence resulting in sub-optimal nitrogen (N_2) fixation is one factor that may contribute to its ongoing niche status. The perceived benefits of sainfoin flowers for honey bees, as well as the non-bloating and antimethanogenic properties of its herbage, are some of the factors prompting renewed interest in the species. Inquiries for commercial sainfoin rhizobia inoculum in the early 2020's initiated a revival of the sainfoin mother culture by the Australian Inoculants Research Group (AIRG) in NSW, following several decades of low commercial demand. Ambiguity with the identity of the commercial strain used for sainfoin in Australia soon became apparent, prompting an investigation of the competency of rhizobia strains for sainfoin. This study reports results of greenhouse screening of eleven rhizobia strains with five Onobrychis accessions. Later experiments evaluated the efficacy of the commercial inoculant strain CC1099 with the cultivar Othello, in laboratory, glasshouse and field experiments. Strain CC1099 (NA806-1) was described as a fast-growing, Gram negative, motile isolate, and identified as Rhizobium sp. via 16S rRNA sequencing. Of the eleven strains tested, only CC1099 was found to form effective symbiosis. Even so, it was restricted to only two sainfoin genotypes, cv. Othello and accession APG38723, and was sub-optimal compared to the nitrogen control. In more recent experiments, effective symbiosis with cv. Othello was not observed until several weeks post inoculation with CC1099. No plant growth response to inoculation was observed 25 weeks after planting in either field environment at Cowra or Wagga Wagga, NSW, despite high rates of lime being applied to half the plots. Results from these experiments point to a delayed onset of symbiotic function or failure in the only Australian commercial sainfoin cultivar, Othello, inoculated with the commercial strain, CC1099 and its derivatives. Subsequent whole-genome analysis identified strain CC1099 as Rhizobium gallicum, sharing over 98% average nucleotide identity (ANI) with other members of this species. Genomics has also revealed the absence of symbiotic nitrogen fixation pathway genes, providing the likely explanation for the strain's underwhelming performance in plant experiments. Further research is necessary to improve the initiation of symbiotic function in sainfoin if this species is to have a greater role in Australian agriculture, potentially through sourcing improved rhizobia strains, either from alternative historical sources of CC1099, or from new elite strains identified internationally.*

Notes:

Formulating pasture mixes with low CH_4 emission potentials – Grass/herb-annual legume mixes

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Abstract: *Enteric methane (CH_4) emission from livestock is the single largest contributor of greenhouse gas emissions in agriculture in NSW, accounting for 75.6% of total emissions. The aim of this study is to screen a range of commercially available annual legume species with anti-methanogenic properties to formulate highly productive pasture mixes (grass/herb-legume mixes), which would improve livestock productivity and reduce enteric CH_4 emissions. Three field experiments were conducted at Wagga Wagga, Cowra and Tamworth, NSW from 2022 to 2024. A total of 33 pasture mixes were evaluated for readiness of establishment, species compatibility, persistence and productivity over 3 years. Pastures were sampled in spring at species level from each plot. The oven-dried samples were analysed for feed quality and pasture mineral composition. The freeze-dried samples were analysed for the plant secondary compounds and* in vitro *gas production to investigate the mechanism and potential of CH_4 reduction. Volatile fatty acids, pH and ammonia concentrations were also tracked through* in vitro *fermentations. Results showed that biserrula had the greatest* in vitro *CH_4 reduction (79%), followed by French Serradella (21%). However, biserrula is poorly adapted in heavier textured soils with poor animal performance due to its low palatability. Serradella may have a broader area of adaption. Chicory was the most productive species across environments, its persistence, compatibility with other annual legumes and CH_4 reduction potential are explored in this study.*

Corresponding Crop & Pasture Science paper:
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Notes:

Evaluating the methane production and nutritional quality of pasture forages under current and future climate conditions

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Abstract:

Context: Improved pasture nutritional quality and reduced enteric methane production under current and projected climate conditions are essential for the meat and livestock industry. Legumes and forbs could provide changes in the ruminant fermentation process, as a result of their high nutritional quality and diverse secondary chemistry.

Aims: This study evaluates the in vitro methane production associated with temperate (Medicago sativa, Onobrychis viciifolia, and Biserrula pelecinus) and tropical (Desmanthus virgatus) legumes and a forb (Cichorium intybus) grown under factorial rainfall and warming treatments (Wet and Dry; Ambient and Elevated).

Methods: Plants were grown in large polytunnels at Western Sydney University's Pastures and Climate Extremes (PACE) field facility, receiving simulated Wet (La Niña) or Dry (El Niño) rainfall regimes, under ambient or elevated (+3°C) temperatures. Following a full year of climate treatments, biomass samples were collected in spring and summer, freeze dried and analysed using near infrared spectroscopy (NIRS) to determine nutritional quality and associated in vitro methane production.

Key conclusions: This study highlights species-level differences in nutritional quality and associated methane emissions for four forage legumes and a forb that are suitable for temperature pastures in southeastern Australia. Temperature and rainfall-related changes in plant nutritional characteristics have the potential to modify enteric methane production, so understanding relationships between climate and plant biochemistry are crucial for achieving reductions in greenhouse gas emissions in the livestock and dairy industries.

Notes:

Modelled potential distribution of C4 pasture species in current and future climates for Australia, with focus on southern Australia

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Abstract: *Climate change is driving increased temperatures impacting the water cycle and other climate variables. In southern Australia, cool season rainfall has declined while, summer rainfall has remained relatively stable or increased slightly. Future projections indicate these trends will continue. Grazing systems in southern Australia are dominated by C3 species and under climate projections the summer-early autumn feed gap is predicted to increase. Perennial C4 species are responsive to summer rainfall and may be a suitable addition to grazing systems although their potential distribution is not known. Our aim was to model and compare the potential distribution of six C4 pasture species under a historic baseline climate (1981–2010) and 2050 (2035-2065) climate scenario (using two global climate models; MIROC-H, CSIRO-MK 3.0) for Australia, with a focus on southern Australia. The study combined the potential climatic suitability with soil pH and landuse suitability. Climatic suitability of Cenchrus clandestinus, Chloris gayana, Digitaria eriantha, Megathursus maximus, Panicum coloratum var. makarikariense and Desmanthus virgatus was modelled using CLIMEX. Based on where these species are currently sown, these species occupy only a small proportion of their potential, especially in southern Australia. Future climate scenarios suggest that the potential distribution of these species will decrease in northern Australia. However, in southern Australia, suitability will increase if temperatures increase (MIROC-H) but decrease if rainfall decreases significantly also (CSIRO-MK). Our study indicates that these species could be sown in new areas, but field testing is required to confirm their persistence, productivity, interaction with soils, and position in the landscape.*

Notes:

Elevated atmospheric carbon dioxide levels and seasonal rainfall change the mineral composition of temperate pasture grasses

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Abstract

Context: Elevated atmospheric carbon dioxide (CO_2) concentrations are known to decrease the concentration of many mineral elements in grain crops. However, the effect of elevated CO_2 on the mineral composition of pasture grasses, and the interaction between elevated CO_2 and changes in seasonal rainfall has not been extensively studied.

Aims: This study aimed to understand the influence of elevated CO_2 and seasonal rainfall on mineral composition of temperate pasture grasses.

Methods: Pasture was sampled in spring and autumn under current CO_2 concentrations (400 ppm) and predicted future concentrations (550 ppm) and three water regimes (adequate in all seasons; limited in spring and excess in summer; limited in summer and excess in spring and autumn) from the TASFACE experiment in Tasmania, Australia. These were then analysed for macronutrient (N, P, K, S, Ca, Mg) and micronutrient (Cu, Zn, Mn, Fe, B, Mo, Co, Si, Al) concentrations using ICP-MS.

Results: In addition to seasonal variation in mineral concentrations, we found concentrations of P, S, Mn, Cu increased in at least one season under elevated CO_2 whilst Ca, Mg, Co decreased. Concentrations of K, Cu, Mo and Si were lower when spring rainfall was excessive and autumn rainfall was limited.

Conclusions: Concentrations of some important minerals for animal health appear to be influenced by likely future climatic conditions of either elevated CO_2 or changes in seasonal rainfall.

Implications: Changes in pasture mineral composition via changes in atmospheric CO_2 and rainfall suggest that mineral supplementation may need to change to fulfil animal requirements

Notes:

Student Competition Papers

Rooting Patterns in Companion Planted Pasture Species

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Abstract: *This study examined root development in perennial ryegrass, white clover, and chicory using a rhizobox system to understand how companion planting affects rooting patterns. Plants were grown as single plants, double (two plants of the same species) or mixed species (two plants of different species), in acid-washed sand with Hoagland's solution applied so nutrients were not limiting. Images collected at day 47 were analysed using a deep learning algorithm and image processing software. Results showed species-specific responses to companion planting. For single-planted treatments, chicory produced greater root volume than perennial ryegrass and white clover, indicating a comparative advantage in root production of the deep-rooted herbs. In double and mixed species plantings, only mixed-planted perennial ryegrass had higher root volume primarily in the top 10 cm, and greater root volume at 10-30 cm depth in double plantings. At the species level, seminal root angles were wider in perennial ryegrass and chicory than in white clover, indicating different water and nutrient uptake potential. Perennial ryegrass exhibited narrower root angles under double planting than single or mixed planting, which led to a greater volume of roots formed at depth and is likely related to the response to greater competition for moisture. White clover had wider seminal root angles when mixed-planted with chicory than when planted with other species, suggesting reduced water stress. Chicory showed similar seminal root angles across all treatments, indicating less rooting pattern plasticity. These findings demonstrate that companion planting can significantly influence seminal root angle in a species-specific manner.*

Key words: Multi-species, complementary, legume, herb, competition

Introduction

Pasture is an essential part of the Australian dairy industry, which provides affordable and accessible feed for cattle on farm and supports 60% of the feed requirement of dairy cattle in Victoria (Nelson *et al.* 2022). Monoculture perennial ryegrass (*Lolium perenne*) is a common choice for a pasture system, being successful but limited by N input and varying seasonal yield (Chapman *et al.* 2008). Multi-species pastures provide a potential solution through their more stable forage yield (Deak *et al.* 2007) and drought resistance (Skinner *et al.* 2004). Previous studies have observed species-specific responses to neighbouring plants that alter the amount and direction of root growth (Chen *et al.* 2012). Therefore, assessing the root interactions between species within multi-species pastures is important to understand the underlying interactions within this system. Quantifying root development is important for evaluating plant performance. Greater root volume and greater seminal root angle have been observed to lead to greater nutrients and moisture withdrawal (Atkinson *et al.* 2014). Reduced root diameter allows the formation of more roots.

Our study examined root volume and its distribution with depth, average root diameter and seminal root angle to assess the performance of selected pasture species to understand the interactions between the functional groups of the multi-species pastures. This study investigated how companion planting affects rooting patterns. We hypothesised that a) chicory would have a greater root volume than perennial ryegrass and white clover; b) there would be species-specific responses to companion planting of pastures.

Material and methods

This study was conducted in the glasshouse of the University of Melbourne, Parkville campus, from December 2024 for 47 days. The glasshouse was a controlled environment, with natural lighting, an average temperature of 21.1 °C and a relative humidity of 69.5%. The results presented in this study were gathered during the establishment period of this rhizobox experiment, and the experiment continued after these data were obtained. Rhizoboxes (25l*3w*50h cm) that allow free drainage at the bottom were filled with 2mm sieved acid-washed sand and were irrigated to field capacity one day before sowing. One species from each of the functional groups was chosen to represent the rooting conditions of that group, with perennial ryegrass (*Lolium perenne* 'Base AR37') representing grasses, white clover (*Trifolium repens* 'Legacy') was chosen for legumes, and chicory (*Cichorium intybus* 'Puna II') for herbs. Plants were sown into the treatments of single (only one species), double (two from the same species) and mixed (two different species) planting boxes. They were sown at approximately 1 cm depth into the sand to maintain a moist environment through germination. White clover was inoculated with Group B inoculant (Peat) before sowing (New Edge Microbials Pty Ltd). Irrigation was provided every day for the first 2 weeks, then three times a week, and Hoagland's solutions were applied twice a week to sustain the moisture and nutrient needs for the pastures. The experiment was conducted for 47 days till the image acquisition. Photos were taken in a custom-made box with controlled lighting and position of the box to ensure consistent quality, using a compact high-resolution camera (Sony A7CR, with Sony FE 50 mm F1.4 GM). Photos were then corrected for distortion and cropped to size, then segmented for roots with a deep learning neural network (Smith *et al.* 2020). Segmented images for boxes containing two plants were processed with Adobe Photoshop 2025 to separate the roots of each plant by visual assessment. All segmented images were then processed by RhizoVision Explorer v2.0.3 (Seethepalli & York, 2021) using the algorithms described by Seethepalli *et al.* (2021) to acquire root volume and root diameter measurements. Seminal root angles were acquired from the segmented images using SeminalRootAngle software (Smith *et al.* 2024).

All data were assessed for normality using the Anderson-Darling test. Variables showing significant deviations from normal distribution were log or square root transformed to achieve normality. All statistical analyses were performed using Minitab software (version 21.4.2). Differences between treatment groups were evaluated using one-way ANOVA followed by Tukey pairwise comparisons.

Results

The root system produced by each plant was analysed separately to assess the impact of the companion species on root development. When comparing the systems of single planting, the total root volume of perennial ryegrass and white clover was significantly less than chicory ($p<0.05$). The root volume of single-planted chicory was not significantly different to all double and mixed-planted boxes ($p<0.05$). Vertical analysis of root distribution revealed no significant difference ($p<0.05$) when comparing single-planted white clover and chicory to the double and mixed planting boxes. However, when comparing single-planted perennial ryegrass to the double and mixed-planted boxes, mixed planting significantly ($p<0.05$) increased the root volume at the top 10 cm by 111%, while double planting resulted in significantly greater ($p<0.05$) root volume (by 137%) below 10 cm depth (Figure 1).

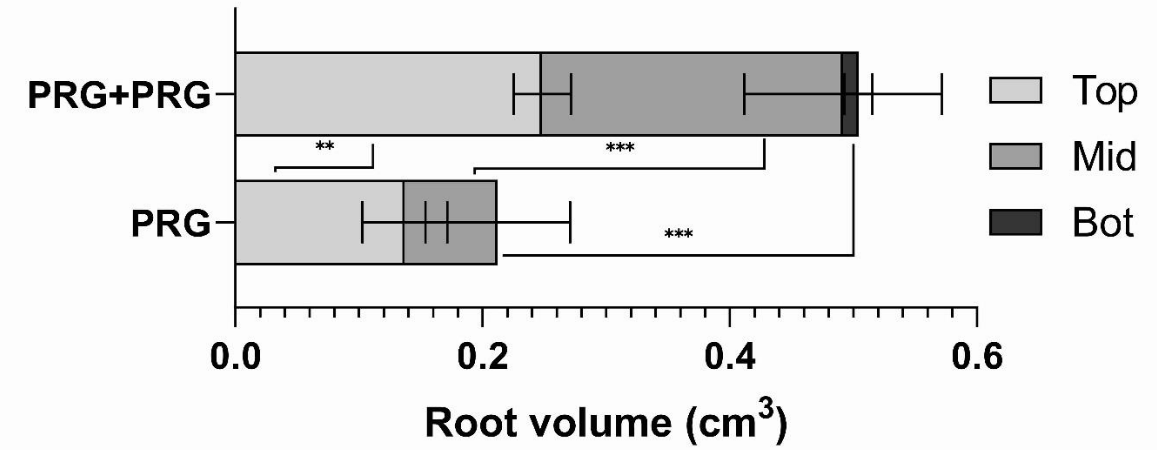

Figure 1. Root volume of single (PRG) and double-planted (PRG+PRG) perennial ryegrass, from three layers of the rhizobox. Top, 0-10 cm depth. Mid, 10-30 cm depth. Bot, 30-50 cm depth. *** $p<0.05$; ** $p<0.1$. Significance was determined through Tukey's pairwise comparisons

For each species across all three planting combinations, white clover produced significantly less ($p<0.05$) root volume (0.37 cm^3) than chicory (0.69 cm^3) and perennial ryegrass (0.58 cm^3). Companion planting did not affect the root volume of white clover or chicory, but for perennial ryegrass in mixed planting, growing with white clover resulted in a significantly greater root volume ($p<0.1$) (0.82 cm^3) than single-planted perennial ryegrass (0.38 cm^3). As for average root diameter, the average root diameter of white clover (0.57 mm) was significantly greater ($p<0.05$) than chicory (0.53 mm) and perennial ryegrass (0.53 mm). Companion planting did not affect perennial ryegrass and chicory, but white clover significantly increased in root diameter ($p<0.05$) when planted with perennial ryegrass (0.59 mm) compared with single-planted ones (0.55 mm).

Seminal root angles of perennial ryegrass and chicory were significantly ($p<0.05$) wider than those of white clover (Figure 2). When comparing the different planting conditions within each species, double-planted perennial ryegrass had significantly ($p<0.1$) narrower seminal root angle than single and mixed-planted treatments. In comparison, white clover had significantly ($p<0.1$) wider seminal root angle when mixed-planted with chicory than those white clovers that were single, double-planted or mixed-planted with perennial ryegrass. The differences between the seminal root angles of chicory were not significant ($p<0.05$) across all treatments (Figure 2).

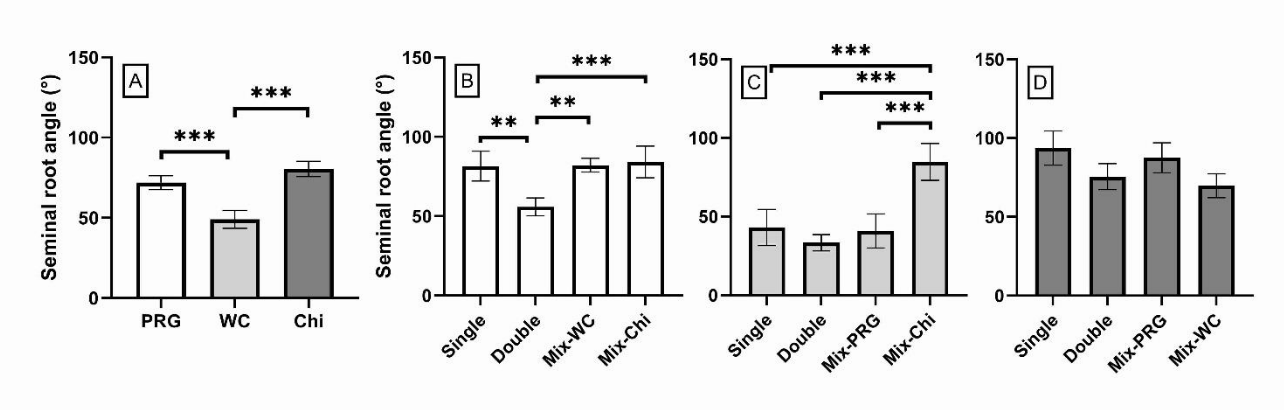

Figure 2. Bar charts of the seminal root angles of perennial ryegrass (PRG), white clover (WC) and chicory (Chi), illustrating: the overall seminal root angle of the three species (A); the seminal root angle of perennial ryegrass (B), white clover (C) and chicory (D) under different planting conditions. Single, planted alone. Double, with the same species. Mix-, with the indicated species. *** $p<0.05$; ** $p<0.1$. Significance was determined through Tukey's pairwise comparisons

Discussion

White clover produced less root volume than chicory and perennial ryegrass but had a larger average root diameter at the species level. A previous study also found that white clover had a larger average root diameter than pasture grasses (Evans, 1977). These thicker roots likely lead to reduced root volume, as a higher input is required to grow to the same length. Chicory had a lower average root diameter at this stage than white clover, indicating a rooting strategy of greater exploration through longer and thinner roots. The preference for forming thicker roots at establishment likely led to a reduced competitiveness for nutrients at the seedling stage, which was pointed out by Lane *et al.* (2000). White clover's narrower seminal root angle than the other two species might also have contributed to this lack of competitiveness, indicating a lesser potential to explore the soil profile.

Single-planted chicory produced the highest volume of roots of the three species, which were comparable to roots produced by two plants within one box. As a plant with a strong tap root system, chicory is reported to have the ability to reach greater depths of the soil profile (Sanderson *et al.* 2005). This experiment showed that, in addition to the advantages in rooting depth, chicory also forms a large volume of roots that can explore the soil profile faster than perennial ryegrass and white clover, leading to greater nutrient and moisture acquisition (Atkinson *et al.* 2014).

Mixed-planting significantly increased perennial ryegrass root volume in the top 10 cm, while double-planting increased root volume in deeper soil and had narrower seminal root angles. With a similar amount of addition of nutrients and moisture, this change in rooting pattern is related to the presence of neighbouring plants. Rooting deeper in monoculture and shallower in mixture was also found in another pasture grass (*Anthoxanthum odoratum* L.), attributed to the recognition of the neighbouring species (Mommer *et al.* 2010). Changes in the seminal root angle with different companions are likely related to the root response to water availability via hydropatterning (Giehl & Von Wirén, 2018). A narrower seminal root angle is likely associated with reduced water availability, such as the competition between the two perennial ryegrass plants from this experiment. On the other hand, white clover developed wider root angles with chicory in mixed planting. A previous field study pointed out that chicory may improve the moisture availability at the surface soil via hydro-lifting (Sanderson *et al.* 2005), which explains our observation. The seminal root angle of chicory did not change across the different companion species, indicating its soil exploration strategy is a less plastic response.

Conclusion

This preliminary experiment found that white clover produced the least volume of root overall, while chicory produced the largest volume of root when planted alone. Companion planting in pastures has altered the root growth of each species differently. Competition within perennial ryegrass in double-planted boxes has improved the root deposition in the deeper part of the soil with a reduced seminal root angle, likely a result of competition for moisture. Observation of the seminal root angle revealed that perennial ryegrass and chicory have greater potential for nutrient and moisture uptake due to their wider root angle. A wider seminal root angle was found in white clover when planted with chicory, likely due to the hydro-lifting mechanism of chicory that increased the available water. These species-specific changes in the rooting pattern from interactions with other plants suggests the possibility of root adaptation to niche environments by different functional groups in a multi-species pasture, and including deep-rooted herb species (e.g., chicory) could improve the establishment of the clovers.

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Notes:

Sowing resilience: adapting red clover for future climates and pastoral systems

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Abstract: *The future of agriculture depends on the seeds we plant today. As climate change intensifies, developing resilient cultivars is key to sustaining productivity in pastoral systems. A species that can help strengthen traditional pastoral mixtures is red clover (Trifolium pratense L.). Through its ability to fix nitrogen, red clover can enhance soil fertility and produce forage high in protein and digestibility. With its large taproot system, red clover can support biomass production through periods of water deficit. Integrating multiple breeding tools, this PhD-based manuscript provides a brief overview of key results on how bioclimatic variables, geography, and population structure shape the genetic diversity and adaptive tendencies of a diverse red clover germplasm panel. While the evaluation in multi-site multi-year field trials of key morphological and physiological traits identified their associated heritabilities and variance components, highlighting the potential benefit of the inclusion of such untapped germplasm material into breeding programs. The insights from these studies support adaptive breeding strategies that can develop productive, stress-resilient forages that enhance pastoral sustainability under future climatic challenges.*

Keywords: red clover (*Trifolium pratense* L.), germplasm, genetic diversity, morphological traits

Introduction

Globally, red clover (*Trifolium pratense L.*) is an important temperate pastural species able to produce quality forage high in digestibility and protein (Taylor and Quesenberry 1996). Originating from Eastern Mediterranean and now found throughout temperate regions worldwide, red clover can be used to enhance soil fertility and strengthen pastural mixes by supporting biomass production during water deficient periods (Annicchiarico *et al.* 2015). With ever-changing environmental conditions bringing more extreme weather events farmers require cultivars capable of maintaining productivity. The challenge for plant breeders, therefore, is to develop cultivars able to produce high-quality biomass while simultaneously persisting under a range of factors such as climates, soil types and grazing regimes.

By utilizing material currently stored in genebank collections through the incorporation of wild germplasm material from diverse geographical backgrounds into breeding programs, a wider range of genetic diversity capable of supplying adaptive traits can be accessed (Egan *et al.* 2021, Taylor and Quesenberry 1996). With limitations on the scale of evaluation and efficiency of characterising large quantities of novel germplasm, alternative methodologies are needed to provide a route to harnessing this genetic diversity to produce adaptive, productive cultivars. The objective of this manuscript was to briefly highlight key findings from three published thesis chapters on methodologies used to identify and incorporate genetic diversity, population structure and the evaluation of trait performance for novel red clover germplasm in temperate environments.

Methodology

The backbone of this research was a panel of 92 geographically diverse red clover populations sourced from the Margot Forde Genebank (AgResearch Grasslands Research Centre, Palmerston North, New Zealand) based on collection site information. Increased weight was given to selecting populations from drier regions. In total, 15 countries were represented with populations selected from Armenia (11 ecotypes); Azerbaijan (8); Bosnia & Herzegovina (1); Croatia (1); Czech Republic (1); Georgia (5); Greece (8); Italy (6); Morocco (1); Portugal (8); Russia (12); Spain (8); Tajikistan (10); Turkey (11); and United Kingdom (1) (Figure 1). This panel was assessed for both morphological and physiological traits in multiple trials comprising a water stress glasshouse pot experiment, with three treatments (water deficit, waterlogged and control) (Heslop et al., 2023), and multi-year mixed sward grazing field trials at Lincoln, Canterbury, NZ and Aorangi, Palmerston North, NZ, both temperate locations (Heslop *et al.* 2025a, Heslop *et al.* 2025b). Additionally, using molecular marker data derived from genotyping-by-sequencing (GBS), integrated models based on genomic, phenotypic and environmental information were developed to assess genetic diversity, genetic relationships and, for key traits, variance components and genotype-by-environment (G x E) interactions. Landscape genomics assessment based on redundancy analysis was used to identify key relationships between environmental bioclimatic variables and trait expression and plant performance. More descriptive methodologies are available in the associated manuscripts (Heslop *et al.* 2023, Heslop *et al.* 2025a, Heslop *et al.* 2025b).

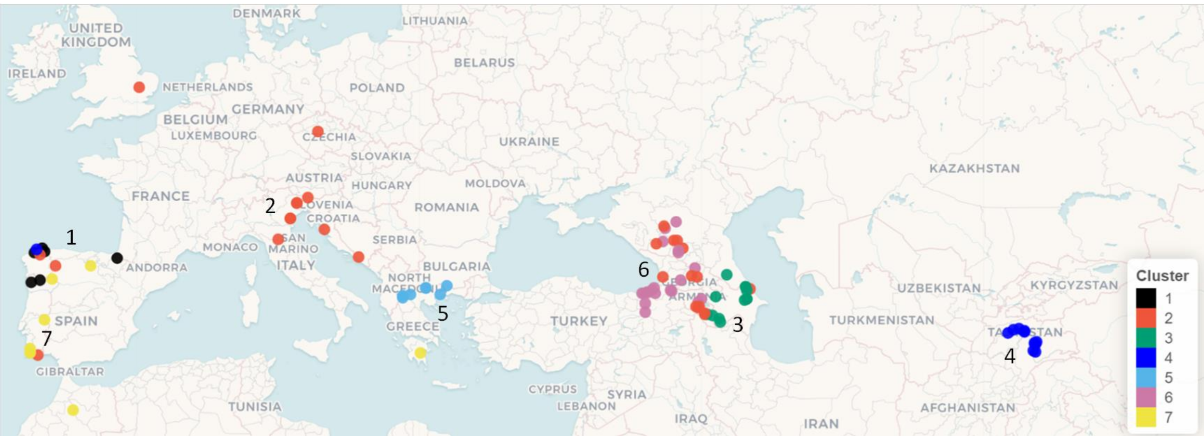

Figure 1. The geographic location of each germplasm population along with colour coordinated cluster groupings based on an optimum of seven cluster using DAPC analysis. Derived from Heslop *et al.* 2025a.

Geographically aligned population structure and varied levels of genetic diversity observed

To obtain a deeper understanding of the adaptive potential of the 92 populations, GBS data from pooled individuals representing each population were used in discriminant analysis of principal components (DAPC) analysis to characterise population structure, expected heterozygosity and gene flow (Heslop *et al.* 2025a). Interpretation of DAPC analysis grouped the 92 populations into seven clusters which aligned with their geographical sources and showed high gene flow (Figure 1). Country of origin not only influenced the genetic structure but also the expected heterozygosity, a measure for genetic diversity within populations, which ranged from 0.08 to 0.17.

Observed trait variations, response and heritability

In a controlled glasshouse experiment, seven of the 92 populations were used to observe 12 morphological and physiological traits in response to water deficit and waterlogged conditions to identify key contributions to plant coping mechanisms (Heslop *et al.* 2023). Water deficit negatively impacted all aboveground morphological traits, highlighted by decreases in total dry matter (40%), leaf number (50%) and leaf thickness (50%) compared to the controls. An increase in root to shoot ratio indicated a shift to maintaining root biomass, a trait attributed to plant water deficit tolerance. A reduction in photosynthetic activity under waterlogging was detrimental to plant performance, highlighted by decreases in root dry mass (83%), total dry matter (30%) and leaf number (34%). This experiment underlined the need for trait improvement for red clover under both water deficit and waterlogging.

In multi-year multi-location field trials, key morphological and physiological traits were characterised for diversity and for understanding plant performance and persistence for the 92 populations (Heslop, et al., 2025a). The variance components and genotype-by-environment (G x E) interactions were assessed, with high heritability ($h^2 > 0.70$) found for key traits including plant biomass, growth habit, leaf size, plot density, plot height, survival, lamina area, chlorophyll content. For a subset of 12 populations, further analysis identified key relationships between root structure and both plant persistence and plant production, with plants exhibiting either an expansive or compact root structure or a mixture of both (Heslop *et al.* 2025b). While neither singular root system resulted in better persistence or production a combination of both, a feature found in the cultivar lines, appeared to be of advantage in the temperate environments. This highlighted importance of either selecting for a singular root system that is adaptive over multiple environments or selecting plants with the ability to adapt structures depending on the environment. Integrating phenotype, genotype and source ecogeographic information of these diverse populations through redundancy analysis (RDA) characterised the influence of environmental variables on trait expression, plant performance and persistence. The key bioclimatic variables shaping the plant responses were precipitation, temperature, and isothermality – an indicator of temperature stability across a year (Heslop *et al.* 2025a). Additionally, single nucleotide polymorphisms (SNPs) were identified that were associated with these environmental variables and could be used as genetic markers to stack genomic regions associated with adaptive traits in breeding programmes.

How can this information be used?

The assessment of genetic diversity and the genetic relationships within the 92 populations showed an abundance of potential diversity available that is currently untapped. The information captured for the diversity and heritability of key morphological and physiological traits under water stress and multi-year, multi-location grazing trials can be used to focus future breeding programs with the inclusion of identified populations that possess traits of interest. All of methodologies used in this study provided different insight and usefulness, which was emphasized through the quantifying of interactions between traits, genotypes and genotype by environment (G x E) variances for important physiological traits.

Key interactions between plant performance and persistence were identified in both trials, in particular insights into root morphology and the relationship between above and below ground traits. This highlighted the need for further screening under water stress conditions with focus needed on selecting for adaptive root structures that can maintain production under such conditions. The identification of influential relationships between environmental variables with plant and trait performance along with the discovery of the associated potential adaptive genetic markers shows promise of being an effective selection tool. While further investigation is needed to test accuracy and efficiency of SNP markers identified through landscape genomics, the alignment between genetic diversity and ecogeographic information, as well as some SNPs mapping to genes associated with abiotic stress response, suggests these markers may be of value as an additional tool for selecting germplasm.

Conclusion

Key messages from these studies show genetically and eco-geographically diverse germplasm can perform well agronomically when tested in similar environments to their collection sites, whereas populations from different environments did not always perform so well. This emphasizes while this material is genetically valuable, the next logical step is to incorporate this novel genetic diversity into locally adapted material for assessment. Although resource intense, the potential benefits from capturing new traits include maintaining potential genetic gain and developing cultivars adaptative to future climatic challenges. This study highlights by leveraging genetic resources we can develop adaptive, productive cultivars that enhance pastoral sustainability for the future.

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Notes:

Investigating how the hard seed breakdown pattern and competition from an established perennial grass affects the timing of white clover seedling regeneration

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Abstract: *Maintaining a stable white clover (Trifolium repens L.) population in the high rainfall permanent pasture environment is challenging due to summer water stress, resulting in a decline in plant population over time. The role of seedling regeneration in maintaining the sward has received less attention in recent breeding programs than improving the survival of mature plants. The effect that hard seed breakdown patterns have on white clover regeneration remains largely unknown.*
Most white clover seedlings emerge in late autumn/early winter when temperatures are suboptimal for white clover growth. It is hypothesised that seedling emergence in white clover is often restricted to the late autumn/early winter period due to the combination of higher soil water status and lower evaporative demand, resulting in reduced competition for water and, as a result, improved seedling survival. The presence of a companion perennial grass increases the competition for soil water, which may lead to increased seedling mortality in white clover.
A modified Nelder wheel design will be used to examine the interaction between the timing of hard seed breakdown and how differing levels of competition from an established perennial grass affects the surface soil water and whether water availability is a contributing factor in the timing of seedling emergence and survival under field conditions. Strategies to conserve surface soil water to enable earlier seedling establishment will also be tested.

Key words: systematic planting design, phalaris, pasture mixture, post-drought recovery

Introduction

White clover (*Trifolium repens* L.) is an aerial-seeding, short-lived perennial pasture legume that can flower prolifically and produce large quantities of seed under favourable conditions (Clark *et al.* 1992). Seedling regeneration is periodically required in white clover, particularly after periods of drought; however, it is not a reliable process (Archer & Robinson, 1989). In white clover, the pattern of hard seed breakdown is relatively unknown. A recent study of the hard seed breakdown in white clover cv. Trophy demonstrated that softened seed was available much earlier in the year (Munday *et al.* 2025) than when established seedlings can be expected, indicating factors other than seed supply may restrict the timing of establishment. Archer and Robinson (1989) have previously suggested that water stress is the primary cause of seedling mortality in white clover, although few field studies have been conducted to support this.

Seedling regeneration in white clover is observed to occur in late autumn/early winter, which is later than ideal for emergence due to low temperatures for white clover growth (Archer & Robinson, 1989). White clover is known to have slow seedling root growth. When grown under non-limiting conditions it takes 12-13 days to get roots to a depth of 5cm (Evans, 1973). When grown in soil under limited water, rooting depth can be one third of that under ideal soil water conditions (Foulds, 1978). Dear and Cocks (1997) demonstrated that seedling survival of subterranean clover (*T. subterraneum* L.) regenerating in a perennial grass sward was greater in late autumn when the surface soil water content stayed above the permanent wilting point for 14 consecutive days. This could also potentially apply to swards containing white clover, explaining the late autumn/early winter seedling regeneration observed in the field. Management options to maintain surface soil water are limited. Heavy grazing to reduce the transpiration of perennial grasses is one option, with Dear *et al.* (1997) demonstrating that surface soil water was higher when phalaris was defoliated. Another possible option for maintaining a higher level of surface soil water is the use of surface litter (Tozer *et al.* 2016).

It is hypothesised that seedling emergence in white clover is often restricted to the late autumn/early winter period due to the combination of higher soil water status and lower evaporative demand, resulting in reduced competition for water and, as a result, improved seedling survival. The presence of a companion perennial grass increases the competition for soil water, which may lead to increased seedling mortality in white clover. This study examines the interaction between the timing of the hard seed breakdown and the drying of the surface soil water following a rainfall event in determining the timing of seedling establishment for white clover. The role of a perennial grass in drying the surface soil water will also be examined, as well as management options to maintain surface soil water.

A modified Nelder wheel design (Nelder, 1962) was selected for this study as it is a space-efficient design for testing multiple plant densities (Figure 1). Individual plants or seeds are spaced at specified positions along spokes radiating out from the centre. Each position is an experimental unit, and each spoke is a replicate (Parrott *et al.* 2012).

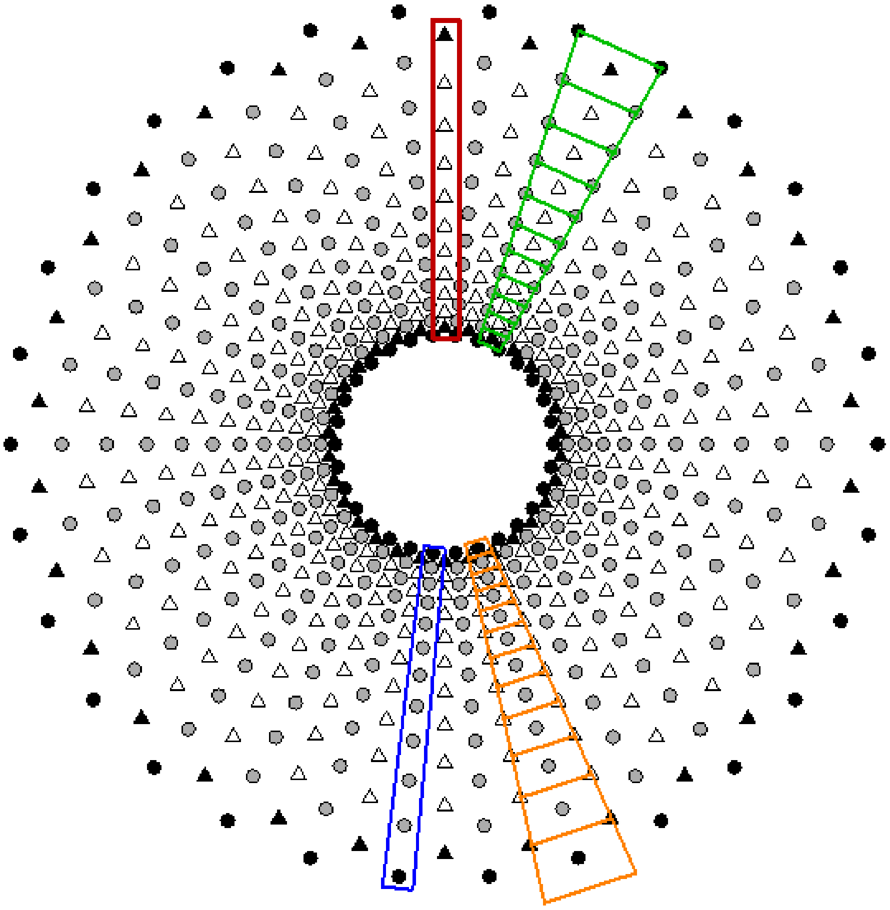

Figure 1. Diagram of the modified Nelder wheel planting design. Phalaris positions are represented by solid circles (●) and white clover by open triangles (Δ). Black filled symbols indicate border rows that are not sampled. Coloured outlined areas represent sampling and calculated growing areas: red (white clover replicate), green (white clover growing area), blue (phalaris replicate) and orange (phalaris growing area)~~.~~

Approach

Experimental design

Modified Nelder wheels will be planted in two contrasting environments in NSW. The first near Orange (33°19'02"S 149°04'52"E), elevation 922 m, with an average annual rainfall of 924 mm, and the second near Cowra (33°48'11"S 148°42'07"E), elevation 381 m, with an average annual rainfall of 595 mm. Annually, the long-term average maximum air temperature at Cowra is 5.7°C higher than at Orange on a monthly basis. The modified design consists of two Nelder wheels that are superimposed on top of each other, and offset by 6 degrees, with each wheel consisting of an individual species, similar to the design used by Snaydon and Howe (1986). The benefit of this design is that each position is centrally located from the surrounding positions that provide the competition. The combined wheels (henceforth referred to as a single wheel) will be 5.95 m in diameter, with 60 spokes (30 spokes per species), and each spoke will contain 12 positions. The position spacings noted in Table 1 were calculated using the formulae of Parrot *et al.* (2012) and selected to cover the range of phalaris densities anticipated to persist under dryland conditions (Dear *et al.* 2007).

Three wheels will be planted at each site. Two wheels will include both phalaris and white clover, while the third wheel will consist of only the white clover component. The wheels with phalaris and white clover are to be divided in half, allowing different treatments to manipulate surface soil water to be applied in each half. As a result, each white clover position will be replicated 13 times per treatment, and each phalaris position will be replicated 14 times. The wheel sown only with white clover will also be divided in half, with different treatments applied to manipulate surface soil water in each half and each position will be replicated 14 times. The phalaris will be allowed to establish for ~9 months before defoliation treatments are applied. At each site, phalaris in half of the wheel will be cut to 5 cm in height and 20 cm in height in the other half. All herbage will be removed from both defoliation treatments. In addition, one phalaris wheel at each site will have 500 kg DM/ha of litter spread across the entire surface. Litter will be comprised of cereal straw that has passed through a combine harvester.

The white clover will be introduced into the wheels at the same time the phalaris defoliation treatments are imposed (late January). At each white clover position, 250 non-scarified seeds will be placed on the soil surface, which approximates the number of seeds from one mature inflorescence (Munday *et al.* 2025). The use of non-scarified seed will enable the hard seed breakdown pattern to dictate the availability of germinable seed over time. The introduction of the white clover seed in late summer will facilitate the natural softening of the hard seed coat under field conditions. The white clover-only wheel will also be sown at this time in the manner described above. It will also be divided in half, with half the wheel receiving the litter treatment. The use of a white clover only wheel enables examination of the effects of intraspecific competition on seedling mortality (Hill & Gleeson, 1988).

Table 1. Radius distance, distance between positions, plant density and growing area for phalaris and white clover positions in a modified Nelder wheel

	Phalaris				White Clover		
Radius number	Radius (cm)	Distance between positions (cm)	Plant density/m^2	Growing area (m^2)	Radius (cm)	Distance between positions (cm)	Growing area (m^2)
Border	70.2		77.1	0.013			
Border					74.8		0.015
R1	79.5	9.4	60.0	0.017			
R2					84.8	10.0	0.019
R3	90.2	10.6	46.7	0.021			
R4					96.2	11.3	0.024
R5	102.2	12.1	36.3	0.028			
R6					109.1	12.9	0.031
R7	115.9	13.7	28.3	0.035			
R8					123.6	14.6	0.040
R9	131.4	15.5	22.0	0.045			
R10					140.2	16.5	0.052
R11	148.9	17.6	17.1	0.058			
R12					158.9	18.7	0.067
R13	168.9	19.9	13.3	0.075			
R14					180.1	21.2	0.086
R15	191.4	22.6	10.4	0.097			
R16					204.2	24.1	0.110
R17	217.0	25.6	8.1	0.124			
R18					231.5	27.3	0.141
R19	246.0	29.0	6.3	0.160			
Border					262.5	31.0	0.182
Border	278.9	32.9	4.9	0.205			

Materials

Phalaris cv. Holdfast seeds will be sown into seedling trays in the glasshouse (~ 23°C) at Cowra. After emergence, trays will be thinned to one plant per cell. Trays will be removed from the glasshouse 2 days before being transplanted into the field. Variability in seedling size will be confined to individual replicates, with seedlings sorted by size before transplanting. The use of transplanted phalaris seedlings is to minimise missing positions through seedling mortality. The white clover seed is to be sourced from a 0.1 ha seed block of cv. Haifa adjacent to the trial site at Orange. To ensure seed dormancy remains intact, mature inflorescences will be harvested by hand, and the seed will be gently rubbed out of the florets to prevent scarification.

Sampling

Following the placement of the white clover seed in the wheels, surface soil water readings (0-5 cm) will occur the day after a significant rainfall event (~30mm) and then every 2-3 days until 14 days post rainfall, after Dear and Cocks (1997). Surface soil water readings will be taken with a ML2x Thetaprobe and a HH2 water meter (Delta-T Devices). Germination counts will be performed 7, 14 and 28 days following the rainfall event. Three key times will be targeted for measurements including late summer–early autumn, mid-autumn, and late autumn. If naturally occurring rainfall is not received at the targeted times, irrigation will be used to simulate a rainfall event.

Discussion and Conclusion

Nelder wheels were initially tested with vegetable crops (Nelder, 1962), but have since been more widely used in forestry and silvopastoral experiments where only a single wheel is used (Pachas *et al.* 2018). They have seen limited use with pasture species. A Nelder wheel design was used in a study on tall fescue (*Festuca arundinacea* Schreb.) (Hill *et al.* 1991). The wheel had 16 spokes and was divided in quarters for application of different treatments. When dividing the wheel, spokes that border two different treatments must be excluded from the analysis. This reduced the number of replicates in a wheel to 3. Three wheels were then used to increase the number of measurable spokes to 9. In the Nelder wheel design of Snaydon and Howe (1986), which studied perennial ryegrass (*Lolium perenne* L.), eight spokes were used per wheel, and there was no replication of wheels. This suggests a minimum of eight spokes (replicates) is required when using pasture species. The modified design used in the current study to implement competition dynamics was selected over more commonly used alternating spoke or alternating position designs. These modifications were made to place the white clover positions at the furthest possible point from the surrounding phalaris positions. This point is considered to offer the best opportunity for establishment and the greatest seedling growth rates (Ross & Harper, 1972). The increasing size of the white clover positions as they move further from the centre is important. Barrett and Silander (1992) observed that in a pasture sward a gap size of 10 cm in diameter recruited the highest number of white clover seedlings compared to gap sizes that were smaller or larger. Barrett and Silander (1992) suggest that the 10 cm gap produces a favourable microclimate for regeneration to occur.

It is expected that heavy defoliation of the phalaris will result in earlier white clover establishment compared to phalaris managed under more lax defoliation. However, once past the final germination count at day 28, the number of white clover plants persisting under the phalaris with the lax defoliation treatment may be greater than that under the heavy defoliation (Curll & Gleeson, 1987).

A limitation of the Nelder wheel design is that it is sensitive to plant mortality. If plant mortality occurs in a position, the surrounding positions must be excluded from analysis due to potential edge effects from reduced competition. When establishing a Nelder wheel it is recommended to use a method of establishment that reduces the likelihood of missing positions (Parrott *et al.* 2012) and increase the number of replicates in anticipation of unavoidable plant mortality.

In summary this study is expected to show that: i) successful establishment of white clover does not occur unless surface soil water following a rainfall event stays above the permanent wilting point for more than 14 consecutive days; ii) establishment occurs earlier and in greater numbers where the phalaris density is low, with establishment becoming progressively slower and less successful as phalaris density increases; iii) heavy defoliation of the phalaris increases surface soil water and increases establishment when compared to phalaris under a more lax defoliation management; and iv) the impact of litter on white clover regeneration is expected to be minimal.

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