Australian Grassland Association Inc.

PASTURE LEGUMES FOR SUSTAINABLE PRODUCTIVE SYSTEMS

University of Western Australia

4-6 July 2023

AUSTRALIAN GRASSLAND ASSOCIATION INC.

Pasture Legumes for Sustainable Productive Systems

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Program

Time	Author/Speaker	Title
DAY 1 - Tuesday 4th July		
8:15-9:00 AM	Registration	
	Session 1. Welcome and setting the scene. CHAIR: Megan Ryan	
9:00-9:10 AM	**Opening Address - AGA President – Rowan Smith**	
9:10-9:40 AM	Kadambot Siddique	Overview of Western Australian agriculture
9:40-11:00 AM	Various presenters.	Pasture research in WA – series of presentations from relevant organisations: • Clinton Revell (DPIRD) • Hayley Norman (CSIRO) • Phil Nichols (UWA) • Rob Harrison (DPIRD, representing Murdoch University)
11:00-11:20	**MORNING TEA**	**CHAIR: Keith Pembleton**
11:20-12:05	Derrick Moot	A review of legume research and extension in New Zealand (1990-2022)
12:05-12:35	Student Competition. Finalists: • Gerelee Enkhbat – Diversity for waterlogging tolerance and other agronomic traits in *Trifolium subterraneum* L. ssp. *Yanninicum* • Jonathan McLachlan – The influence of CO_2 concentration and soil phosphorus supply on the growth and nodulation of two *Desmanthus* spp. genotypes • Victoria Clarke – Enhancing pasture legume performance in a changing climate: the case for super-nodulating varieties	
12:35-1:35 PM	**LUNCH**	
1:35-1:40	**Major Sponsor presentation: DLF Seeds (Nathan Tognela)**	
	Session 2. Legume and mixed pastures for changing and variable climates.	**CHAIR: Hayley Norman**
1:40-2:10 PM	Rebecca Stutz	Legumes for lambs: perennial options for summer finishing pastures in southern New South Wales
2:10-2:40 PM	Rebecca Haling	Variation in flowering time and flowering date stability within a cultivar of French serradella
2:40-3:10 PM	Laura Goward	Flowering responses of serradella (*Ornithopus* spp.) and subterranean clover (*Trifolium subteranneum* L.) to vernalisation and photoperiod and their role in maturity type determination and flowering date stability
3:10-3:30 PM	**AFTERNOON TEA**	**CHAIR: Daniel Kidd**
3:30-4:00 PM	Rowan Smith	Strawberry clover (*Trifolium fragiferum* L.): current status and future role in Australian agriculture
4:00-4:30 PM	Matthew Newell / Neil Munday	Hard seed breakdown patterns of serradella (Ornithopus spp) in two contrasting environments of south-eastern Australia
4:30-5:00 PM	Richard Hayes	Legume persistence for grasslands in tableland environments of south-eastern Australia
5:00 PM	**Housekeeping: dinner venue, time etc.**	
6:00-11:00 PM	**Symposium dinner – Frasers Restaurant, Kings Park** **60 Fraser Avenue, Kings Park West Perth, WA 6005** https://www.frasersrestaurant.com.au/ **Guest speaker** Digby Growns (Senior Plant Breeder, Botanic Gardens and Parks Authority – Kings Park)	

DAY 2 – Wednesday 5th July	CHAIR: Clinton Revell

Field tour (8:00am – 6:00 pm):

- 8:00 depart from UWA
- Featuring visits to:
  - UWA Shenton Park field station: clover breeding and subclover seed harvesting.
  - Phil Barrett-Lennard property at Gingin: see serradella, panic and Serradella, and couch pastures on sand, plus new sub clover varieties with phalaris and fescue,
  - Zac Roberts property at Dandaragan: see French and Yellow serradella, panic, tedera, and sub clover pastures used in a well-run sheep enterprise.
- Return to Perth at approximately 6:00pm.

Dinner at WA Rowing Club, 171 Riverside Drive Perth. https://www.warowingclub.org/

DAY 3 - Thursday 6 July		
8:40 AM	**Day 2. Opening and housekeeping for the day**	
8:45 AM	**Session 3. Developing new pasture legumes. CHAIR: Brendan Cullen**	
8:45-9:15 AM	Daniel Real	Successful creation of seedless (sterile) leucaena germplasm developed from interspecific hybridisation for use as forage
9:15-9:45 AM	David Peck	A simplified protocol to apply speed breeding techniques to temperate annual pasture legumes
9:45-10:00 AM	N. K. Nazeri	Identification of promising accessions of subterranean clover (*Trifolium subterraneum* L.) from six years of genetic resource regeneration activity
10:00-10:15 AM	Gerelee Enkhbat	Prolonged waterlogging tolerance in *Trifolium subterraneum* ssp. *yanninicum*
10:15-10:30 AM	Alan Humphries	Advances in Genebank information systems to identify diversity in pasture genetic resources
10:30-11:00 AM	**MORNING TEA**	**CHAIR: Phillip Nichols**
11:00-11:15 AM	Carmen Teixeira / Derrick Moot	Flowering time of subterranean clover (*Trifolium subterraneum* L.) in Australia and New Zealand
11:15-11:30 AM	J. Guo / Derrick Moot	The effects of climate change on flowering of subterranean clover (*Trifolium subterraneum* L.) in New Zealand
11:30 -11:45 AM	Clinton Revell	SerraMax yellow serradella (*Ornithopus compressus* L.) – a new annual pasture legume for livestock and cropping systems in southern Australia
11:45-12:00 PM	Rowan Smith	Yellotas: A unique yellow serradella cultivar with potential for permanent pasture environments
12:00-12:15	T. Dorji	The potential of pasture legumes for sandy alkaline soils of South Australia’s low-rainfall regions: a case from on-farm trials
12:15-1:15PM	**LUNCH**	
	Session 4. Future directions. CHAIR: Laura Goward	
1:15-1:45 PM	Warwick Badgery	Reducing enteric methane of ruminants in Australian grazing systems - a review of the role for temperate legumes and herbs
1:45-2:00	Yi Zhou	Inoculation of novel soil bacteria improves the establishment of lucerne cultivars
2:00-2:30 PM	Richard Smith	Some known unknowns, unknown knowns, and potential unknown unknowns associated with pasture legumes for the dairy sector: a northeast US perspective
2:30-3:20 PM	Facilitated by Rowan Smith and Brendan Cullen	Future directions and priorities for pasture legume research and development – panel session
3:20-3:30 PM	Rowan Smith	Symposium close

Contents

Welcome Address

Hello and welcome everyone to the 6th Symposium of the Australian Grassland Association (AGA).

Firstly, I wish to acknowledge that The University of Western Australia is situated on Noongar land, and that the Noongar people remain the spiritual and cultural custodians of their land and continue to practise their values, languages, beliefs and knowledge.

In some ways we are going back to the future with our theme being focussed on 'Pasture legumes for sustainable, productive systems'. Our first in the series, 'The Australian Legume Symposium' was held in Melbourne in 2012. Other previous symposiums have focussed on perennial grasses, livestock productivity, soil constraints, and most recently online, 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.

In my relatively short time in pasture research, I have been warmed by the humble nature of pasture scientists, many providing mentorship, and the general willingness to collaborate. With so few of us left, these collaborations often come down to personal relationships between individuals rather than heads of organisations.

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.

There are two CSIRO pasture scientists who have recently retired that I would like to make mention of. Richard Simpson has made a significant contribution in plant nutrition and pasture production, including studies across a range of pasture legumes. Richard Culvenor is best known for his work with perennial grasses and in particular his breeding work with phalaris. Both have contributed enormously to improving our knowledge of pasture species and their adaptation and suitability to Australian conditions. We thank them for their efforts, congratulate them on their achievements and wish them well in retirement.

I believe one of the great strengths of the symposium is that it encourages full paper submissions that are peer reviewed and published in a special issue of 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 the special issue which, contains 10 papers that can be found in Crop and Pasture Science now.

It's a full program starting with a keynote introduction from Kadambot Siddique on Western Australian Agriculture, followed by local updates from research organisations. After morning tea we will hear from Derrick Moot on pasture legume research in New Zealand, followed by the student competition sponsored by the AW Howard Trust.

The following sessions will follow the more traditional format of research presentations on: Legume and mixed pastures for changing and variable climates; Developing new pasture legumes; and Future directions in pasture research. Wedged in between we have a field trip tomorrow and I will provide more details of that later in the day.

I would like to thank our sponsors DLF Seeds (Major), Cropmark Seeds (Field Tour), DPIRD (Student Prizes) and 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, as well as venues and field tours with the local WA team of Clinton Revell, Phil Nicholls and Daniel Kidd. Carol Harris for her efforts with registration, Keith Pembleton with the student competitions, Beth Penrose with auditing, Brendan Cullen with the special issue and program, and Kevin Reed for his guidance.

We trust you will all have an enjoyable symposium.
Rowan Smith
President of the Australian Grassland Association

A review of legume research and extension in New Zealand (1990–2022)

D.J. Moot

Dryland Pastures Research Group, Lincoln University, Canterbury 7647, New Zealand

Abstract: *Legumes have underpinned transformational change on New Zealand sheep and beef farms over the last 30 years. This was through an emphasis on ewe nutrition based on lucerne or red clover dominant pastures, and increased use of subterranean and white clovers on uncultivatable hill country. Pre- and post-weaning lamb growth rates have increased and enabled earlier slaughter of heavier lambs. The farm systems results include greater numbers of hoggets mated, higher lambing percentages and greater ewe efficiency (kg lamb weaned/kg ewe mated). Extension packages to support legume use have compared growth rates of resident and legume-based pastures, economic analyses of successful farms and management packages for the most appropriate legume in different environments. Over the same period, the dairy industry rapidly expanded in cow numbers and area onto flat irrigated land on the Canterbury Plains. The nitrogen deficiency of perennial ryegrass was overcome by a linear increase in nitrogen fertiliser use. Environmental concerns from this intensification have led to a legislated nitrogen cap of 190 kg/ha.year. This, coupled with a recent trebling in urea price, has returned attention to increasing the white clover content of these pastures. Nitrogen applications can be minimised by using diverse pastures sown with a legume, herb and <8 kg/ha of perennial ryegrass. Work on other legumes, including annuals and those with condensed tannins, has to date failed to increase their use in most pastoral settings, with the exception of the perennial lupin which is adapted to high-aluminium soils in the South Island High Country.*

Corresponding Crop & Pasture Science paper:
Moot DJ (2022) A review of legume research and extension in New Zealand (1990–2022). *Crop & Pasture Science*. https://doi.org/10.1071/CP22237

Notes:

Legumes for lambs: perennial options for finishing pastures in southern New South Wales

R.S. Stutz[1], R.A. Culvenor[1] and J. De Faveri[2]

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Abstract: *High quality, summer-active pastures could improve profitability of lamb meat production on the southern tablelands of New South Wales by facilitating finishing over summer, with legumes critical for enhancing the nutritive value of pasture mixes. Perennial legumes available for south-eastern Australia are summer-active but vary in their ability to withstand moisture stress and grazing. We tested pure swards of 12 cultivars across 8 legume species in replicated small-plot trials at Goulburn and Bombala, assessing productivity, persistence and warm-season nutritive quality over 2-3 years. Lucerne (Medicago sativa), the standard summer-growing perennial legume for meat production in south-eastern Australia, was clearly the most productive species during summer and outperformed the clovers (Trifolium spp.) in terms of persistence and productivity throughout most of the trial period at both sites except for autumn 2021 after high rainfall in March. However, lucerne was susceptible to waterlogging at Goulburn. Caucasian clover (T. ambiguum) was also highly persistent at both sites, while talish (T. tumens) and strawberry (T. fragiferum) clovers were more persistent than white (T. repens) and red (T. pratense) clovers. White clover recovered strongly under high rainfall after drought while red clover established rapidly but showed less capacity for post-drought recovery. Caucasian x white clover was the least productive species. Alternative clover species were sometimes slightly lower in nutritive quality than white clover, but differences were small except for red clover which was sometimes distinctly lower. Further assessment is warranted of talish clover, caucasian and strawberry clovers in pastures on the southern tablelands.*

Corresponding Crop & Pasture Science paper:
Stutz RS, De Faveri J, Culvenor RA (2023) Legume options for summer-active pastures in a temperate rainfall environment of south-eastern Australia. *Crop & Pasture Science*. https://doi.org/10.1071/CP22406

Notes:

Variation in flowering time and flowering date stability within a cultivar of French serradella

Rebecca E. Haling[1,3], Laura Goward[1,2], Adam Stefanski[1] and Richard J. Simpson[1]

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[2]Tasmanian Institute of Agriculture, University of Tasmania, Private Bag 98, Hobart, Tas. 7001, Australia.
[3]Corresponding author. Email: rebecca.haling@csiro.au

Abstract: *In Australia, serradellas (Ornithopus spp.) have proven adaptation to the climate and sandy, acid soils of WA and north-west NSW. Most cultivar selection has been undertaken in these regions (particularly in WA). Opportunity exists to expand serradella use onto heavier, duplex soil types in permanent pasture environments of south-eastern Australia. However, this requires cultivars with appropriate flowering times and flowering-date stability (i.e. flowering at the same date in spring regardless of timing of the autumn break). Initial studies indicated that a widely-used French serradella (O. sativus) cv. Margurita exhibits flowering date instability in the NSW southern tablelands. This may have implications for feeding value, seed production and, consequently, cultivar persistence. Variation in flowering date and flowering-date stability within cv. Margurita was examined. Individual seed lines (sourced from commercial seed) were sown 21 March 2019 (Canberra, ACT) and were monitored for time to first flower. A subset of plants exhibiting 'early' or 'late' flowering were netted and their seeds collected. In 2020, 20 plants from each selection were sown in Canberra in late March and early May to represent an "early" and "late" break of season (n=3). In the early-sown treatment, "early-flowering" selections typically reached the median date of first flower (50%-flowering) from mid- to late-August, while "late-flowering" selections reached 50%-flowering early- to mid-September. For the late-sown treatment, "early-flowering" selections initiated flowering from mid- to late-September, while "late-flowering" selections flowered mid-September to early-October. The "early" selections exhibited greater flowering-date instability than "late" selections and flowered particularly early when sown early. This indicates diversity within cv. Margurita for flowering-time control (i.e. vernalisation and/or photoperiod requirements). The Perth environment, in which the cultivar was selected, may not allow expression of this variability and instability. Additional selection of cultivars in new "target" environments is warranted to ensure cultivars are suitably adapted to these environments.*

Corresponding Crop & Pasture Science paper:
Haling RE, Goward L, Stefanski A, Simpson RJ (2022) Variation in flowering time and flowering date stability within a cultivar of French serradella. *Crop & Pasture Science*. https://doi.org/10.1071/CP22222

Notes:

Predicting subterranean clover and serradella flowering date and flowering date stability in southern Australia

L. Goward [1], R. Haling [1], R. Smith [2], B. Penrose [3] and R. Simpson [1]

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[2] University of Tasmania, Mt. Pleasant Laboratories 165 Westbury Road, Launceston, TAS 7250
[3] University of Tasmania, Sandy Bay Campus, Hobart, TAS 7001

Abstract: *Serradellas are temperate annual pasture legumes, with many cultivars exhibiting unstable flowering dates: i.e., flowering date shifts when autumn germination date is changed. This has potential risks for seed production and, consequently, persistence because early germination can result in flowering during periods of high frost risk. Controlled-environment experiments were used to determine the flowering time responses to vernalisation and photoperiod among subterranean clover, yellow serradella and French serradella cultivars. The time to first flower (TFF) of early-maturing cultivars was insensitive or weakly responsive to photoperiod (PPD) and required 0-1 week of vernalisation at 5℃ (VRN) to minimise TFF. In contrast, late-season types required longer VRN and longer PPDs to minimise time to flowering. A phased phenology model was developed to predict TFF. The first (sowing-emergence) and last (floral initiation-first flower) phases of the model were driven by thermal time. The middle phase (emergence-floral initiation) also progressed with thermal time, but flowering was delayed when VRN and PPD requirements were not met. The node at which floral initiation occurred in field locations was determined using a first flowering-node x PPD x VRN response surface constructed for each genotype using data from the controlled-environment experiment. Thermal time to flower was then determined from the rate of node appearance. The model required specification of a critical PPD at which the dominant influence of VRN on TFF was reduced and then replaced by a dominant influence of PPD; maximum time to flower; minimum time to flower and, for some cultivars, a residual VRN response under long days. The results inform selection of cultivars for given environments so that appropriate flowering times and flowering date stability is achieved.*

Corresponding Crop & Pasture Science paper:
Goward LE, Haling RE, Smith RW, Penrose B, Simpson RJ (2023) Flowering responses of serradella (*Ornithopus* spp.) and subterranean clover (*Trifolium subterraneum* L.) to vernalisation and photoperiod and their role in maturity type determination and flowering date stability. *Crop & Pasture Science*.
https://doi.org/10.1071/CP22366

Notes:

Strawberry clover (*Trifolium fragiferum* L.): underutilised or just unproductive and unsuitable?

R.W. Smith[1], B. Penrose[2], A. Humphries[3], C. Harris[4], M. Rogers[5], P. Nichols[6], G Li[7] and R. Hayes[7]

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[7] New South Wales Department of Primary Industries richard.hayes@dpi.nsw.gov.au

Abstract: *Strawberry clover (Trifolium fragiferum L.) is periodically raised as an alternative perennial legume for temperate regions of Australia. It is tolerant of waterlogging, yet it's ability to persist through periods of moisture stress has possibly been overlooked. Strawberry clover can spread via stolons and is mildly salt tolerant, additional characteristics that are sought after in resilient perennial pastures. Yet there have been just 4 registered cultivars to date in Australia and the most popular cultivar is Palestine, which was the first to be released in 1938. Furthermore, it's distribution has largely been confined to niche areas. This paper reviews the taxonomy and breeding system, morphology, distribution and ecology and subsequent transfer to other countries. It reviews the suitability of strawberry clover in perennial pasture systems in the med-high rainfall and irrigated temperate zones of Australia, with reference to future climates. It aggregates knowledge from a range of experimental research over the years, identifying strengths and weaknesses of the species. Furthermore, the paper suggests growth suitability rules for strawberry clover based on previous evaluations and maps suitability of strawberry clover across southern Australia. It highlights the breeding focus, commercialisation and marketing required to supersede cv. Palestine and lists the germplasm available in the Australian Pastures Genebank and the origin of that material.*

Corresponding Crop & Pasture Science paper:
Smith RW, Penrose B, Langworthy AD, Humphries AW, Harris CA, Rogers ME, Nichols PGH, Hayes RC (2023) Strawberry clover (*Trifolium fragiferum*): current status and future role in Australian agriculture. *Crop & Pasture Science*. https://doi.org/10.1071/CP22301

Notes:

Hard seed breakdown patterns in cultivars of serradella (Ornithopus spp) in two contrasting environments of south-eastern Australia

Matthew T. Newell[1,4], Rebecca E. Haling[2], Richard C. Hayes[3], Adam Stefanski[2] and Richard J. Simpson[2]

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[3] NSW Department of Primary Industries, Wagga Wagga, NSW, Australia.
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Abstract: *Serradellas (Ornithopus spp) are aerially seeding forage legumes which have been used extensively in mixed cropping areas of southern Australia. Serradellas offer several potential benefits including bloat free grazing for ruminants, adaptation to acid soils and high phosphorus acquisition efficiency. These attributes have increased the interest in their use as alternative pasture legumes in the permanent pasture zone of south-eastern Australia. However, a combination of initial level of hard seededness and the pattern of hard seed breakdown are important traits for the productivity and persistence of temperate annual legumes in the permanent pasture zone. There is a lack of understanding of the hard seed breakdown patterns of serradellas in these environments. This study quantified the pattern of hard seed breakdown among 13 serradella cultivars in comparison to five cultivars of subterranean clover (Trifolium subterraneum L.) in the southern tablelands (Canberra, ACT) and central slopes (Cowra NSW) districts of eastern Australia. Few of the serradella cultivars exhibited a hard seed breakdown pattern similar to the subterranean clover cultivars; the subterranean clovers had high initial hard seed levels but softened significantly over the first three months of the study (i.e. autumn period). All but three of the French serradella (Ornithopus sativus) cultivars tested were found to be completely soft-seeded. The yellow serradella (O. compressus) cultivars generally had a high initial level of hard seed and associated very slow break down pattern. The exception being cv. Yellotas, which displayed rapid seed softening leaving only a low level of residual hard seed after 12 months. Implications for cultivar choice and long-term persistence in these novel environments is discussed.*

Corresponding Crop & Pasture Science paper:
Newell MT, Haling RE, Hayes RC, Stefanski A, Li GD, Simpson RJ (2022) Hard seed breakdown patterns of serradella (*Ornithopus* spp.) in two contrasting environments of south-eastern Australia. *Crop & Pasture Science*. https://doi.org/10.1071/CP22199

Notes:

Pasture legume persistence in Tableland environments of south-eastern Australia

Richard C. Hayes[1,6], Matthew T. Newell[2], Rebecca E. Haling[3], Carol A. Harris[4], Richard A. Culvenor[3], Guangdi D. Li[1], Warwick B. Badgery[5], Neil Munday[5], Andrew Price[1], Rebecca E. Stutz[3] and Richard J. Simpson[3]

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[2] NSW Department of Primary Industries, Cowra, NSW, Australia.
[3] CSIRO Agriculture and Food, Canberra, ACT, Australia.
[4] NSW Department of Primary Industries, Glen Innes, NSW, Australia.
[5] NSW Department of Primary Industries, Orange, NSW, Australia.
[6] Corresponding author: richard.hayes@dpi.nsw.gov.au

Abstract: *This study reports on the persistence of a broad range of self-regenerating annual and perennial pasture legumes from 18 separate field experiments conducted since 2012 across a diversity of Tableland environments. The management of these experiments was lenient compared to standard commercial practice, with legumes sown at a high seeding rate in the absence of a companion perennial grass and only grazed or mown occasionally. Despite this lenient management, most legumes failed to persist at most sites. Of the annual legumes, only a small number of yellow (Ornithopus compressus L.) and French serradella (Ornithopus sativus Brot.) cultivars persisted adequately, although generally not as well as the best-performing subterranean clover (Trifolium subterraneum L.) cultivar, Goulburn. White clover (T. repens L.) was the most consistent of the perennial legumes, although lucerne (Medicago sativa L.) and strawberry clover (T. fragiferum L.) performed well at a small number of sites where the soil was deeper and less acidic. The narrow range of available cultivars in most of the alternative species is seen as a constraint to their adaptation in many Tableland environments. A close examination of subterranean clover and serradella species, where a good range of cultivars existed for testing, highlighted the seed characteristics important for persistence in these environments and potentially presents a model of the traits that might be targeted in other species to improve their persistence. Given the lack of viable long-lived perennial legumes, we suggest that this same model might also be applied to some perennial species such as white clover which could persist through seedling recruitment, to extend its boundary of adaptation and ensure persistence beyond inevitable periods of drought.*

Corresponding Crop & Pasture Science paper:
Hayes RC, Newell MT, Li GD, Haling RE, Harris CA, Culvenor RA, Badgery WB, Munday N, Price A, Stutz RS, Simpson RJ (2023) Legume persistence for grasslands in tableland environments of south-eastern Australia. *Crop & Pasture Science*. https://doi.org/10.1071/CP22277

Notes:

Successful creation of seedless (sterile) leucaena germplasm developed from interspecific hybridisation

D. Real[1,4], C. Revell[1], Y. Han[1,2], C. Li[2], M. Castello[1] and D. Bailey[3]

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[2] Western Crop Genetics Alliance, College of Science, Health, Engineering and Education, Murdoch University, Murdoch, WA 6150
[3] Department of Biology, New Mexico State University, Las Cruces, NM, 88003, USA
[4] Corresponding author. Email: daniel.real@dpird.wa.gov.au

Abstract: *The legume shrub leucaena (Leucaena leucocephala (Lam.) de Wit.) is considered an environmental weed in many parts of Australia, although it is highly regarded as a cattle fodder. The Department of Primary Industries and Regional Development WA with support from Meat & Livestock Australia is creating a leucaena variety that is sterile (without seeds), which poses no weed risk to the environment. Leucaena is a genetically diverse genus from Central America with five tetraploid and 19 diploid species. A conventional breeding strategy was used to create interspecific triploid hybrids that are typically sterile or have much reduced viable seeds. During 2019/20 and 2020/21, more than 2000 inter-specific hand-crosses from 45 different combinations of diploid mothers by tetraploid pollen donors and their reciprocals have created over 3000 hybrid plants. Elite parent accessions were selected from gene banks in Australia, USA, Colombia and locally collected accessions from naturalised populations. Molecular markers have been developed to confirm successful crosses and flow cytometry to confirm the ploidy level. The plants are being evaluated for flowering behaviour, seed production and herbage production across two environmentally diverse sites in Western Australia, Carnarvon and Kununurra. An elite set of triploid germplasm has been identified for further evaluation for their edible biomass production, forage quality, methane emissions, insect tolerance, mimosine level and facility to vegetative propagate. Another strategy to create a sterile Leucaena is utilizing gene editing technology (CRISPR) to edit out flowering genes with the particular candidate of sterile apetala (SAP).*

Corresponding Crop & Pasture Science paper:
Real D, Revell C, Han Y, Li C, Castello M, Bailey CD (2022) Successful creation of seedless (sterile) leucaena germplasm developed from interspecific hybridisation for use as forage. *Crop & Pasture Science*. https://doi.org/10.1071/CP22281

Notes:

Overcoming embryo dormancy in annual medics (Medicago spp.), subterranean clover (Trifolium subterraneum) and alternative annual pasture legumes to assist speed breeding

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Abstract: *Freshly ripened seed of annual medics (Medicago spp.), subterranean clover (Trifolium subterraneum) and alternative annual pasture legume species have embryo dormancy which delays the growth of the next generation by up to six months and is thus an impediment to growing the next generation in breeding programs and basic science studies. In a series of experiments, treatment combinations were identified that achieved high (>90%) seed germination within five days of picking freshly ripened seeds (defined as when pods first lose their green colour) from seven genera (Medicago, Trifolium, Trigonella, Mellilotus, Ornithopus, Astragalus and Biserrula). For highly dormant genotypes the method involved drying freshly picked pods overnight at 45 °C, extracting and scarifying seed, placing seed on filter paper in a petri dish along with non-dormant seed contained within a PVC ring, imbibing seed with 4 μM benzylaminopurine, sealing the petri dish and incubating in the dark at 15 °C. The method was simplified for genotypes with low dormancy. Very highly dormant genotypes required the additional treatment of removing the testa. The method is an effective way of breaking seed dormancy in the ten pasture legumes species from the seven genera tested and may be applicable to other legume species and genera. The rapid breaking of seed dormancy assists speed breeding and makes it less laborious, and time bound than prior methods. The dormancy breaking methods will also assist basic science studies of annual pastures legume species.*

Corresponding Crop & Pasture Science paper:
Peck D, Humphries A, Ballard R (2023) Development of methods to overcome physiological seed dormancy of temperate annual pasture legumes to assist speed breeding. *Crop & Pasture Science.*
https://doi.org/10.1071/CP22314

Notes:

Identification of promising accessions of subterranean clover (*Trifolium subterraneum* L.) from six years of genetic resource regeneration activity

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Abstract: *Subterranean clover (Trifolium subterraneum L.) is one of the most widely sown annual pasture legume species across southern Australia. Over 9000 accessions of subterranean clover are conserved in the Australian Pastures Genebank (APG) in Adelaide, South Australia. One of the functions of the APG is to regenerate accessions of forage species that have low seed stocks or low seed viability. During the period 2016 to 2021, over 1000 accessions of subterranean clover from the subspecies of subterraneum, yanninicum and brachycalycinum were grown in unreplicated small plots in the field in Western Australia to replenish seed stocks. Basic morphological data was recorded for confirmation (and correction) of historical descriptions and to fill gaps in the database. Additional information was collected on distinguishing leaf markers, winter vigour and days to flowering. A comparison of seed yield and winter vigour with a well-established benchmark cultivar cv. Dalkeith (T. subterraneum ssp subterraneum) has identified a cohort of promising accessions that merit further field evaluation.*

Keywords: subterranean clover, Australian pasture genebank, forage, germplasm

Introduction

The conservation of plant genetic resources is increasingly important for Australian and world agriculture as plant breeders seek traits to meet the challenges of a changing climate and animal production systems. Furthermore, urbanisation, landscape degradation and political instability are making it increasingly difficult to collect pasture and forage germplasm from native grasslands in many countries (Smith *et al.* 2021). Pasture legume germplasm in Australia is now conserved in the Australian Pastures Genebank (APG) located in Adelaide, South Australia (Hughes *et al.* 2017; Smith *et al.* 2021) . Key functions of the APG include germplasm acquisition, conservation and distribution. Conservation has required the development of regional (state-based) seed regeneration programs with priorities based on a combination of the species most relevant to Australian agriculture and the quality of seed stocks (Hughes *et al.* 2017). Western Australia, through the Department of Primary Industries and Regional Development (DPIRD) was a major contributor of *Trifolium* species to the APG and manages one of the regeneration programs. It has focussed primarily on subterranean clover (*Trifolium subterraneum* L.) germplasm, given the importance of this species to the livestock and grains industries of southern Australia as a source of high-quality forage and for its ability to fix atmospheric nitrogen (Nichols *et al.* 2012, 2013).

Since the early 1900's, over 50 cultivars of subterranean clover have been registered in Australia across the three recognised sub species of *subterraneum*, *yanninicum*, and *brachycalycinum*. Nichols *et al.* (2013) reviewed the history of genetic improvement of subterranean clover and suggested a range of new breeding objectives including increased phosphorous-use efficiency, reduced methane emissions from grazing ruminant livestock, increased persistence and autumn–winter productivity, increased nutritive value (particularly of senesced material), increased nitrogen fixation ability, and tolerance to low-cost broadleaf herbicides. Over the last five years, the regeneration program has provided an opportunity to explore some aspects of the wider genetic diversity of subterranean clover held in the APG collection.

Methods

Nearly 1200 accessions of subterranean clover sourced from the APG for the purpose of seed stock regeneration were grown during the period 2016 to 2021. Selection of these lines was based on the following factors: i) they were accessions with low seed quantity (<5g) and potentially low viability, ii) they had a maturity less than 140 days to flowering, and iii) they were not represented in medium term storage. Collectively, there were 897 accessions of ssp. *subterraneum*, 258 accessions of ssp. *brachycalycinum* and 18 accessions of ssp. *yanninicum*.

The experimental program was conducted at the DPIRD South Perth field plots with latitude 31.99 ^{o}S and longitude 115.88 ^{o}E. The plot area was typically rotary hoed in late February and watered weekly to encourage as much weed germination for control prior to sowing. Basal dressings of 100 kg/ha 3:1 superphosphate/potash (6.3% P, 12.4% K) were applied in mid-April. The plot area was again rotary hoed a day before the transplant of seedlings.

At the beginning of each growing season, APG provided the seeds for each accession (varying between 5 to more than 1000). Seeds were soaked in deionised (DI) water overnight. Imbibed seeds were kept on moist filter paper in Petri dishes for 3-4 days until germinated. Germination was generally sufficient without scarification. Seedlings with 1-2cm long roots were then transplanted into 78 cell Preforma reusable trays (Australian Seed) prefilled with propagation plugs. Trays of seedlings were transferred to an airconditioned glasshouse (20-21 deg C) and were watered twice a day for 3-4 weeks. After two weeks, a dense peat suspension of Group C *Rhizobium trifolii* was hand watered onto the seedlings. Seedlings were also fertilised weekly using a liquid fertiliser (Thrive®). A week before transplanting the seedlings to the field, seedling trays were moved outside for acclimatization. For each accession, a maximum of 30 healthy seedlings with 4-5 trifoliate leaves were transplanted into micro-plots, each comprising 3 m double rows (30 cm apart) with a 1.5 m buffer between plots and a 1m buffer end to end. This spacing allowed plants to grow without competition for light and moisture. Accessions with low numbers of healthy seedlings (less than 10) were transplanted into 25 cm diameter pots (20 cm depth) filled with Baileys® premium grade potting mix (data not reported). There was no replication of accessions in this experimental design, however Dalkeith was the commercial cultivar that was used each year as a common comparator.

Red-legged earth mite (*Halotydeus destructor* Tucker) and blue green aphids (*Acyrthosiphon kondoi* Shinji) were controlled during the growing season with label rates of chlorpyrifos insecticide. Plants were fertilised throughout the growing season with Thrive® liquid fertiliser and during flowering with liquid potash (Manutec). Hand weeding was conducted within and between rows during the growing season. Supplementary overhead irrigation was used in spring (up to late October) to maximise seed production.

A range of morphological characteristics were recorded but only days to flowering (date when 50% of plants have at least one open flower), and sward vigour in winter (relative to cv. Dalkeith given a score of 100). Accession descriptors based on leaf marks and pigmentation were checked against database records and low numbers of off types removed. In some cases, descriptors were lacking or poorly matched – in these instances plants were retained with an updated characterisation. In early to mid-summer (December to January), each accession was assessed for complete burr development according to maturity and rate of seed set. When fully senesced, the microplots were harvested by collecting the top material, and digging buried burrs directly underneath for sieving. Only intact burrs were collected, and loose seed was discarded to reduce the possibility of background contamination. This plant material was broken up by hand and passed through a Venables thresher to extract the seed. Seed samples were cleaned with an aspirator and small gravity table to remove remaining plant residues, before weighing and packaging for return to the APG.

Results

Amongst this cohort of germplasm, the group from subspecies *brachycalycinum* on average flowered later than ssb. *subterraneum* or ssp. *yanninicum* (Figure 1). Mean seed production (yield) was similar for all three subspecies ($P<0.001$), with the highest yields were found in ssp. *subterraneum*. It should be noted that the low yield of some accessions might be due to having less than 30 plants in the microplot plot, or perhaps where seed quality was poor (data not recorded). However, the accessions that have high yields are the main focus of this study.

The spread of seed yield and flowering time data within the cohort of T. *subterraneum* ssp *subterraneum* is presented in Figure 2. Accessions to a maturity of about 160 days to flowering were able to express their seed yield potential under irrigation. The mean flowering time of cv. Dalkeith across years was 107 days (minimum 92 days and maximum 114 days). Mean seed yield for cv. Dalkeith was 118.4 g (range 66.3 - 262.5 g). Eleven accessions were found to have similar or greater seed yields compared with the highest seed yield of cv. Dalkeith (Figure 2). Mean winter vigour ratings tended to be higher for early flowering accessions and many accessions had a higher winter vigour rating than cv. Dalkeith (Figure 2).

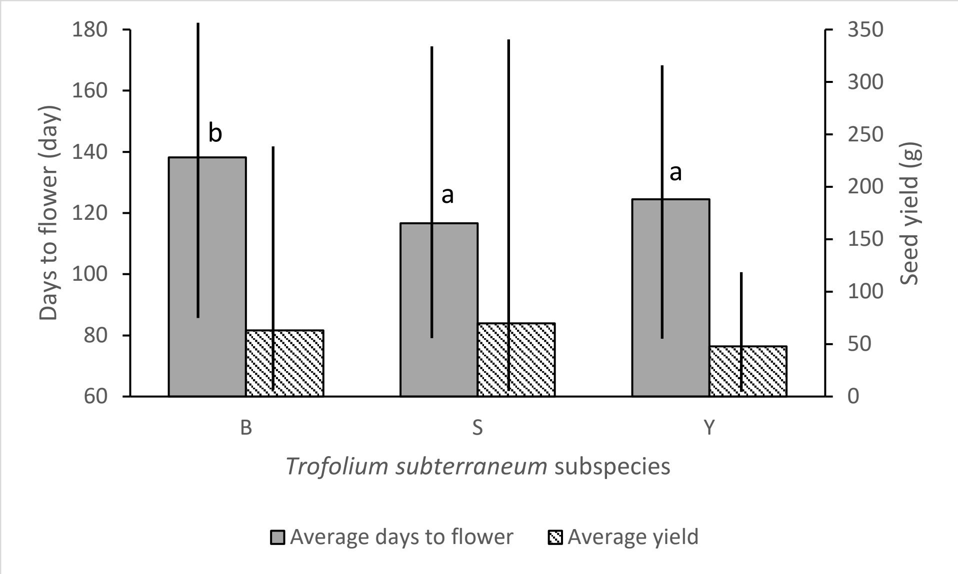

Figure 1. Mean flowering time of the cohort of *Trifolium subterraneum* (ssp. s*ubterraneum (S)*, ssp. *brachycalicinum (B), ssp. yanninicum (Y))*. Grey bars show the mean days to flower (days) and the patterned bars show mean seed yield (g/plot). The solid lines show the range of data. Days to flower bars that show a common letter are not significantly different according to Fisher's protected LSD test (P<0.001). Mean seed yields were not significantly different.

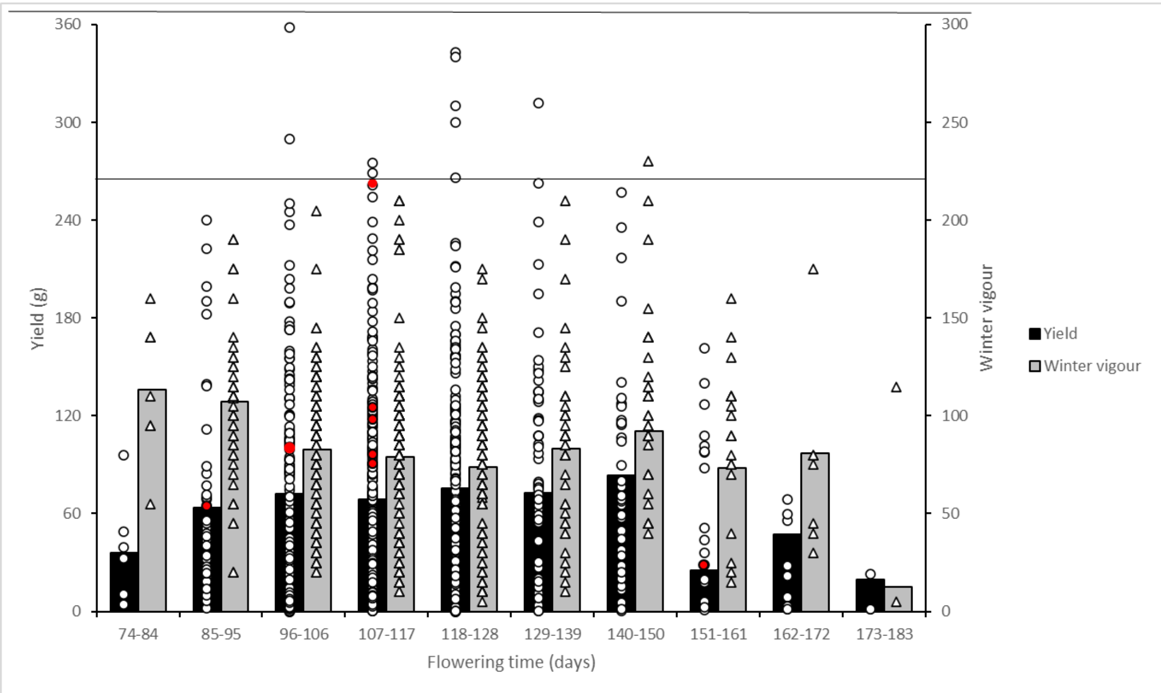

Figure 2. Seed yield (g/plot) (circles) and winter vigour rating (triangles) of individual accessions of *Trifolium subterraneum ssp. subterraneum* grouped according to 10-day intervals in flowering time (days from sowing to appearance of the first open flower on 50% of plants). Mean seed yield (g/plot) (black bars) and mean winter vigour (grey bars) are shown for each flowering interval. Filled circles are cv. Dalkeith points and the horizontal line is the highest seed yield obtained for cv. Dalkeith.

Discussion and conclusions

The early growing season productivity of subterranean clover pastures is a function of plant density and seedling vigour. Rossiter (1966) has shown that in terms of persistence, the success of a strain of subterranean clover, either in competition with other strains or species, is related to its capacity to produce seeds in swards. Seedling regeneration is therefore directly linked to the ability to produce and maintain high seed banks, moderated by the level of seed dormancy and dormancy release. The top 10 accessions of ssp *subterraneum* for seed yield are presented in Table 1 and interestingly four of those were collected from naturalised pasture in Australia, the remainder from the Mediterranean Basin. Four accessions were similar, or earlier flowering, than cv. Dalkeith. High winter vigour was not a feature of this cohort. The top ten accessions across all subspecies for winter vigour were also not well correlated with seed yield (Table 2) but interestingly, half originated from Morocco.

While clearly not systematic, this study has identified a number of subterranean clover accessions that appear to have a combination of desirable flowering times and high seed production. The commercial cultivar Dalkeith remains one of the most widely used subterranean clovers and is a recognised benchmark for the species in WA. Selected accessions with seed yields and winter vigour similar or higher than cv. Dalkeith are therefore worthy of further field evaluation, either for potential commercialisation or as parents in a breeding program. There is also potentially useful later flowering germplasm for higher rainfall environments. Closer evaluation of subterranean clover accessions from Morocco appears warranted.

Table 1. Top ten accessions of T. sub. sub. with the highest seed yield (g/plot) and their flowering time, winter vigour (where measured) and country of origin (nat = naturalised). Baseline winter vigour of cv,Dalkeith = 100.

APG Accession No.	Yield (g/plot)	Days to flower	Winter vigour	Country of origin
79836	358	103	75	Morocco
82794	343	119	-	Aust (nat)
82177	340	119	95	Italy
80063	312	135	-	Greece
82755	310	120	15	Aust (nat)
83265	300	122	60	Italy
82721	290	105	105	Aust (nat)
82541	275	110	-	Spain
1340	269	113	-	Aust (nat)
80059	266	121	-	Greece

Table 2. Top 10 accessions with high winter vigour and their flowering time, seed yield (g/plot) and country of origin (ssp-subspecies S = subterraneum, B = brachycalycinum). Baseline winter vigour of cv. Dalkeith = 100.

APG Accession No.	ssp	Winter vigour	Days to flower	Yield (g/plot)	Country of origin
82954	S	310	85	40	Morocco
73949	B	215	89	128	Morocco
82886	S	210	111	87	Morocco
82925	S	210	109	60	Morocco
82861	B	210	122	16	unknown
79709	B	210	81	13	Greece
82981	S	210	117	10	Morocco
81929	B	205	88	26	Cyprus
11771	S	205	102	9	unknown
81184	S	200	114	136	Israel

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We acknowledge the financial support provided to the APG from Meat and Livestock Australia (MLA), the Grains Research and Development Corporation (GRDC), Australian Wool Innovation (AWI), Dairy Australia and AgriFutures, together with the support and resources for the Regeneration Program provided by the South Australian Research and Development Institute (SARDI) and the Department of Primary Industries and Regional Development (DPIRD) in Western Australia. We also acknowledge the occasional technical staff including Billy Boyd, Timur Talukder and Richard Bennett for their tireless input into the field program.

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Notes:

Prolonged waterlogging tolerance in *Trifolium subterraneum* ssp. *Yanninicum*

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Abstract: *This study investigated tolerance to an extended period (72 days) of waterlogging, relative to free-draining controls, among five Trifolium subterraneum ssp. yanninicum varieties comprised of three commercial cultivars (Yarloop, Trikkala and Riverina) and two breeding lines (YM038 and YM039). After 49 days of growth in pots, waterlogging and control treatments were imposed for 35 days in a controlled glasshouse environment and prolonged for a further 37 days when pots were transferred to outside benches. After 72 days, waterlogging severely reduced shoot DW (P≤0.001; estimated mean 49% of controls). Significant (P≤0.05) variation was evident among varieties, as shoot DW was least affected for YM038 (67% of control) compared to YM039 (36%), Yarloop (40%), Trikkala (47%) and Riverina (53%). In conclusion, waterlogging prolonged for extended periods severely reduced shoot DW but variation for prolonged waterlogging tolerance exists within ssp. yanninicum.*

Key words: subterranean clover, prolonged waterlogging, pasture legume, genotypic variation.

Introduction
Annual plants in regions with Mediterranean-type climates can experience waterlogging from excessive rainfall during the winter-spring growing season and from increasingly intensive and erratic rainfall as a result of climate change (Chapman et al., 2012). Moreover, plants can suffer ‘transient waterlogging’, in which the adverse effects of waterlogging persist after the water recedes, particularly in Mediterranean-type environments when the upper soil level dries but the subsoil remains waterlogged (Malik et al., 2002).

Subterranean clover (subclover, *Trifolium subterraneum* L.) is the most widespread pasture legume in southern Australia, comprising three subspecies: *subterraneum*, *brachycalycinum* and *yanninicum* (Nichols et al., 2013). Of these, ssp*. yanninicum* is broadly used in medium and high rainfall areas of southern Australia, particularly on soils prone to waterlogging, where it is more productive and has greater persistence than the two other subclover subspecies. Genotypic differences within ssp. *yanninicum* have been found for tolerance to waterlogging for periods of up to 35 days (Enkhbat *et al.* 2021; 2022), but little is known about genotypic differences under longer periods of waterlogging. Such information is needed to identify genotypes with improved tolerance, and most importantly, high persistence under current and predicted future climate scenarios.

Methods
Plant materials
Seeds of five varieties of ssp. *yanninicum* were grown at the University of Western Australia Shenton Park Field Station in 2020 to obtain fresh seeds. These comprised three widespread cultivars (Yarloop, Trikkala and Riverina) and two breeding lines (coded YM038 and YM039).

Plant growth
Seeds were scarified and sown on 25 March 2021 into plastic pots (200 mm diameter) filled with a mixture of Gingin loam:potting mix (3:2) in a controlled temperature glasshouse (20/15°C). *Rhizobium leguminosarum* bv. *trifolii* strain WSM1325 was applied and pots were fertilised weekly with commercial ‘Thrive’ fertiliser. Before the imposition of treatments, plants were grown for 49 days after sowing (DAS) under free-draining conditions and watered daily.

Experimental design and treatments
The experiment had a factorial randomised design with two factors, comprising two treatments and five varieties, in three replicates. Each pot, consisting of three plants, was an experimental unit. The treatments, which commenced at 49 DAS, were: (i) a control (free-draining with daily watering); and (ii) waterlogging (WL). Waterlogging was produced by placing pots inside non-draining buckets of the same external diameter and maintaining the water level 10 mm above the soil surface. After 35 days of treatment (84 DAS) in the glasshouse, pots were transferred to outside benches, where they were subjected to winter rainfall and grown for another 37 days (121 DAS), giving a total treatment period of 72 days. Shoots were then harvested by cutting at the soil surface, oven-dried at 60 °C for four days and weighed.

Statistical analysis
Data are graphed as mean with standard error (+SE) using SigmaPlot 14 (Systat Software, Inc.). Data were analysed using two-way ANOVA in R software (version 3.6.3) to assess the effects of varieties, treatments and their interactions with response variables.

Results and discussion
Tolerance to prolonged waterlogging over the 72-day period is defined as the shoot dry weight (DW) under waterlogging, relative to the well-drained conditions (control). For shoot DW, significant main effects for treatment and variety occurred (Fig.1), but there were no significant interactions. The extended period of waterlogging severely reduced shoot DW ($P \leq 0.001$) for all varieties to an estimated mean of 49% of controls.

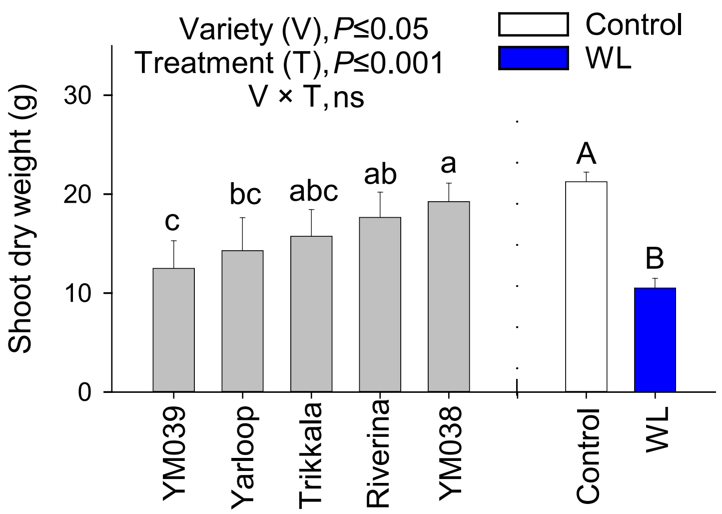

Figure 1. The effect of variety and treatment prolonged for 72 days on shoot dry weight for *Trifolium subterraneum* ssp. *yanninicum* (mean + s.e.; *n*=3). The results of two-way ANOVA with the variety (V) and treatment (T) main effects are presented as there was no significant interaction V × T: n.s., not significant. Different lower- and upper-letters denote significant differences among ssp. *yanninicum* varieties and treatments (*P*=0.05; Fisher's LSD test).

This reduction is more severe than in previous glasshouse experiments of ssp. *yanninicum* with shorter durations of waterlogging. After waterlogging for 35 days (commenced at 28 DAS) Enkhbat *et al*. (2021) demonstrated reduced shoot DWs from 57-62% of well-drained controls in cultivars Meteora, Trikkala and Yarloop. Shoot DWs among 32 diverse ssp. *yanninicum* genotypes also ranged from 66-120% of well-drained controls after waterlogging for 28 days (commenced at 21 DAS) (Enkhbat *et al*. 2022). The severe reduction of shoot DW in the present experiment is likely to be associated with the extended period of waterlogging, as the adverse effect of waterlogging becomes more pronounced as waterlogging extends for longer periods (Ponnamperuma, 1984, Striker et al., 2005). It should be noted that while the effects of extended waterlogging are likely to be indicative in this experiment, the absolute levels of reduction are not conclusive, as plants were grown in an outside environment in the later half of the treatment, in which the effects of waterlogging might have interacted with other environmental factors that could exacerbate the effects of waterlogging.

Significant genotypic variation ($P \leq 0.05$) was evident among ssp. *yanninicum* varieties to prolonged waterlogging, with YM038 having the highest relative shoot DW (67% of control), compared to Riverina (53%), Trikkala (47%), Yarloop (40%) and YM039 (36%). Earlier Enkhbat *et al*. (2022) demonstrated high variation in tolerance to shorter durations of waterlogging among genotypes of ssp. *yanninicum*. It is noteworthy that the highest tolerance to waterlogging was observed in breeding line YM038, rather than commercial cultivars, indicating the potential to increase tolerance to prolonged waterlogging over current cultivars. The magnitude of shoot DW differences varied little among the three cultivars tested, but significant shoot DW differences were demonstrated between YM038 and YM039 (Fig. 2). Several morphological differences were also observed visually between YM038 and YM039. The highly waterlogging tolerant YM038 grew more vigorous superficial roots and maintained leaf size and had lower reductions in petiole length. In contrast, leaf size and petiole lengths of YM039 were extensively reduced with yellowing or senesced leaves and petioles (Fig. 2). Such varietal differences in biomass production and persistence have been observed in field trials subjected to prolonged waterlogging, but further testing is needed in commercial paddocks to confirm.

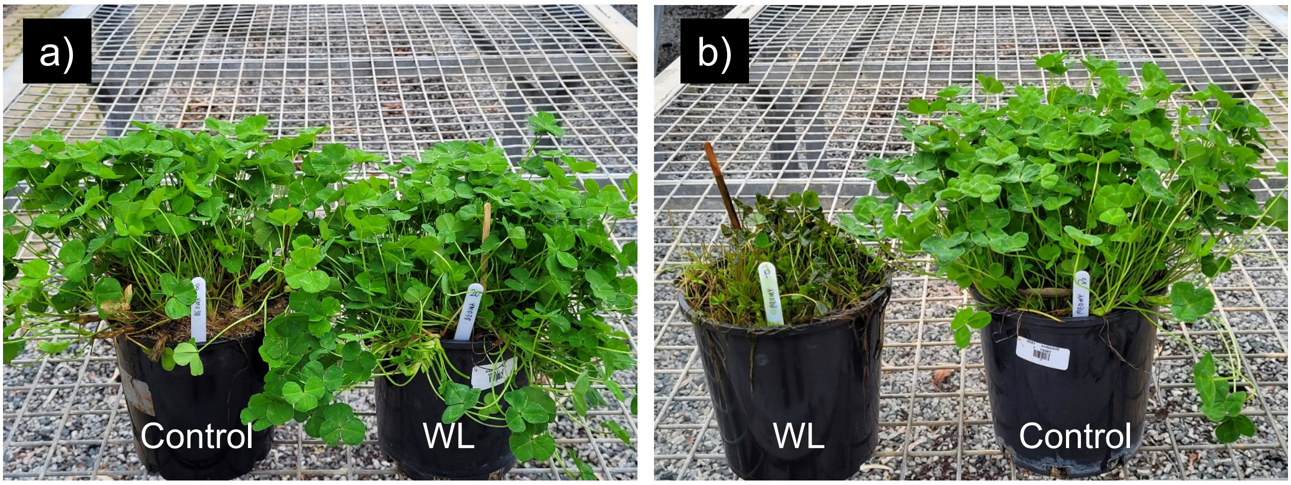

Figure 2. The effect of waterlogging prolonged for 72 days on shoot production for (a) YM038 and (b) YM039.

Conclusions

All five varieties of ssp. *yanninicum* in this experiment were severely impacted by prolonged waterlogging for 72 days. Differences among varieties were found, with breeding line YM038 being the most tolerant.

Acknowledgments

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Notes:

Advances in Genebank information systems to identify diversity in pasture genetic resources

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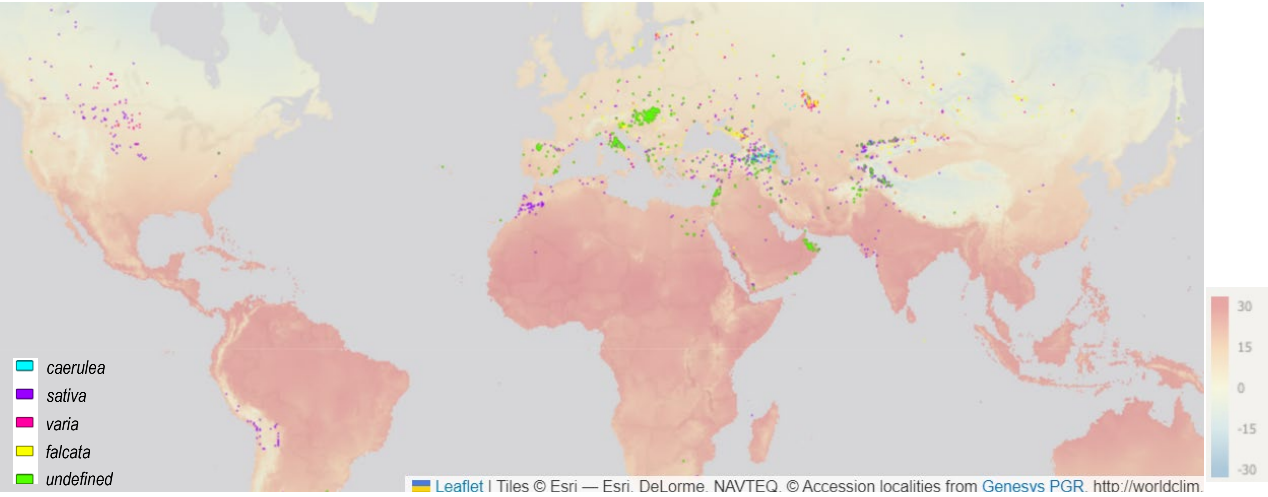
Abstract: *Information systems that support genetic resource collections are continually being improved, offering new methods to search for genetic diversity. Since its inception in 2015, the Australian Pastures Genebank (APG) has built a GRIN-Global database (https://apg.pir.sa.gov.au/gringlobal/search, June 2023) that contains passport information, an online shop and inventory. The Standard Material Transfer Agreement (SMTA) is now also digital, using the Food and Agriculture Organisation (FAO) easy SMTA (https://mls.planttreaty.org/itt/), reducing the time it takes to order and receive germplasm. The APG loaded its database onto Genesys (https://www.genesys-pgr.org/), an online platform that combines information on global plant genetic resources onto a single website. Mapping features and climate overlays within Genesys allow users to view the geographical distribution and climate adaptation of accessions that are returned from a search, perform simple ecogeographical analyses, and refine germplasm lists based on any combination of these criteria. The APG will process seed orders directly from either GRIN-Global or Genesys. APG staff and collaborators will continue to build on these information systems, loading new and historic observation data, and publishing core collections to make pasture genetic diversity more accessible to users. This paper provides examples of how GRIN-Global and Genesys can be used to define the limits of species adaptation and identify accessions with diversity for key climate adaptation traits.*

Keywords: Forages, Genebank, Climate, Adaptation, Core collection

Utilising Genesys to Identify lucerne with adaptation to Diverse Climates

Genesys is an online platform for managing information on global plant genetic resources, assembling databases from individual genebanks at one location. The mapping feature of Genesys displays the global distribution of collection origins for accessions that are georeferenced, proving excellent information on the known geographical distribution of a species (Figure 1). This feature allows the user to refine their search for accessions based on latitude, longitude, and elevation. The georeferenced data is also overlayed onto www.worldclim.org datasets, which further allows accession lists to be refined using a range of bioclimatic variables (rainfall, temperature, seasonality etc.).

We used Genesys to summarize the geographical and climatic adaptation of lucerne (*Medicago sativa*) based on collection origins (Figure 2). The results show that lucerne has been collected from environments with elevation between -36 and 3913m, average annual precipitation of 153 – 2384 mm and between latitudes of 13.7 – 68.3 degrees (within the arctic circle). Alfalfa is adapted to extreme cold and hot environments, with the average minimum and maximum temperatures of the coldest and warmest months -44.3 and 42.8 °C respectively (Figure 2). The accessions that have been collected from origins that represent extremes of the geographical and climatic adaptation are available as a subset, and can be viewed and requested as a group through https://www.genesys-pgr.org/subsets/0367d084-95c8-4d26-85d1-c14b98ebbb7b



M. sativa subspecies*	APG	Total
caerulea	182 (122)	432 (172)
sativa	1,165 (161)	7,886 (1,130)
varia	412 (223)	2,239 (478)
falcata	303 (205)	1,396 (610)
glomerata, tunetana, viscosa	57 (27)	180 (40)
Undefined	576 (32)	7,603 (64)
Total	**2,695 (770)**	**19,736 (2,494)**

*Information collated from Genesys-PGR and GRIN-Global databases

Figure 1. The number and distribution of Medicago sativa L. subsp. accessions held at the APG and other international genebanks, listed on Genesys (November 2022). Numbers in parenthesis indicate the number of georeferenced accessions. Background displays annual mean temperature, provided by www.worldclim.org.

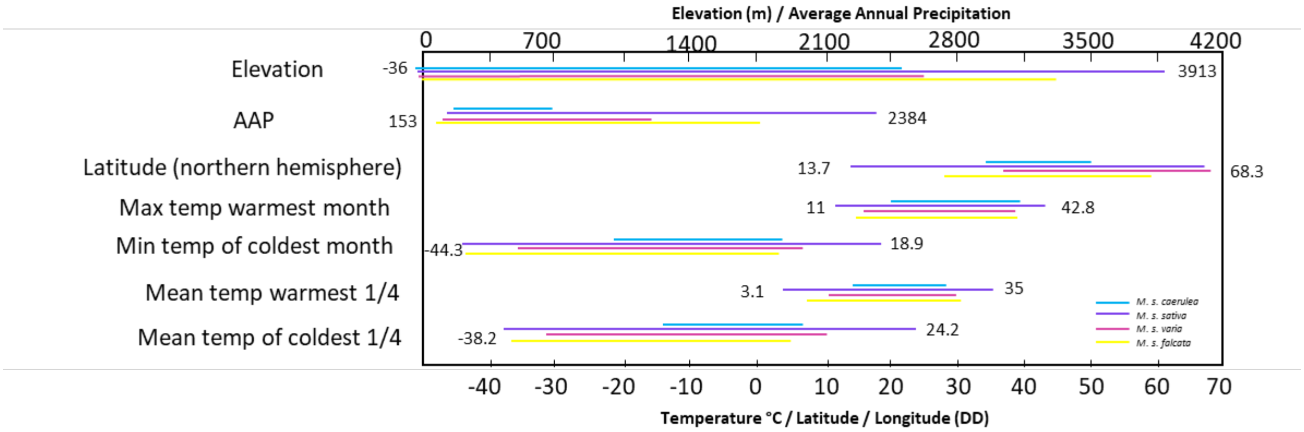


Figure 2. Adaptation of Medicago sativa L. subsp. accessions [wild, (natural, semi natural), traditional landrace and unclassified] to different environments based on elevation, average annual precipitation (AAP), northern hemisphere latitude, and bioclimatic variables that include the maximum temperature of the warmest month, minimum temperature of the coldest month, mean temperature of warmest quarter, and mean temperature of the coldest quarter. Extreme values at species level shown for each variable.

The subset includes 28 lucerne accessions including 14 subsp. *sativa*, 4 nothosubsp. *varia*, 1 subsp. *caerulea* and 9 subsp. *falcata*. The subset also contains 2 *M. sativa* subsp. *falcata* accessions collected from the extreme mildest winter temperature for this sub species, where the minimum temperature of the coldest month was at least 3 ºC. The lucerne climate adaptation subset will now be characterised for key phenotypic traits and molecular diversity. Data and results from all evaluations of this subset will be available at Germinate 3, https://ics.hutton.ac.uk/cwr/alfalfa/#/home and from GRIN-Global.

Ecogeographical analysis in Genesys

Genesys can be used to quickly find germplasm collected from similar environments to an area selected on a map. Users simply mouse click on the show climate button (◉) and then click on the map, bringing up the climate for that location. Accessions collected from similar climates can then be displayed, further refined, and ordered (from participating genebanks such as the APG). This feature can be used to find germplasm that is adapted to an area that has the same climate as a future climate for an agricultural zone (i.e., where there is predicted to be geographical climate shift, (Nidumolu *et al*. 2022). It can also be used to identify new potential candidate species that are adapted to a climatic zone. An instructional video on how to perform an ecogeographical analysis in Genesys is available at [Using Genesys - A Crop Trust Tool - YouTube].

Core Collections

Core collections developed using phenotypic and molecular techniques for subterranean clover (*Trifolium subterranean,* Nichols et al 2013), biserulla (*Biserrula pelecinus*, Ghamkhar et al 2012) barrel medic (*Medicago truncatula,* Ellwood *et al.* 2006) and hyacinth bean (*Lablab purpureus,* Pengelly and Maass 2001) are now available as a subset on Genesys, and can be ordered as a group (added to the shopping cart as a single click) at Genesys (https://www.genesys-pgr.org/subsets). Core collections typically capture >90% of the diversity held in a species and are therefore an excellent starting point for investigating diversity. However, diversity for rare genetic traits may not be represented in the core collections.

Observation data

The APG is uploading both new and historic (collected over the last 50 years) observation data onto GRIN-Global. Available data is dependent on the pasture group and information collected. For annual *Medicago* and *Trifolium* spp. there are more than 20,000 accessions with observation data for important agronomic traits such as flowering time, forage and seed yield. The observation data can be searched under the Descriptors heading, at https://apg.pir.sa.gov.au/gringlobal/descriptors. Datasets for each descriptor in a pasture group can also be downloaded as a .csv file. For example, the range in values for flowering time in annual medic, for 17,582 accessions is shown in Table 1. The flowering time of annual pasture species is one of the most important traits, because it determines the plants adaptation to a specific rainfall zone. The availability of this information online makes the collection much more valuable, by allowing users to refine species search lists for traits of interest. There are 4.5 million data points of historic phenotypic observation data, which will begin to become available in GRIN-Global in 2023. The availability of observation data combined with ecogeographical selection tools in information systems such as Genesys, provide the key tools required to identify wild diversity that will support the development of climate adapted forages over the next 50+ years.

Table 1. Number of days from wetting up to 10% plants with a flower (10% First Flower Date) in 17,582 accessions of annual *Medicago.*

Days to 10% flowering	Number of Accessions
37 - 57.4	2
57.4 - 77.8	281
77.8 - 98.2	1874
98.2 - 118.6	6276
118.6 – 139	7498
139 - 159.4	1986
159.4 - 179.8	92
179.8 - 200.2	15
200.2 - 220.6	0
220.6 – 241	1

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Notes:

Flowering time of subterranean clover (*Trifolium subterraneum* L.) in Australia and New Zealand

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Abstract: *Subterranean clover (sub clover) is an important winter annual legume species commonly used to provide lactation feed to livestock in winter-spring months. A key physiological aspect to be considered in sub clover management is flowering time. In this paper the sub clover flowering time in Australia and New Zealand was systematically quantified based on a compilation of previous published datasets and field observations. The mean time to 50% flowering was 56 days shorter for the Australian (116 days, ranging from 44 to 213 days) than for the New Zealand datasets (172 days, ranging from 114 to 271 days). On a thermal time basis this ranged from 628 to 2600 °Cd. The values also differed between countries, being lower in Australia (1259 °Cd) than in New Zealand (1551 °Cd). From field observations, the number of days and thermal time from sowing to flowering ranged from 67 to 261 which correspondent to 771 to 2330 °Cd across sowing dates and cultivars. By combining all the datasets, it was established that when in an increasing photoperiod (Pp), 'Early' and 'Late' cultivars had constant thermal time requirements. In a decreasing Pp, a single slope (370 °Cd/h) explained the increase in thermal time with 'Early' cultivars having a maximum value of 1700 °Cd at ~13.5 h while 'Late' cultivars flowered with nearly 2500 °Cd.*

Key words: annual legume, *Trifolium subterraneum*, development, re-analysis.

Introduction
Subterranean clover (*Trifolium subterraneum* L.; sub clover) is an important winter annual legume commonly used to provide high-quality feed to livestock in winter-spring months. It accumulates biomass during autumn-winter, which are periods of low pasture growth in temperate regions. It also fixes atmospheric nitrogen that becomes an input to the pastoral system. The species originates from temperate Western Europe and the Mediterranean basin (Ghamkhar *et al.* 2014) and now is grown worldwide, with large areas under cultivation in Australia and New Zealand (Abdi *et al.* 2020). Its long-term persistence depends on its annual ability to regenerate a sufficiently high plant population by reaching flowering and setting seeds to re-establish following the summer dry period. Thus, a key physiological aspect to be considered in sub clover management is flowering time. Defoliations can be timed to maximise the number of inflorescences and seed set (Brambilla *et al.* 2017). Sub clover development rates towards reproduction are expected to accelerate in long photoperiods, which further regulates the control of flowering time by temperature (Evans *et al.* 1992; Pecetti *et al.* 2020). This paper (i) systematically summarizes the seasonality of sub clover flowering time from cultivars of different subspecies and maturity groups ('Early' and 'Late' cultivars) based on a compilation of previous published datasets from Australia and New Zealand; and (ii) quantifies the thermal time requirement to flowering from published datasets and field experiments in response to the mean photoperiod and its direction of change.

Methods
Published datasets
These data were obtained from 15 peer-reviewed publications gathered using search engines Google Scholar and Web of Science using the key words: subterranean clover, flowering time, *Trifolium subterraneum*, reproduction, seed set, reproductive, development, phenology, Australia, New Zealand. Data points were directly extracted from tables and text or digitally sampled from graphs. The variables obtained from the publications were year, location, country, latitude, longitude, cultivar name, sowing date and flowering date. The datasets compiled yielded a total of 369 independent datapoints from Australian and New Zealand experiments with seven locations in Australia and six locations in New Zealand (Figure 1 A). A total of 39 subterranean clover cultivars from the three subspecies were gathered (Teixeira *et al.* 2020).

Field experiment data
The field experiment was located at Iversen Field at Lincoln University, Canterbury, New Zealand (43°38'S, 172° 28'E, 11 m a.s.l., Figure 1 A). The seeds were outsourced from commercial Australian companies or from distributors in New Zealand. 'Antas', 'Denmark', 'Leura' and 'Monti' were supplied by Seedmark, Seed Technology & Marketing Pty Ltd. 'Narrikup' was supplied by Seed Force Ltd and 'Woogenellup' was sourced from the Field Research Centre, Lincoln University seed collection. These cultivars were selected because they have been introduced into the New Zealand seed market and represent a range of flowering groups (medium and late), growth habit (prostrate and semi-erect) and a range of hardseededness based on

Australian information. To create a wide photoperiod range seeds were sown from winter 2015 to autumn 2016 at ~ 45-day intervals over eight sowing dates. Details of the experimental design has been reported (Teixeira *et al.* 2021). Flowering was evaluated at 3–5-day intervals on the first runner of five marked plants.

Flowering quantification and weather files

The difference between sowing and flowering dates provided the estimate of number of days to flowering. For both published and field observations, flowering was defined as when "50% of plants had their first open flower (R3, or stage 12 BBHC scale) in the phenological scale (Enriquez-Hidalgo *et al.* 2020; Teixeira *et al.* 2021). Australian weather files were sourced from the Australian Data Archive for Meteorology and New Zealand weather data were sourced from New Zealand's National Climate Database. For the field experiment at Lincoln, air and soil temperatures were recorded on site. Mean daily thermal time was calculated using the daily maximum and minimum air temperatures and assumed a smooth sinusoidal pattern across the day (Jones and Kiniry 1986). Changes in duration and direction of photoperiod influence the time to flower initiation of clovers (Nichols *et al.* 2009) . Thus, photoperiod direction was considered as either "descending" (autumn to winter when the photoperiod declined from ~12.5 to 10 hours) or "ascending" (winter to mid-spring when photoperiod increased from ~10 to 15 hours (h).

Analysis

Box plots for the chronological time (days) and thermal time (°Cd) to flowering show medians (lines in box middle) and interquartile ranges (25 and 75% as box edges). Analysis of the datasets was performed with the statistical language R version 3.6.3. Linear regression coefficients for the thermal time to 50% flowering (°Cd) and photoperiod were generated with package "broom" and function "glance.lm" (R Core Team 2020).

Results

Studied cultivars and subspecies

The subspecies *subterraneum* was the most common (60%), followed by *yanninicum* (15%) and *brachycalycinum* (2%). 'Mt Barker' was the most frequent cultivar, present in 73% of the experiments, followed by 'Tallarook' and 'Seaton Park' in 60% of the experiments. Some non-commercial lines and Mediterranean ecotypes were included (Smetham *et al.* 1994).

Flowering time - published datasets

The flowering time ranged from 44 to 271 days across all reanalysed experiments (Figure 1 B).
The average time to 50% flowering was 56 days shorter for the Australian datasets (116 days, ranging from 44 to 213 days) than for the New Zealand datasets (172 days, ranging from 114 to 271 days).
On a thermal time basis the period from sowing to 50% flowering ranged from 628 to 2600 °Cd. The values also differed between countries, being lower in Australia (1259 °Cd) than in New Zealand (1551 °Cd).

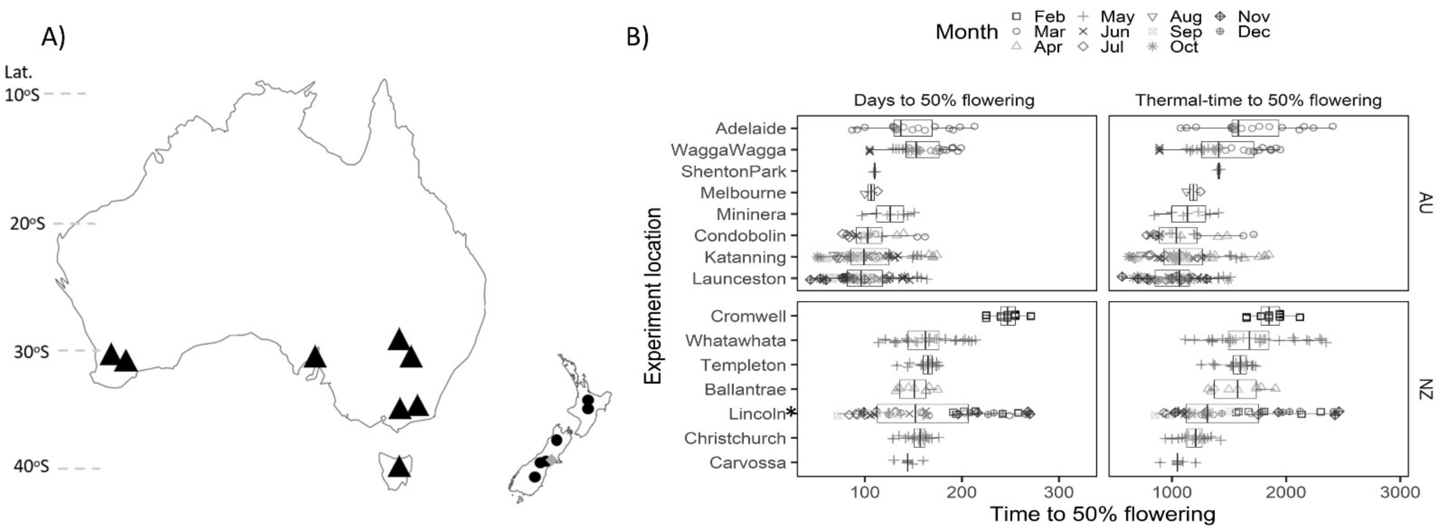

Figure 1. (A) Location of published experimental data from Australia (▲) and New Zealand (●) and the field experiment (♦) on Iversen Field, Lincoln University, New Zealand. (B) The average time (days and thermal time °Cd) to 50% flowering for experimental locations in Australia and New Zealand. Lincoln* indicates 50% flowering measured in the field experiment at Lincoln, New Zealand.

Flowering time – field experiment
The number of days and thermal time from sowing to flowering ranged from 67 to 261 days which corresponded to 771 to 2330 °Cd across sowing dates and cultivars (Figure 1 B). Cultivars only differed ($P<0.01$) in summer to mid-autumn sowing dates (November-March). 'Early' cultivars ('Narrikup' and 'Monti') flowered 25±5.4 days (239±69.1 °Cd) before the 'Late' cultivars.

Flowering time in relation to photoperiod
Data points from published experiments and the field experiment were combined in a meta-analysis to establish unifying relationships (Figure 2) between time to flowering and photoperiod. For all cultivars tested in the field experiment, flowering occurred when the absolute photoperiod was between 12.8-16.5 h for 'Antas', 13.3 and 16.6 h for 'Denmark', 14.3-16.6 h for 'Leura', 13.4-16.5 h for 'Monti', 12.5-16.6 for 'Narrikup' and 12.9-16.3 for 'Woogenellup'. By combining all the datasets, it was established that in an increasing Pp, 'Early' and 'Late' had constant thermal time requirements and at decreasing Pp, a single slope (370 °Cd/h) explained the increase in thermal time with 'Early' cultivars having a maximum value of 1700 °Cd at ~13.5 h while 'Late' cultivars flowered after ~2500 °Cd.

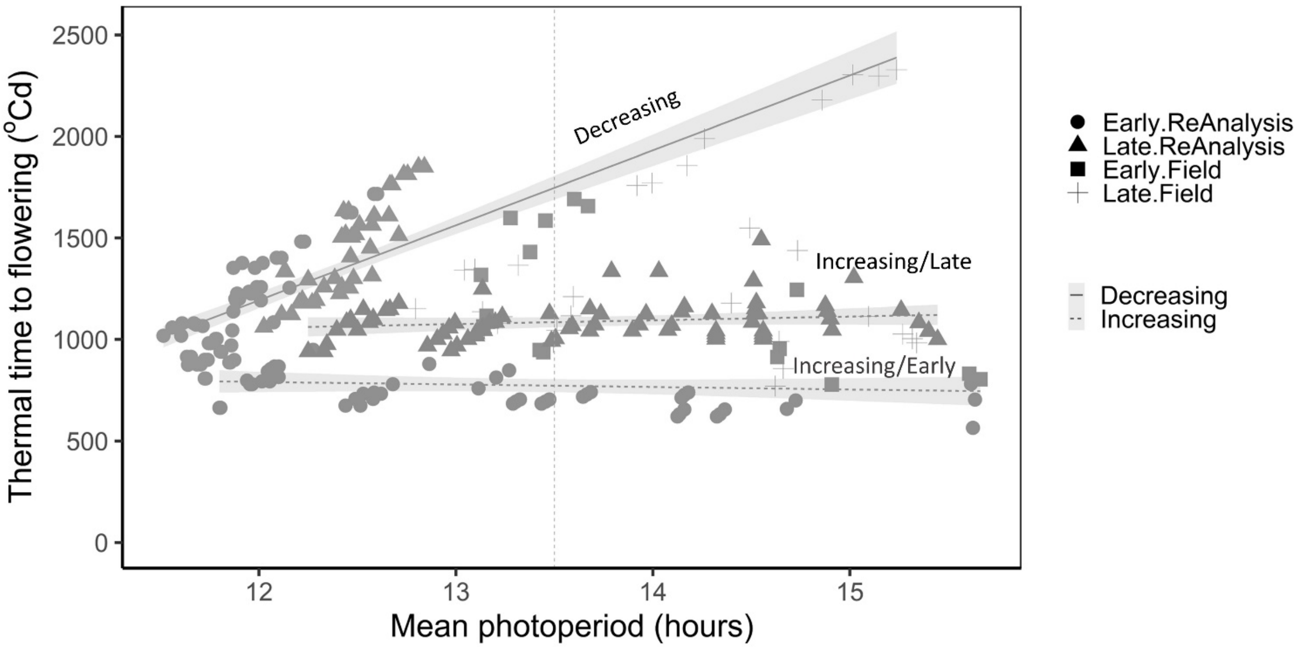

Figure 2. The estimated thermal time to 50% flowering from the published datasets and field experiment in response to the mean photoperiod direction (Pp_direction). Coefficients for groups are a constant of 565 and 770 °Cd for 'Early' and 'Late'- cultivars at any value of decreasing Pp. At increasing Pp, a unifying linear increase from 11 h to 16.5 h at a rate of 550 °Cd/h was found for both 'Early' (lower dotted line) and 'Late' cultivars (R^2=0.75, upper dotted line) with a maximum value of 1200 °Cd for 'Early' cultivars. The regression (solid line) is: TT (°Cd) = -3237(±275.8)+369(±22.1)Pp, R^2=0.75, p<0.001. The grey bands are the 95% Confidence interval= 0.95-0.99.

Discussion
A strong seasonality was found in Australian datasets where similar cultivars were sown in the same location but on different days of the year. In both countries, flowering of subterranean clover occurred later for autumn-sown crops (April) than early-spring sown crops (October). Autumn sown plants flowered after ∼180 days while early spring sown crops flowered 50 days after sowing. The extent and seasonal pattern indicated a separation of cultivars into two maturity groups. For example, 'Early' flowering cultivars ('Nungarin', 'Trikkala' and 'Dalkeith') showed a maximum thermal time requirement to flower of ∼1400 °Cd while 'Late'-flowering cultivars ('Karridale', 'Larisa', 'Meteora', 'Mt Barker', 'Seaton Park' and 'Woogenellup') had a maximum thermal time requirement of ∼2000 °Cd.

The clear contrast between 'Early' and 'Late' cultivars highlights the flexibility available to farmers who can select genotypes according to local environments. For example, in a New Zealand dryland scenario, it would be unreasonable to sow late-cultivars in late spring (late-October/November) as the plants would have insufficient time to reproduce before the summer drought. These would only provide vegetative growth. Typically, in New Zealand sub clover is sown in and then re-establishes in autumn. The equations developed using the re analysed datasets and the measurements from the field experiment enabled an estimate of the flowering date of 'Early' and 'Late' cultivars for different locations.

Conclusions

Based on the quantitative parameters developed in this investigation it is now possible to predict the flowering time of groups of cultivars in any given environment, and from any sowing date.

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Notes:

The effects of climate change on flowering time of subterranean clover (Trifolium subterraneum L.) in New Zealand

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Abstract: *The flowering time of subterranean clover was simulated and quantified using a thermal time-based model under different greenhouse gases and aerosol pathways over the 21st century known as the Representative Concentration Pathways (RCPs). Twenty years of historical (1979-1998), mid (2039-2058) and end (2079-2098) of the century, daily minimum, maximum air temperature (°C) and rainfall (mm) data from Virtual Climate Station Network were used to estimate the flowering time shift in three northern and southern New Zealand districts. The results show an early shifting under the 'worst-case' pathway (RCP8.5, which requires emissions continue to rise throughout the 21st century). The flowering date of 'Early' cultivars shifted (P<0.001) ~ 12± 3.1 days across the districts by the end of the century under RCP8.5. Overall, the shifts in flowering time were lower (2.0 - 12 days) on mid-century RCP's than on end of century RCP's (2.0 - 26 days). In northern areas of New Zealand flowering time showed little change with opportunity for grazing management that would allow seed set to be managed on-farm. To mitigate future warming effects may require greater use of supplementary feed to manage longer durations of summer dry conditions. The genetic diversity currently available within different maturity subterranean clover cultivars is likely to be adequate to cope with the magnitude of climate change estimated for New Zealand conditions.*

Key words: development, forage legume, genotypes, phenology, *Trifolium subterraneum*.

Introduction
Subterranean clover (*Trifolium subterraneum* L.) is a winter annual legume pasture commonly used in summer dry environments in New Zealand and Australia. Subterranean clover provides grows feed in spring than perennial legumes before it flowers and sets seed to survive the summer dry as a buried seed to regenerate each autumn. Thus it provides high protein feed for livestock from autumn to late spring. Climate predictions to the end of the century suggest that mean global temperature will increase by 1.8–4.0 °C (range 1.1–6.4 °C), depending on the greenhouse gas emission scenario. This will be accompanied by changes in rainfall patterns and an increase in climate variability (IPCC 2013). These changes have implications for pasture legumes growth and development. The time of flowering is a critical stage of development in the life cycle of most plant species, including subterranean clover, and is when seed set begins. It is also important for adaptation to the abiotic stresses of temperature and water deficit, and to biotic limitations within the growing season. Adaptation to moderate changes in climate that influence temperature, season duration, and sowing dates, as well as the occurrence of abiotic stress, can be achieved by selecting genotypes with appropriate flowering times and crop durations (Berger *et al.* 2017). Farmers and plant breeders have successfully selected/manipulated life cycle duration and phenology of subterranean clover to maximize the array of environments in which plants grow (Nichols *et al.* 2007) as well as their yield (Olykan *et al.* 2019) under current climates. The question for this research is how to plan for future climate change. In this study, the flowering time of subterranean clover was simulated and quantified using a thermal time-based model under different greenhouse gas and aerosol pathways over the 21st century known as Representative Concentration Pathways (RCPs).

Methods
Computer modelling study component
The modelling component of this study requires the initial definition of land "suitable for subterranean clover" in New Zealand (CRS: WGS84; resolution: 0.05° * 0.05°). Temperature and rainfall are major drivers of spatial patterns and physiological responses in plants (Tait *et al.* 2016). The modelling component was described previously (Guo *et al.* 2022). Future scenarios known as representative concentration pathways (RCPs) used were RCPs which include one mitigation pathway (RCP2.6, which requires removal of some of the CO_2 presently in the atmosphere), two stabilization pathways (RCP4.5 and RCP6.0), and one pathway ('business as usual') with very high greenhouse gas concentrations by 2100 and beyond (RCP8.5). The mitigation pathway RCP2.6 requires removal of some of the CO_2 presently in the atmosphere. The stabilization pathway RCP4.5, requires CO_2 emissions to start declining by approximately 2045 to reach roughly half of the levels of 2050 by 2100. RCP 6.0 includes continuous global warming where CO_2 levels rise to 670 ppm by 2100 making the global temperature increase by about 3-4°C by 2100. RCP8.5 represents the worst-case scenario with CO_2 emissions continuing to rise throughout the 21st century (IPCC,

2013). The RCP-past represents the simulated historical data which were generated by the same general circulation (GCM) model. Both historical (Past RCP, 1979-1998) and future climate (RCP's) predictions created from the same model were compared. The mid and end indicated on result graphs represent a 20-year period in mid (2039-2058) and end (2079-2098) of 21st century. The 0.05° by 0.05° resolution daily minimum, maximum air temperature (°C) and rainfall (mm) data from Virtual Climate Station Network (NIWA 2020) were used.

Phenological component

The life cycle of subterranean clover starts when seed is drilled or over sown in autumn (March- April in New Zealand), or buried seeds germinate. This was estimated to occur after a rainfall event of ~20 mm (Lucas *et al.* 2005) and the accumulation of ~36±6.0°Cd (Teixeira *et al.* 2019). Flower development was quantified as when 50% of plants had their first flower fully open (R3, or stage 12 BBHC scale) (Enriquez-Hidalgo *et al.* 2020). The date of flowering required different functions for cultivars previously classified as 'Early' or 'Late' maturity groups based on field trials (Teixeira *et al.* 2020). This included the total period of a decreasing photoperiod in autumn from emergence or natural plant re-establishment. Time to first flower was then estimated by unified regression which considered photoperiod and thermal time accumulation based on cultivar maturity grouping from field experiments in Australia and New Zealand and field experimental data from 2015 to 2017 at Lincoln University, Canterbury New Zealand (Teixeira *et al.* 2021, *Ibid*). The estimated change in the number of days for three districts in the North (Far North, Hastings and Ruapehu) and South (Tasman, Ashburton and Central Otago) Islands in New Zealand (Figure 1) according to mid and end century RCP2.6; RCP4.5; RCP6.0 and RCP8.5 scenarios are presented. Data processing and analyses were performed using R version 3.6.3 (R Core Team 2020).

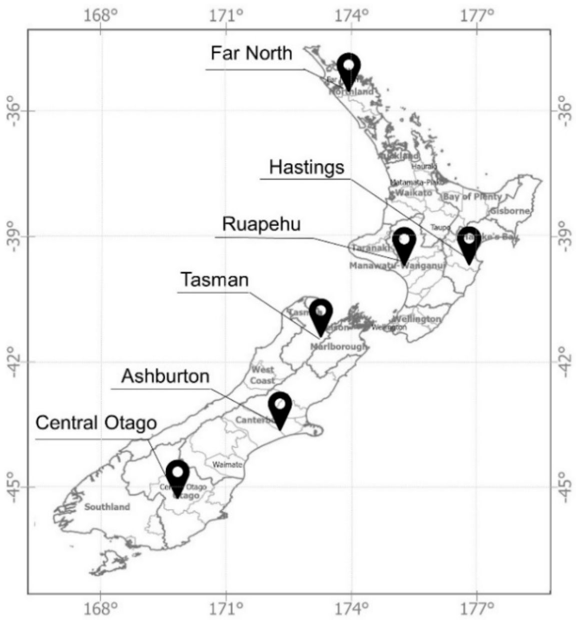

Figure 1. Location of the six New Zealand districts selected to estimate the effects of climate change in flowering time of 'Early' subterranean clover cultivars.

Results

The flowering seasons were expected to stay around the same date but shift to slightly earlier dates under the mitigation pathway (RCP2.6), and under the stabilization pathway (RCP4.5) respectively. There was a notable shift to earlier flowering under the 'worst-case' pathway (RCP8.5, Figure 2). The earliest flowering (50% of the plants had their first visible flower) date was projected to occur on August 02 in the Far North district (North Island) and the latest on October 17 (±2.5 days) with the baseline climate (Figure 2). The flowering date of 'Early' cultivars shifted ($P<0.001$) ~12 ±3.1 days across the districts in New Zealand by the end of the century under RCP8.5. Overall, the shifts in flowering time were lower (2.0 - 12 days) on mid-century RCP's than on end of century RCP's (2.0 - 26 days). Overall, the shifts in flowering time were on average lower (6.0 ±2.2 days) in northern districts than in southern districts (10 ±2.5 days). The estimated changes in flowering time were extreme when considering RCP 8.5 at the end of century. When comparing the baseline climate (RCP-past) to the worst future climate (end-RCP8.5), the 'Early' cultivars hastened their flowering by 16.0 ± 3.5 days. Among the districts, the overall projected changes in flowering time were lower (3.0 ±1.0 days) in the Far North district than in the Central Otago district (13.0 ±3.2 days).

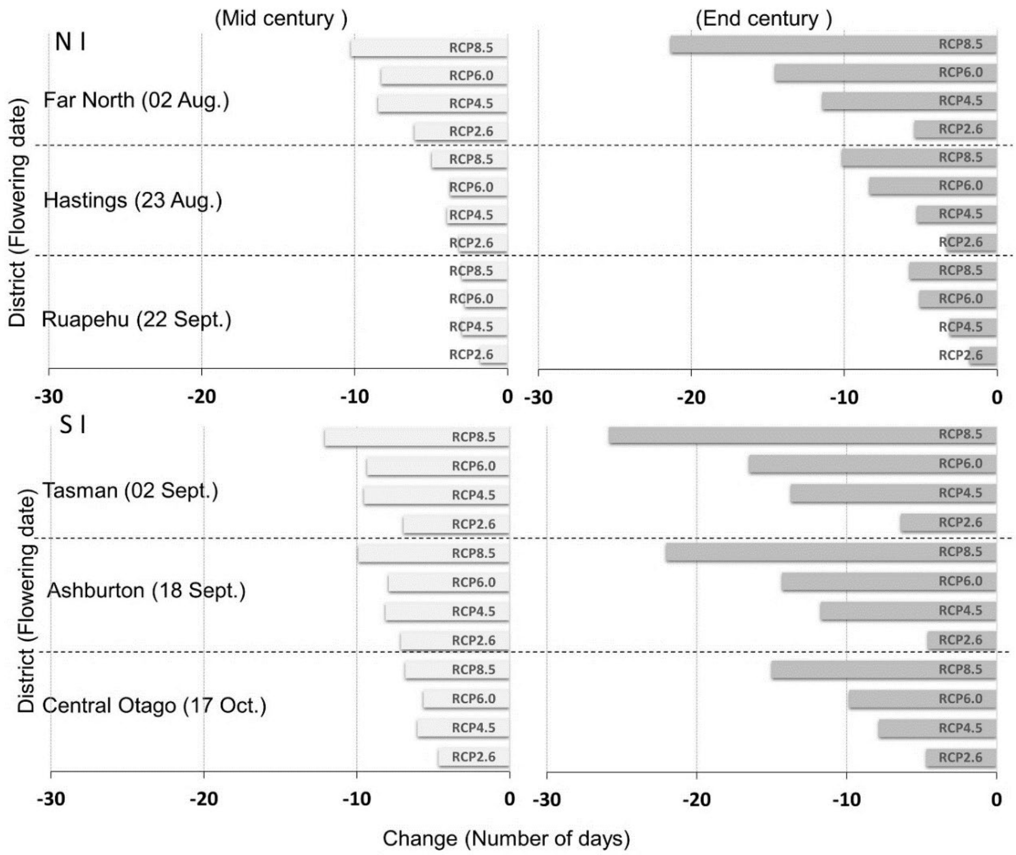

Figure 2. Projected number of days change for three districts in the North (NI) and South (SI) Islands in New Zealand for 'Early' subterranean clover cultivars to reach first flower (R3) according to Representative Concentration Pathways (RCPs) mid and end century RCP2.6; RCP4.5; RCP6.0 and RCP8.5 scenarios. Dates in parentheses indicate the projected dates considering historical climate.

Discussion

As expected, the increase in temperature predominantly accelerated the flowering of subterranean clover. None of the scenarios tested resulted in delayed flowering in New Zealand. This suggests that earlier flowering genotypes will be required in all environments. These may be introduced through commercially available cultivars. For example, in northern district's 'Early' cultivars such as 'Monti', 'Bindoon', 'Trikkala' in the future extreme climate change scenarios would flower too quickly and have a very short life cycle. They might be replaced by mid-Late flowering cultivars (e.g. 'Woogenellup', 'Mt Barker', 'Rosabrook'). Late cultivars such as 'Tallarook', 'Denmark' and 'Leura' will gain more relevance in warmer areas in a future warmer climate. Alternatively, depending on the rate of climate change in the future, genotype changes may occur naturally within the existing population. For example, environments that impose early terminal drought stress will cause natural selection for plants within that population that have early flowering and short lifecycles as a drought escape mechanism. In contrast cold, higher spring rainfall environments would select for delayed phenology. These trends have been demonstrated in a wide range of wild and domesticated Mediterranean annuals (Nelson *et al.* 2010). The physiological process is related to water stress due to rainfall alterations which causes stomata closure resulting in temperature increase within plant tissues and cells, and consequently, augmented thermal time accumulation and hastening development (Porter and Semenov 2005). The larger shifts in the South Island compared with the North Island relates to the temperature change predictions (Inouye 2022) which reflect projections that New Zealand southern temperate areas will experience more temperature increases compared with the northern districts.

Such temperature interactions for subterranean clover have practical implications on farm. In New Zealand subterranean clover is predominantly used in summer dry regions to provide high quality lactation feed in permanent pastures before the onset of summer dry conditions. This study suggests the period of available grazing between the shortest day and the onset of flowering will be reduced in future. Failure to recognise this by farmers, through continued grazing once flowers have appeared, will result in less seed being set each year and a consequent reduction in the total amount of seed in the seed bank. This may result in less seed for regeneration the following autumn and thus a diminishing of the subterranean clover component of these pastures. The warmer temperature that accelerated the time to flowering can also be expected to promote total pasture production through winter (Revell *et al.* 2012). The consequence may be an earlier lambing date to match the earlier growth and reproductive development of subterranean clover.

Conclusions

The results from this study show the genetic diversity currently available within subterranean clover cultivars would seem adequate to cope with the magnitude of change estimated in New Zealand conditions. In the Northern areas of New Zealand, flowering time is expected to be less affected than in southern regions. However, in both situations grazing management will need to change to maximise seed set.

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Notes:

SerraMax yellow serradella (Ornithopus compressus L.) – a new annual pasture legume for livestock and cropping systems in southern Australia

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Abstract: *SerraMax (Breeder's code 87GEH72.1a) is an early flowering cultivar of yellow serradella (Ornithopus compressus L.) with a unique seed dormancy characteristic. The breakdown of hard seed is rapidly accelerated by shallow burial, resulting in higher germination percentages in regenerating pastures compared to the alternative yellow serradella cultivars available in the market cvv. Yelbini, Charano and Santorini. This particular trait makes SerraMax an ideal candidate to be used with summer and twin sowing techniques to establish serradella pastures at low cost. It is suitable for acidic and neutral soils in 300 to 500mm annual average rainfall zones where it will be either sown alone for rotation with crops or into subtropical perennial grass-based pastures. It can be harvested in most situations with conventional harvesters and has an intermediate level of dehulling efficiency. The main use of SerraMax is expected to be in pod form with a small percentage of dehulled seed traded in the market. SerraMax is protected by Plant Breeders Rights.*

Key words: Pasture establishment, regeneration, mixed (cereal-livestock) farming

Introduction

Sown pasture legumes and weedy fallow remain common phases within crop sequences in the low to medium rainfall cereal growing regions of southern Australia (<500 mm annual average rainfall). They occupy between 20% to 60% of arable land use, depending on region and farm enterprise mix, and account for 80% of the "break" or non-cereal crop area in Western Australia (WA) (Lawes *et al*. 2009). In any one year up to 5m hectares fall into this land use category in southern WA. Yellow serradella (*Ornithopus compressus* L.) was developed as a pasture legume for sandy, acidic soils (Bolland and Gladstones 1987, Revell 1992). The most recent cultivars Santorini, Charano and Yelbini were released over 20 years ago. However, the high cost of seed processing (dehulling), and the delayed germination patterns in some cultivars when the seed undergoes natural breakdown of the hard seed in the field (Taylor and Revell 2002 has restricted large scale adoption of the species. The release in the last two decades of several cultivars of hard seed French serradella (*O. sativus* Brot.) cvv. Margurita, and Fran$_2$o, with much lower seed costs have further contributed to reduced uptake of yellow serradella. These cultivars are relatively easier to harvest and have hard seed breakdown patterns suitable for use with low-cost planting techniques such as summer sowing and twin sowing (Nutt *et al*. 2021). However, these cultivars are not as resilient as yellow serradella to survive drought and insect infestation, such as native budworm (*Helicoverpa punctigera* Wall.) when used in the low-rainfall regions of the WA wheat belt.

The selection of SerraMax was aimed at developing a cultivar that retains the resilience of yellow serradella, whilst incorporating a lower level of hardseedness and an absence of the delayed germination pattern – thereby allowing its use with summer and twin sowing establishment techniques or in long pasture phases together with C4 perennial grasses.

Origin and development

SerraMax (breeders code *87GEH72.1a*) was collected in May 1987, on the island of Santorini in the Aegean Sea by Dr. M. Ewing and Dr. J. Howieson of the then Western Australian Department of Agriculture (now Department of Primary Industries and Regional Development). Eight single plants were grown from seed collected at the site in 1989 by the Australian Trifolium Genetic Resource Centre. SerraMax was initially selected for its early maturity, rapid germination and pod characteristics that suit bulk handling with conventional machinery and evaluated for field performance at several trial sites in WA and NSW from 1991 to 2011. The role for SerraMax became clearer over time, particularly following research that identified the role of shallow burial in creating the conditions for hard seed breakdown (dormancy release) in yellow serradella (Revell *et al.* 1998, Taylor and Revell 2002). The marked burial response in SerraMax is a striking feature of this variety. The selection of one plant from a mixed population qualifies as a plant breeding process under the IP Australia definition of plant breeding. Plant Breeders Rights (PBR) protection for SerraMax was sought in 2017 and full protection is expected to be listed in 2023.

SerraMax is similar in appearance to other cultivars of yellow serradella. It has a semi-erect growth habit when un-grazed, with the first flowers appearing on the fifth/sixth to the ninth node of the main branches depending on location and time of sowing. Each leaf has up to 15 leaflet pairs with 5-7 leaflets on the floral leaf. The inflorescence is composed of 2 to 3 flowers on a 5 cm peduncle. The most distinctive features are pods that are light brown in colour, relatively straight with a small beak, flat in cross section and readily break into individual segments consistently at the pod segment wall without exposing the seed (Table 1). There are usually 7 to 10 seeds or segments per pod and approximately 152,000 seeds as pod segments/kg or 344,000 seeds/kg when dehulled. Flowering time is about 95 days in Perth, almost 3 weeks earlier than Margurita French serradella.

Table 1. Maturity and pod characteristics of cv. SerraMax compared to other common yellow serradella cultivars.

Cultivar /Character	SerraMax	Charano	Paros	Yelbini
Days to flower*	95	96	100	82
Full pod length (mm)	48.5	56.4	59.3	55.0
Beak length (mm)	3.9	9.3	9.3	7.9
Curvature**	1.2	1.7	2.4	1.5
Segmentation***	4.0	1.7	0.9	1.4

*Days from germination to first open flower from a mid-May sowing in Perth, WA.
**Curvature is calculated from full pod length (non-linear) divided by longest diameter (longest linear length, usually from stalk to the bottom curve of the pod).
***Segmentation rated as 4 = readily and always breaks between segments with no seed visible. 0 = does not readily break into segments and the break will occur past the pod joint, exposing the seed.

Field performance

Productivity

Field experimentation in WA has measured herbage dry matter (DM) production in spring of un-grazed plots of SerraMax up to 8 t DM/ha and compares well with currently available cultivars of yellow serradella, French serradella, and subterranean clover (*Trifolium subterraneum* L.). SerraMax is a prolific seed producer consistently producing pod yields of over 1 t/ha (Tables 2, 3, and 4). The actual seed content of pod is usually 35% to 40%. SerraMax has a distinctive seed softening behaviour whereby seed on the surface can remain dormant for several seasons (Table 5) but hard-seed breakdown is rapidly accelerated by shallow burial (Revell *et al.* 1998, Taylor and Revell 2002). This softening behaviour suits a 1:1 pasture : crop sequence but two consecutive crop years should be avoided as the seed bank can be exhausted too quickly.

Table 2. Ungrazed herbage dry matter (DM) yield (t DM/ha) and pod/seed yield (kg/ha) in the establishment year (ungrazed) and plant regeneration in the second year (prior to cropping) of yellow serradella cv. SerraMax and cv. Santorini, French serradella cv. Margurita and subterranean clover cv. Dalkeith on an acid duplex loamy sand soil at Pingelly, WA in 1999. Data in parentheses is the standard error of the mean.

Species	Cultivar	Herbage yield (t DM/ha) 5 Oct 1999	3 Nov 1999	Pod/seed yield (kg/ha)	Regeneration (plants/m^2) 28 Jun 2000
Yellow serradella	SerraMax	2.8 (0.3)	6.8 (0.5)	1587 (524)	1242 (356)
Yellow serradella	Santorini	2.1 (0.2)	5.0 (0.3)	1313 (223)	111 (22)
French serradella	Margurita	2.9 (0.3)	5.5 (0.2)	374 (84)	1328 (507)
Sub. clover	Dalkeith	2.9 (0.3)	6.0 (0.2)	181* (81)	506 (19)

*Seed – subterranean clover

Similar to Margurita French serradella, SerraMax yellow serradella is ideally suited to twin and summer sowing establishment (Valentine 2013, Nutt *et al.* 2021) as dormant seed drilled into the soil undergoes a high level of breakdown (over 60% seed softening) within the one summer/autumn period (Table 5). Forage legumes are typically sown after the main cropping program is completed and require the application of a pre-sowing knockdown herbicides to control established weeds. This method can seriously reduce early winter pasture production (Table 4), which is then compounded by the slow growth rate of legumes under cold winter conditions (Nutt *et al*. 2021). Summer sowing and twin sowing techniques may be applied in a number of scenarios, offering early winter grazing in a mixed enterprise farm and lifting the legume component of a pasture with a low legume base due to drought and/or intensive cropping.

Serra Max is better suited to low rainfall areas than Margurita because of its earlier maturity and the ability to establish greater seed banks under stressful conditions, such as an early spring finish and native budworm (*Helicoverpa punctigera* Wall.) infestation.

Table 3. Seedling regeneration (plants/m²), ungrazed herbage dry matter (DM) yield (t DM/ha) and seed/pod yields (kg/ha) of cv. SerraMax and other annual pasture legumes in the year after cropping at Pingelly, WA (2001, third year after establishment). Data in parentheses is the standard error of the mean.

Species	Cultivar	Regeneration (plants/m²) 20 Jun 2001	Herbage yield (t DM/ha) 20 Sept 2001	1 Oct 2001	Pod/seed yield (kg/ha)
Yellow serradella	SerraMax	3700 (1317)	4.5 (0.8)	7.9 (0.1)	1293 (117)
Yellow serradella	Santorini	1333 (895)	3.6 (0.8)	6.3 (0.4)	749 (185)
French serradella	Margurita	4289 (771)	4.0 (0.5)	7.4 (0.1)	70 (6)
Sub. clover	Dalkeith	2778 (768)	2.8 (0.1)	4.9 (0.2)	327* (53)

*Seed – subterranean clover

Table 4. Herbage dry matter (DM) yield in winter and spring (t DM/ha) and pod/seed yield (kg/ha) of cv. SerraMax and other annual pasture legumes sown at contrasting times: as seed at the break of the season (normal) and in pod form at the start of summer (summer sowing) at Mingenew, WA in 2010. Data in parentheses is the standard error of the mean.

Species	Cultivar	Sowing treatment	Herbage yield (t DM/ha) 5 Aug 2010	23 Sept 2010	Pod/Seed yield (kg/ha)
Yellow serradella	SerraMax	Normal sowing (seed)	0.8 (0.1)	4.9 (0.5)	1423 (115)
Yellow serradella	SerraMax	Summer sowing (pod)	2.9 (0.4)	8.7 (0.6)	2175 (384)
French serradella	Margurita	Normal sowing (seed)	0.7 (0.0)	3.6 (0.8)	311 (92)
French serradella	Margurita	Summer sowing (pod)	4.5 (0.1)	9.3 (0.7)	1358 (154)
Sub. clover	Dalkeith	Normal sowing (seed)	0.6 (0.0)	3.6 (0.8)	216* (12)

*Seed – subterranean clover

Harvesting and dehulling

SerraMax in most situations will develop enough height for direct harvesting with a grain harvester, but in low rainfall years SerraMax may require suction harvesting to maximise pod yield, either directly or after a combine harvester pass. The pods of SerraMax are relatively straight with a high level of segmentation (Table 1) and should readily flow in modern machinery. Pod removal (dehulling) to enhance germination of SerraMax is less efficient (intermediate) compared to other commercial serradella cultivars but is a likely requirement for establishing SerraMax seed crops.

Table 5. The hard seed content (%) of cv. SerraMax and other annual pasture legumes at Shenton Park (Perth) 2002. Initial hard seed content was determined in January, seed treatments were left on the soil surface in mesh pockets and sampled during 2002 and 2003. Two more treatments were buried in mesh pockets at a depth of 10 mm in January 2002 and 2003 and sampled in early winter (June) each year. Data in parentheses is the standard error of the mean.

Species	Cultivar	Hardseed (%)				
		Surface			Buried Jan 02	Room temp in 02 Buried in Jan 03
		Initial Jan 02	Final Jun 02	Final Jun 03	Final Jun 02	Final Jun 03
Yellow serradella	SerraMax	94 (2.5)	91 (2.4)	36 (4.6)	40 (3.9)	47 (4.2)
Yellow serradella	Yelbini	96 (1.5)	97 (1.9)	64 (4.8)	65 (4.5)	41 (5.2)
French serradella	Margurita	85 (1.8)	69 (1.4)	29 (2.5)	30 (6.6)	11 (1.0)
French serradella	Erica	91 (1.3)	67 (6.7)	49 (0.9)	71 (2.8)	47 (2.4)

Disease and pest reaction

The only serious diseases currently recognised in yellow serradella are the root and hypocotyl rots caused by *Rhizoctonia solani* and *Pleiochaeta setosa*, generally at seedling establishment. Screening for relative susceptibility to these diseases is not currently undertaken in the development of new pasture cultivars.

Based on observation in field trials, SerraMax is no more susceptible than Santorini or Charano and is more tolerant than annual clovers.
SerraMax is considered tolerant to blue green aphid (*Acyrthosiphon kondoi* Shinji) and cowpea aphid (*Aphis craccivora* Koch) based on field observations and control should not be required in most situations.
SerraMax has some tolerance to red-legged earth mite (*Halotydeus destructor* Tucker) but early control is recommended in seed production crops and when mite populations are high. SerraMax is susceptible to native budworm, however the risk is low compared to other cultivars of yellow serradella due to its early maturity. Regular monitoring is recommended in seed crops from the beginning of pod fill to senescence and infestations should be controlled with an appropriate insecticide. SerraMax is moderately susceptible to lucerne flea (*Sminthurus viridis* L.) and may require chemical control in some situations.

Herbicide tolerance

Based on field observation and limited experimental data, SerraMax has a similar reaction to common herbicides as used on other cultivars of yellow serradella. It is highly susceptible to sulfonyl ureas, phenoxy based herbicides (MCPA, 2,4-D), glyphosate, triazine and paraquat/diquat herbicides.

Conclusions

SerraMax yellow serradella will be suitable for use on acidic sands and sandy loams in low to medium rainfall areas (300 to 500 mm annual average). Large areas of these soils occur in the south-west agricultural areas of WA and in central New South Wales. In WA, SerraMax will complement other serradellas such as Charano and Yelbini yellow serradella and Fran$_2$o and Margurita French serradella in mixtures for low to medium rainfall areas and where regular spray topping is used for control of grass seed set. SerraMax is also expected to be a suitable companion legume for the sub-tropical perennial grass-based pastures of panic grass (*Megathyrsus maximus* (Jacq.) BK Simon & SWL Jacobs) and Rhodes grass (*Chloris gayana* Kunth) in the West Midlands region of WA and for kikuyu pastures on the south coast.

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Yellotas: A unique yellow serradella cultivar with potential for permanent pasture environments

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Abstract: *Yellow serradella (Ornithopus compressus L.) has been identified as a priority self-regenerating annual legume species for permanent pasture environments in south-eastern Australia. However, most yellow serradella genotypes exhibit high levels of hard seed and slow rates of hard seed breakdown, which reduces regeneration density in the years following the year of sowing. One cultivar, Yellotas, exhibits a much faster rate of hard seed breakdown and has been identified as one of only a handful of cultivars of that species with promising persistence in permanent pasture environments. In addition, this cultivar is substantially easier to de-hull than other cultivars of that species, potentially reducing seed costs. In a field evaluation under severe drought conditions, this cultivar was shown to exhibit a high level of tolerance to close grazing. Yet, doubt still exists as to whether cv. Yellotas produces sufficient residual hard seed to withstand periodic drought suggesting further improvement may be required. This paper details the origins of cultivar and observations of its performance under a range of conditions in south-eastern Australia.*

Key words: *Ornithopus compressus*, hardseed, breakdown, regeneration, development

Introduction
Yellow serradella has long been a species of interest for crop and livestock production systems across southern Australia. It is a self-regenerating annual pasture legume adapted to acidic, low-fertility soils, and has a demonstrated ability to tolerate periodic droughts. The species has been used extensively in mixed crop-livestock systems across south-western Australia (WA) and parts of northern New South Wales (NSW), but to date has achieved relatively low levels of utilisation in the higher-rainfall permanent pasture environments of south-eastern Australia. The low penetration into such environments is in-part attributable to a lack of adapted cultivars with proven ability to self-regenerate over the long term. A second contributing factor was the historically high cost of yellow serradella seed compared to other pasture legume options (Nichols et al. 2012). The curled and segmenting characteristics of early cultivars of yellow serradella meant that suction harvesting was required and dehulling was difficult; leading to high seed costs (Nichols et al. 2007). This was later addressed in cultivars such as Yelbini and Santorini, which were selected for non-segmenting straight pods and pod retention meaning they could be direct headed (Nichols et al. 2007).

This paper describes the attributes of a cultivar, cv. Yellotas, developed specifically for use in permanent pasture environments in south-eastern Australia. Although developed two decades ago, the unique traits of the cultivar have scarcely been documented as cv. Yellotas was never registered for Plant Breeder's Rights (IP Australia 2022). Recent investment in legume research for south-eastern Australia by the red meat industry body, Meat and Livestock Australia (MLA), has sparked renewed interest in this unique cultivar.

Yellotas (sometimes misspelt Yellowtas) was developed from an accession introduced to Australia in 1972; CPI 50484. It was maintained by Agriculture Victoria (previously Vic DPI) under the derivation Ham 1038 and later received by Natural Resources and Environment Tasmania (previously the Department of Primary Industries, Parks, Water and Environment Tasmania) and maintained as Tas 349. Yellotas was bred by Eric Hall and Andrea Hurst at the Tasmanian Institute of Agriculture during the early 2000s through a process of 4 cycles of recurrent phenotypic selection. The breeders selected for traits including plant vigour, late flowering and high seed production. Seed attributes are described as having 'a high proportion of soft seed, which develop in straight to slightly curved pods'; differing from other yellow serradella cultivars. This paper reports on field observations comparing the relative performance of cv. Yellotas with other yellow serradella cultivars.

Methods
Initial evaluation in Tasmania
Yellotas was evaluated with a number of breeding lines and established cultivars in two experiments in the early 2000's at Cressy in Northern Tasmania. In May 2003, yellow serradella (11 lines), French serradella (*O. sativus* Brot.; 12) and hybrid serradella (*O. compressus* x *sativus*; 1) were dehulled, scarified and inoculated (a subset reported here) and hand sown into 2 x 2 m plots with 3 replicates at a sowing rate of 20 kg/ha. Fertiliser (0-6-17-7 – NPKS) was applied at sowing at 300 kg/ha. Following promising results in 2003,

a further smaller evaluation was sown in 2004 with 3 breeding lines (one being Yellotas) and three commercial cultivars. These lines were sown into 2 x 1 m plots with 4 replicates, with seed inoculated and fertiliser applied as per 2003.

Year 2 regeneration

An evaluation of pasture legume persistence was recently undertaken in NSW (Hayes et al. 2022). Five cultivars of yellow serradella were included in experiments sown in 2017 at sites near Bigga and Middle Arm, on the NSW Southern Tablelands, and Bombala in the Monaro region, including cvv. Avila, Charano, King, Santorini and Yellotas. Here, we report year 1 seed production and year 2 seedling regeneration of those cultivars. Seed production was assessed at the end of spring in year 1 at each site after legumes had senesced and most of the serradella pod was detached and on the soil surface. A strip of soil 1.0 m long x 0.1 m wide x 0.02 m deep was excavated from each plot, perpendicular to the direction of sowing. Samples were transported back to the laboratory and pod and seed removed by threshing and running over a series of sieves of varying apertures. An assessment of seedling density was taken in autumn of Year 2 using a 1.0 x 1.0 m quadrat, divided into 100 equal cells, placed randomly in the plot and the number of serradella seedlings visible in a diagonal transect of this quadrat recorded. This was repeated at three locations within each plot and the average of those counts converted to plants/m2.

Tolerance of grazing under drought

The experiment at the Middle Arm site described above was continuously grazed by sheep in 2018, which also happened to be a drought year, with total rainfall recorded for that year almost 30% lower than the long-term annual rainfall at that site. These circumstances provided an opportunity to assess the response of the yellow serradella cultivars under heavy grazing during dry seasonal conditions. Three assessments of relative pasture density were taken at that site during 2018 (year 2), including seedling regeneration on 13 March as described above, followed by two assessments of legume frequency on 15 June and 12 November. Legume frequency was assessed using a 0.5 □ 0.5 m quadrat divided into 100 cells of equal size and counting the number of cells in each quadrat containing the base of a serradella plant. Quadrats were placed in three random locations in each plot with values expressed as a percentage (%).

Pattern of hard seed breakdown

Hard seed (impermeable seed coat) content of cv. Yellotas was initially determined in Tasmania from seed grown on weedmats at Mt. Pleasant, Launceston in 2003 concurrently with other experiments. Seed was collected by hand and a known number of pod segments of each line were placed in germination trays where the seed was kept continually moist, replicated twice. The number of seeds that produced a radicle was assessed at 14, 21 and 28 days, with the remainder presumed to be hard seed.

Patterns of seed softening were evaluated in seed grown in 2018 at Cowra in central NSW. Pod segments, totalling 100 of yellow serradella cvv. Avila, King, Pitman, Santorini and Yellotas were placed in separate pouches constructed from plastic coated fibreglass mesh. Pouches were laid flat on the surface of a cleared patch of soil in January 2019, as described in Newell et al. (2022). Initial proportions of hard seed were assessed at the start of the experiment (Day 0) by incubating 100 segments on moist filter paper for 14 days. Pod segments which were firm to touch were considered hard and used to calculate the percentage of hard seed. Progress of seed softening was followed in subsequent incubation tests by retrieving pouches at 30-day intervals for the first five assessments. The sixth and seventh harvest were at 509 and 745 days respectively.

Results

Initial evaluation in Tasmania

Yellotas was one of the best performed yellow serradella lines under dry seasonal conditions in 2003 and 2004 and was superior to cv. Avila in the second year (2004) with higher regeneration densities and herbage yield (Table 1, Experiment 1). Similarly, Yellotas performed better than cv. Avila with higher establishment densities and spring yield in 2004 (Table 1, Experiment 2). These evaluations formed the basis of the selection of the Yellotas cultivar and subsequent seed bulking. It was not registered under Plant Breeder's Rights (IP Australia. 2022) at the time but was later marketed and sold by Tasglobal Seeds and their affiliates and has remained of interest in evaluations and experiments.

Year 2 regeneration

There were no significant site x cultivar interaction (P>0.05) effects detected with either seed production or seedling regeneration. There was less yellow serradella seed produced at the Bigga site (295 kg/ha) compared to either Middle Arm (943 kg/ha) or Bombala (1201 kg/ha; l.s.d at P=0.05 was 302 kg/ha). Averaged across sites, there was little difference between cultivars in the quantity of seed produced in year 1 (P=0.057), with yields ranging from 574-1065 kg/ha. Seedling regeneration in year 2 was lower at Bigga (271 plants/m2) compared to either Middle Arm (1072) or Bombala (1577). Averaged across sites, seedling density was greater in cvv. King (1848 plants/m2) and Yellotas (1832) compared to cvv. Santorini (280) or and Charano (373), and intermediate in cv. Avila (777; l.s.d. at P=0.05 of 1140).

Table 1 Performance of selected yellow serradella (*Ornithopus compressus* L.) cultivars in; Experiment 1 - year of sowing (2003) and first year of regeneration (2004); Experiment 2 - year of sowing (2004) at Cressy, Northern Tasmania, Australia.

	Experiment 1				Experiment 2	
Cultivar	Plant count/m^2 May 2003	Yield kg DM/ha Nov 2003	Regeneration plant count/m^2 May 2004	Yield kg DM/ha Dec 2004	Plant count/m^2 May 2004	Spring yield kg DM/ha Nov 2004
Yellotas	284	2028	428	896	348	1023
Avila	331	601	19	69	58	76
Tauro	161	1144	766	115		

Tolerance of grazing under drought

Overall, there was a decline in seedling density throughout the year as expected (Table 2). Frequency was higher in cv. Yellotas than cv. Avila at all three frequency assessments during 2018, likely an artifact of the initial seedling density.

Table 2 Three assessments of relative pasture density of 2018 sown experiment at Middle Arm, New South Wales.

Cultivar	Seedling density Mar 2018	Frequency Jun 2018	Frequency Nov 2018
Avila	1080	77	43
Charano	373	50	5
King	2060	83	21
Santorini	280	27	5
Yellotas	1567	91	79
l.s.d.	669	18.7	17.1

Pattern of hard seed breakdown

In the Tasmanian experiment in 2003, cv. Yellotas had a hard seed content of 65%, slightly lower than the original parent material of Tas 349, which was 69%, with both much softer than cv. Avila, which was 98%, after 28 days. In the NSW experiment, there were two distinct patterns of seed softening among the cultivars tested (Figure 1). Yellow serradella cvv. Avila, King and Santorini had high proportions of initial hard seed (100%) with very slow seed softening during the first year (~Day 320). However, from mid-summer (Day 340) through the end of autumn 2020 (~Day 500) there was a significant increase in the rate of seed softening (P< 0.001). In contrast, cvv. Yellotas and Pitman were characterised by rapid seed softening during late summer-autumn 2019 (Day 28-138), and again in late-summer-autumn 2020. This resulted in loss of 80-90% of the hard seeds over the two-year experimental period (Day 500).

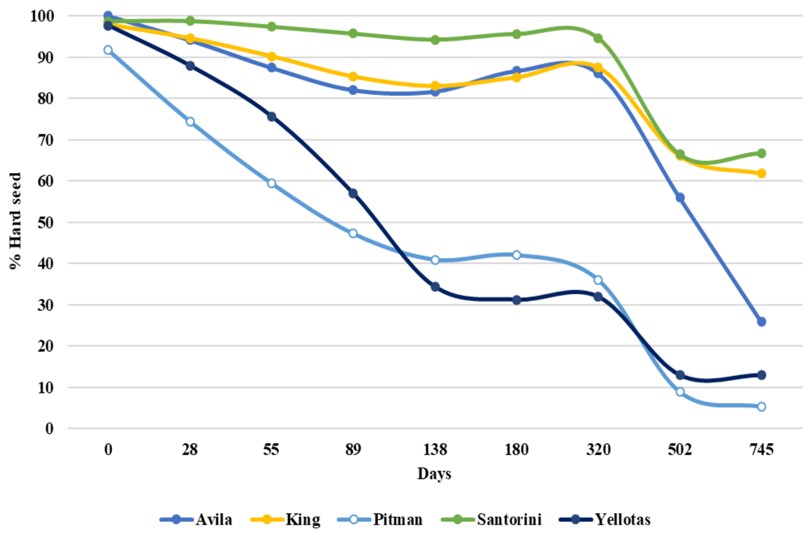

Figure 1. Mean of residual hard seed expressed as a percentage over time for yellow serradella cvv.

Discussion

The combination of characteristics found in Yellotas are unique. It has a hard seed breakdown pattern most similar to cv. Pitman, an old cultivar long recognised for low levels of winter production (Clark and Hamilton 1985). It is late-maturing similar to cv. Avila yet had better 2nd year regeneration and tolerance to grazing in the reported study. The rapid breakdown of hard seed suits environments in the higher rainfall zone where autumn breaks can occur very early (e.g February or March) yet conditions can be cool and moist enough for seedlings to survive. In this respect, cv. Yellotas is most similar to well-adapted subterranean clover cultivars that presently dominate much of the permanent pasture zone of south-eastern Australia (Newell et al. 2022). The ability to tolerate hard grazing under dry spring conditions is an obvious advantage in permanent pastures.

The observations and descriptions of the characteristics of cv. Yellotas in this paper indicate a context and farming systems fit for a cultivar such as Yellotas in the med-high rainfall permanent pasture zone and warrants further evaluation. More recent research identifying serradellas as having a lower phosphorus requirement (Sandral et al. 2019) has reinvigorated interest in this species.

Conclusions

Cultivar Yellotas is a late maturing yellow serradella cultivar with rapid seed softening, grazing tolerance and regenerative abilities suitable for the high rainfall permanent pasture zone. This cultivar, or cultivars with similar traits, have the potential to extend the reach of serradella into permanent pasture environments of south-eastern Australia.

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Notes:

The potential of pasture legumes for sandy alkaline soils of South Australia's low-rainfall regions: a case from on-farm trials

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Abstract: *South Australia's low rainfall regions have a long history of using regenerating annual medics that have contributed soil fertility, cereal disease breaks, livestock feed and other benefits to ley farming systems. However, new medic cultivars and alternative legumes that vary in hard seededness, have deeper roots, more efficient nutrient uptake, resistance to pests, and harvestable seed may provide improved options for increasing farm productivity. We examined a suite of annual legumes Penfield and Emperor, barrel medic (*Medicago truncatula*), Seraph strand medic (*M. littoralis*), Cefalu arrowleaf (*Trifolium vesiculosum*), Bartolo bladder clover (*T. spumosum*) and Frano French serradella (*Ornithopus sativus*) for their productivity and ability to persist and extend growing season window in a low rainfall region. Pasture emergence and establishment rates ranged from 49 to 162 plants/m^2. Dry matter biomass measured on 1 November 2022 ranged between 1.6 to 5.9 t/ha. Seed yield varied from 1.4 to 2.56 t/ha. Feed quality at flowering surpassed the minimum maintenance requirements for ewes. In contrast to adjacent medic pastures, new medics also showed resistance to the severe powdery mildew outbreak coinciding with the abnormally wet spring. Frano and Cefalu grew longer in the season with green foliage until mid-December and thereafter as standing stubble, suggesting feed continuity in dry summers. The results indicate that improved legumes can contribute to a pasture mix, with potential to maximize pasture productivity in a wet year, provide out-of-season feed, protect soil and regenerate after crop or drought cycles.*

Key words: annual legumes, drought, cultivar, powdery mildew, senescence scores

Introduction

Annual medic pastures form an integral component of ley farming systems in low and mid-rainfall regions of South Australia (SA). They provide high-quality feed to livestock, increase land productivity and contribute to the overall profitability of farm enterprises (Carter et al. 1982). Barrel (*Medicago truncatula*) and strand (*M. littoralis*) medics have emerged as the dominant medic species due to their widespread cultivation. Furthermore, significant progress in plant breeding and the subsequent release of new strand medic cultivar Seraph in 2021 and barrel medic cultivars Emperor and Penfield in 2022, with improved agronomic traits, has garnered increasing interest in these species among growers. A range of alternative ley legume species have also been developed in recent decades with traits (higher hard seed levels, deep root systems, more adapted phenology, more efficient nutrient uptake, pest resistance, harvestable seed) that can benefit farming (Loi et al. 2005, Nichols et al. 2012). The recent dryland legume pastures systems project (GRDC 2022) found that French serradella (*Ornithopus sativus*), arrowleaf clover (*Trifolium vesiculosum*) and bladder clover (*T. spumosum*) have the potential to perform in SA and extend the growing season. Most farmers in SA are not familiar with new annual medic cultivars and alternative ley legume species and requested local trials to determine their suitability to the region. Thus, the Pasture Optimization for Drought Solutions (PODS) project evaluated three new annual medic cultivars and three alternative species for their ground cover, biomass production and feed quality (pasture productivity), and ability to grow longer in the season to fill feed gaps using out-of-season rainfalls (drought resilience). This paper presents some of the preliminary results from the PODS' pasture trial in a low-rainfall region of SA. The project had a strong focus on local engagement and extension, to transfer knowledge and uptake of pasture technologies by local farm managers.

Methods

The study was conducted in the Riverland region of SA (34.25168° S, 139.97801° E) in 2022. The mean temperature averages 17.4 °C and mean annual rainfall is around 272 mm in the year. A high annual rainfall of 350 mm was recorded in 2022 with the area experiencing the wettest October on record (BOM 2022). The area has loamy sand with particle distribution of sand (86.25 %), silt 8.95 (%) and clay (4.8%). Soil pH was 7.5 ($CaCl_2$).

The trial used a randomized complete block design (RCBD) with four blocks and six treatments (Table 1). The cultivars (treatments) were assigned at random within blocks of adjacent subjects, each treatment plot (9 x 1.5 m) occurring once per block. The cultivars were sown on 9 June 2022 and plant emergence was assessed on 2 August 2022. Samples were obtained on 1 November 2022. Soil samples were taken from two depths, 0-10 and 10-20 cm, from each treatment plot. Biomass cuts were obtained by clipping plants at ground level from two random 40 x 40 cm quadrats. Senescence scores were recorded following the techniques of Cai et al. (2016) and (Maliro et al. 2007). DM yield was determined by oven-drying the biomass samples at 60 °C to constant weight. Biomass samples were also collected from adjacent companion paddocks. Phytoestrogen levels were determined using 0.5% phosphoric acid in 80% methanol extraction using reverse-phase High Performance Liquid Chromatography (HPLC) and detected by UV and fluorescence. Feed quality analysis was done using Near Infrared Spectroscopy (NIR) at the Forage Lab Australia, Bendigo, Victoria.

Table 3. Cultivars used in the performance evaluation trial at Lowbank, South Australia.

Species	Common name & cultivar	Characteristics	Sowing rate
Medicago littoralis	Strand medic Seraph	Cultivar released in 2021. Resistant to powdery mildew and bluegreen aphids and tolerant to SU herbicide residue.	10 kg/ha
Medicago truncatula	Barrel medic Penfield	New early-season medic with spineless pods, tolerant to SU herbicide residues, resistant to bluegreen and spotted alfalfa aphids and tolerant of high boron levels.	10 kg/ha
	Barrel medic Emperor	New mid-season barrel medic with powdery mildew resistance. Resistant to blue-green aphids and tolerant of high boron levels	10 kg/ha
Ornithopus sativus	Serradella Frano	Hard seeded. Suited to acidic to neutral sandy dunes. Aerial harvested and suited to summer sowing.	10 kg/ha
Trifolium spumosum	Bladder clover Bartolo	Hard seeded, mid-season, aerial harvested, suited to summer sowing.	10 kg/ha
Trifolium vesiculosum	Arrowleaf clover Cefalu	Hard seeded. Deep-rooted and suited to a wide range of soils. Ability to respond to late-season rainfalls. Aerial harvested.	5 kg/ha

The treatment effects were analyzed using linear mixed-effects models (*lme4* package; Bates et al. 2015) in R (Core Team R 2023). The model fitted can be represented as $Y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}$; where Y_{ij} is any observation for which *i* is the treatment factor and *j* is the blocking factor, μ is the overall mean, T_i is the effect for being in treatment *i* and B_j is the effect for being in block *j*. ε_{ij} is the random error. After checking the normality of residuals Kenward-Roger approximations were used for *F* tests of mixed effect model terms. The least significant difference (LSD) test was used to identify statistically different population means.

Results and discussion

Plant emergence averaged 94 plants/m^2 and ranged from 49 plants/m^2 (Cefalu) to 145 plants/m^2 (Seraph) (Table 2). Pasture biomass averaged 4.7 t DM/ha and ranged from 1.6 (Penfield) to 5.9 t DM/ha (Emperor and Seraph) (Table 2). The DM biomass values were high for the area and could be attributed to the wet season. High biomass production can benefit N fixation as 20-25 kg N is fixed for every ton of plant DM (Peoples et al. 1998). The ground cover averaged 71% and varied from 38 (Penfield) to 95 % (Frano) (Table 2). Early senescence and powdery mildew infestation in surrounding pastures, which also affected Penfield, might have contributed to the lower biomass yield and ground cover (Johnson 1948). A high seed yield averaging 1.8 t/ha and varying from 1.4 (Frano) to 2.6 t/ha (Emperor) was estimated (Table 2). The high quantities of seed yield, likely driven by the higher-than-average rainfall during the growing season, indicate sufficient seed banks for subsequent regeneration (Quinlivan 1972).

Table 4. Performance of six cultivars, Plant emergence, dry matter biomass, seed yield and senescence score (1 = green healthy plant, 2=bottom leaves begin to yellow, 3= necrosis 25%, 4 = necrosis 50%,….7= necrosis 75%, 8= necrosis whole plant, 10= plant death) and their nutritional value (Dry matter digestibility, DMD; Crude Protein, CP; Metabolisable energy, ME and Neutral detergent fibre, NDF). Different letters indicate significant difference (P<0.05).

Cultivars	Emergence (Plants/m^2)	Biomass (t DM/ha)	Ground cover (%)	Seed yield (t/ha)	DMD (%)	CP (%)	ME (MJ/kg DM)	NDF (%)	Senescence score
Penfield	93.9^b	1.63^d	37.5^c	2.01^a	53.3^c	12.3^b	7.19^c	55.7^a	8
Seraph	144.7^a	5.93^a	87.5^a	1.47^a	64.8^a	14.2^b	9.20^a	40.1^c	3
Emperor	87.9^b	5.91^{ab}	67.5^b	2.56^a	61.0^{ab}	13.2^b	9.07^{ab}	45.4^{bc}	2
Frano	80.3^b	4.22^c	94.8^a	1.39^a	59.7^b	12.3^b	8.89^{ab}	48.6^{ab}	1
Bartolo	106.8^b	4.81^{bc}	47.5^c	2.56^a	60.8^{ab}	18.2^a	8.83^{ab}	44.8^{bc}	5
Cefalu	49.2^c	4.69^c	92.0^a	1.47^a	58.3^b	11.3^b	8.32^b	51.4^{ab}	1
Sig.(*p*=0.05)	<0.001	<0.001	<0.001	0.67	0.002	0.002	0.001	<0.05	
LSD	29.8	1.12	17.9	1.78	4.36	2.89	0.84	7.65	

Most of the legume cultivars grew longer with lower senescence scores indicating their ability to grow longer in a wet year. The alternative species, the mid-season medic Emperor, and Seraph had later senescence than Penfield which was at an advanced senescing stage on 1 November with a score of 8 (Table 2). Seraph is an early-season cultivar (like Penfield) but was able to keep persisting in a wet spring. Seraph appeared green from the top, however, recorded some necrosis on the bottom half and mature pods (4). Frano and Cefalu had the lowest senescence scores (1) with green foliage and flowering followed by Emperor (2) with bottom leaves beginning to yellow. Senescence in Frano and Cefalu was observed to have begun in mid-December 2022 and remained as dense standing stubble throughout the dry summer (observed in February 2023).

The nutritional value of legumes sampled on 1 November 2022 (Table 2) reported a mean dry matter digestibility (DMD, %) of 60%, with a high of 64.8 (Seraph) and a low of 53% (Penfield). Except for Penfield, all legumes had DMD above the maintenance requirement of 55% (Frischke et al. 2021). Crude protein (CP, %) averaged about 14%, varying from a high of 18% (Bartolo) to a low of 11.3% (Cefalu). All legumes recorded CP% above the maintenance requirement of 8% (Table 2). The metabolisable energy (ME, MJ/kg DM) averaged 9 MJ/Kg ranging from 7.2 (Penfield) to 9 MJ/Kg (Emperor) (Table 2). All legumes except Penfield exceeded the maintenance requirements of ewe at 8 MJ/kg DM (Frischke et al. 2021). Neutral detergent fibre (NDF, %) averaged 48%, with a low of 40% (Seraph) and a high of 56% (Penfield) (Table 2). All legumes exceeded the minimum requirements of 30% NDF (Frischke et al. 2021).

All medic pastures in the district were infested with powdery mildew (PM, *Erysiphe trifolii*) from early October onwards. Penfield (large amount of caliph in its breeding) became covered in PM but was slower to develop PM than the surrounding paddock of Harbinger strand medic, consistent with observations in Ballard et al., (2012). Seraph and Emperor were bred to be PM resistant (IP Australia 2023) and were not found to be affected, as did the alternative species which are not known to be susceptible to PM. PM elevated phytoestrogen levels (coumestrol) in the susceptible cultivars (Figure 1), which can have adverse implications on livestock fertility (Reed 2016).

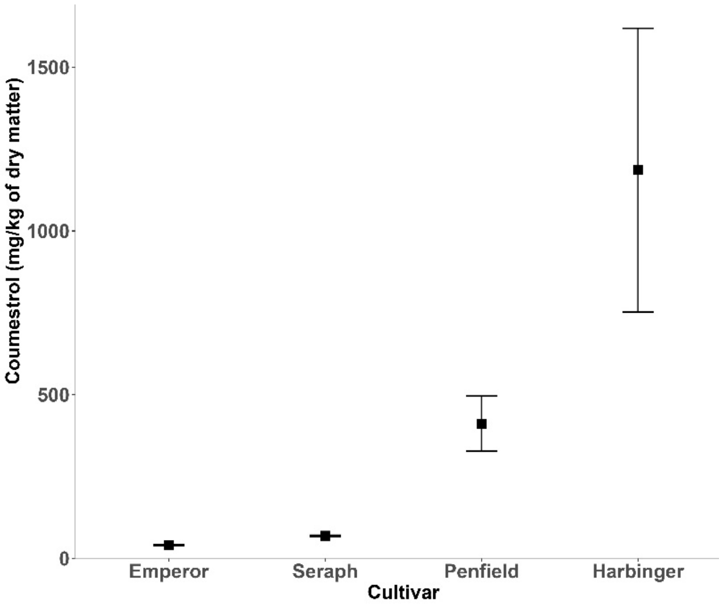

Figure 1 Phytoestrogen (coumestrol) levels (mg/kg DM) detected in the biomass samples of different medic cultivars. Bar represents one standard deviation from the mean.

Conclusions

The legume cultivars produced high dry matter biomass over a longer season, capitalizing on the higher-than-average rainfall and mostly resisting the subsequent PM outbreak that diminished the productivity of most other medics in the region. Medic cultivar Penfield matured early, demonstrating its ability to produce early feed, but senesced earlier than other cultivars tested. Emperor, Seraph and Bartolo showed feed continuity in the mid-season while Frano and Cefalu grew longer in the season, until early summer. The wide range of cultivars trialled offers potential choices for a pasture mix providing flexibility and options for growers to improve their farm productivity and resilience to climatic variability.

Acknowledgements

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Notes:

Reducing enteric methane intensity of ruminant production in Australian grazing systems - a review of the role of temperate legumes and herbs

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Abstract: *In Australia, approximately 70% of the agricultural greenhouse gas emissions are enteric methane (CH_4) and most of these emissions are produced by ruminants grazing on pastures. The introduction of alternate pasture species, including legumes and perennial herbs, has been identified as one of ways to reduce the CH_4 emission intensity from these grazing animals. There are several pathways for pasture species to reduce CH_4 intensity. These include: 1) improving the quality of pasture throughout the year to reduce the time for animals to achieve target weights; 2) anti-methanogenic properties that reduce the production of methane in the rumen (e.g., with species such as Biserrula pelecinus L.); and 3) reducing N limitation in the soil to increase pasture production and enhance soil organic carbon. A system level assessment of the potential benefits of these alternate pasture species is required to identify the magnitude of these multiple benefits and determine whether there are any negative impacts on pasture productivity and persistence compared to currently recommended species and ensure that there are not increases in other GHG emissions, such as nitrous oxide. A literature review will be completed, on the role of new pasture species or increasing the scope of currently used legumes to reduce CH_4 emission intensity in grazing systems. This will highlight opportunities where there is currently evidence for a reduction in greenhouse gases with these alternate species in grazing systems and the potential level of these reductions when implemented. Research gaps that need to be addressed will also be identified.*

Corresponding Crop & Pasture Science paper:
Badgery W, Li G, Simmons A, Wood J, Smith R, Peck D, Ingram L, Durmic Z, Cowie A, Humphries A, Hutton P, Winslow E, Vercoe P, Eckard R (2023) Reducing enteric methane of ruminants in Australian grazing systems – a review of the role for temperate legumes and herbs. *Crop & Pasture Science*. https://doi.org/10.1071/CP22299

Notes:

Inoculation of novel soil bacteria improves the establishment of lucerne cultivars

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Abstract: *Establishing a new pasture can represent a considerable outlay of time and cost. Here, we developed a novel approach to improve lucerne establishment through inoculation of seeds with plant growth promoting bacteria (PGPB). Five public lucerne cultivars and 5 PGPB strains isolated from an Australian agricultural soilbank were evaluated using high-throughput phenotyping facilities based at The Plant Accelerator, at Waite campus. In this system, plants were automatically weighed, watered, and imaged by a 3D camera every 24h for 48 days. Images were analysed using a calibrated algorithm to calculate growth rate. Compared with the control, PGPB strains significantly increased the growth rate, shoot biomass and root biomass of the cultivars with high winter activity, while cultivars with weak winter activity had lower shoot biomass but more root biomass under the PGPB inoculation, although the interaction between cultivar and strain was also observed. At least one PGPB strain significantly improved the nodulation in every lucerne cultivar. Our results demonstrate that the use of PGPB can improve the establishment of lucerne cultivars under controlled conditions, and a high-throughput and lucerne-specific phenotyping system was established for use in future programs.*

Key words: alfalfa, PGPR, microbiome, beneficial microbes, rhizosphere

Introduction

"The most expensive pastures are those that fail to establish". Generally, pasture seeds are small, providing less carbohydrate and nutrients for seedling growth compared with most crops. At the seedling stage, pasture plants have not developed a vigorous root system and canopy for nutrient uptake and photosynthesis, respectively, and are delicate, slow growing, and poorly competitive with weeds. Therefore, pasture establishment is an essential difficult step for growers to ensure high levels of production and persistence.

Undersowing, defined as establishing a pasture by sowing pasture seed together with a crop (cover-crop) has been widely undertaken in south-eastern Australia, and some regions in SE Queensland and Western Australia. While competition for resources between the pasture and the crop is the biggest issue leading to the poor pasture establishment (McCormick *et al.* 2014), using higher pasture seeding rates can increase the success of pasture establishment, but also increase the cost of pasture seeds. Plant-endophytic or free-living soil bacteria with the ability to stimulate plant growth, termed plant growth promoting bacteria (PGPB), can be used to enhance seedling growth without requiring high resource input. The main mechanisms of PGPB-mediated enhancement of plant growth are the ability to modulate levels of plant hormones, i.e., increasing auxin and indole acetic acid (IAA), and decreasing the level of ethylene in the roots of developing plants, thereby increasing the root length and growth (Alemneh *et al.* 2020). The objective of this study is to screen putatively beneficial PGPB and identify those with the greatest efficacy in improving the seedling growth of lucerne cultivars, including daily growth rate, biomass production and nodulation.

Methods

The pot experiment investigated 5 lucerne cultivars and 6 inoculation treatments (5 PGPR strains and one non-inoculated control) with 6 replicates using phenomic accelerator based on The Plant Accelerator, the high-throughput phenotyping facilities, at Waite campus of The University of Adelaide. Lucerne cultivars were Magna995 (winter activity score, 9), Siriver (winter activity score, 9), Sequel (winter activity score, 9), Hunter River (winter activity score, 5), and Aurora (winter activity score, 6). The 5 PGPB strains used here belonged to *Burkholderia* spp. selected from our previous PGPR collection (Alemneh *et al.* 2021). The *in vitro* experiment demonstrated that they were able to produce a high level of IAA to stimulate plant growth, and 1-aminocyclopropane-1-car-boxylate (ACC) deaminase to reduce the level of ethylene in the roots thereby increasing the root length. The soil collected from the cropping field at Roseworthy campus, The University of Adelaide, was used to fill the pots (14 cm diameter and 11 cm height). A testing experiment was conducted first by growing the lucerne plants in the soils to confirm that adequate nodules were formed, so no rhizobia inoculation was required.

Colonies of the PGPR strains were inoculated into yeast-mannitol broth. The cultures were incubated in a shaker incubator (28 °C, 48 h), then measured at a wavelength of 600 nm in spectrophotometry to estimate the cell density. At 5 days after planting (DAP), 1-3 ml inoculant (adjusted to reach 10^6 cells per pot) was added into the young roots. Broth with no bacteria cells was used for the control treatments.

All the pots were placed in the glasshouse of the Plant Accelerator system. The greenhouse was fully automated with conveyor belts that delivered the plants to be automatically weighed, watered to 70% field capacity, and imaged every day. A 3D camera captured plant images under different light wavelengths, the visible and near infrared regions. From these images the Projected Shoot Area (PSA) of the plant was calculated as the sum of the areas, measured in kilopixels, from 3 camera views, comprising two side views and a view from above. Mild logarithmic smoothing was applied to produce the smoothed PSA (sPSA) by employing the R package growthPheno (Brien 2021). The daily change of sPSA was used to indicate plant growth rate. Plants were harvested at 48 DAP. Shoot biomass, root biomass, and nodule number were determined.

A linear mixed model was used for statistical analysis of every trait by the R packages ASReml-R (Butler *et al.* 2020). Normal probability plots of the residuals were inspected to check that the assumptions underlying the analyses were met. For each trait, a Wald F-test was conducted for the interaction effect of strain x cultivar. Least significant differences for $P = 0.05$ [LSD(5%)] were calculated for determining the significance of pairwise differences between treatments.

Results

The smoothed growth curve between sPSA and DAP can not fit the logistic model or any other exponential model (Fig. 1a). Hence, we calculated the relative growth rate (RGR) for every day, and divided the whole growth period into five intervals (Fig. 1b). The first interval covered 10-16 DAPs during which plants initially tended to have a constant RGR. The next interval was 16-23 DAP in which the RGR was increasing, with some plants reaching a peak by the end of the interval. Then, in 23-32 DAPs, the RGR was decreasing, while in 32-42 DAPs the RGR started to flatten out so that it was constant in DAPs 42-48. So the growth rate within each of the five intervals was calculated, and used to compare the PGPB strains effect in different lucerne cultivars. Compared with the non-inoculated control, the strain A084F significantly increased the growth rate of Magna995 during 32-42 DAP (Fig. 1c). The growth rate of the cultivar Siriver during 42-48 DAP was higher when inoculated with the PGPB strain A007F and A028F, while for the other lucerne cultivars, the responses of growth rate to PGPB were not significant.

After 48 days growth, the inoculation of the strain A028F significantly increased the shoot biomass of the cultivar Siriver by about 22% compared with the control (Fig. 2a), and the root biomass of Siriver was also promoted by A028F and A007F (Fig. 2b). For the cultivar Hunter River and Aurora, the application of A028F, A084F and A086F reduced shoot biomass, but increased the root biomass. The shoot growth of Magna995 and Sequel were not influenced by PGPB strains, while there was a rise of the root biomass with inoculation of A028F and A084F. Compared with the control, at least one PGPB strain significantly improved the nodulation in every lucerne cultivar (Fig. 2c), in particular, the strain A028F was able to increase the nodule number for every cultivar by 123%-333%.

Discussion

There was a significant interaction between PGPB strain and lucerne cultivar on plant growth, possibly because the survival and activity of the inoculated bacteria differed in the rhizosphere and enodpshere microhabitat which was shaped by the genetic variation of root exudations among the host cultivars (Micallef *et al.* 2009). PGPB improved lucerne growth by different mechanisms. For example, PGPB increased both shoot and root growth for the cultivars with high winter activity (Magna995, Siriver and Sequel), while for the less winter-active cultivars (Hunter River and Aurora), PGPB modulated the biomass allocation within the plant by limiting aboveground growth but enhancing the belowground growth. PGPB promoted the nodulation to a greater extent than the other measured traits such as growth rate and biomass production, indicating that PGPB might closely interact with the other beneficial microbes in the soil including the symbiotic rhizobia to favour the lucerne growth.

Conclusions

The findings of present study confirm that the Plant Accelerator is an effective system to perform high-throughput phenotyping for the lucerne germplasm. The inoculation of the novel soil bacteria can improve the growth rate, biomass production and nodulation of lucerne cultivars during the establishment, and the beneficial effect on the winter-active cultivars is more remarkable.

Acknowledgments

This study was funded by the Early-Career Research Grant of the AW Howard Memorial Trust.

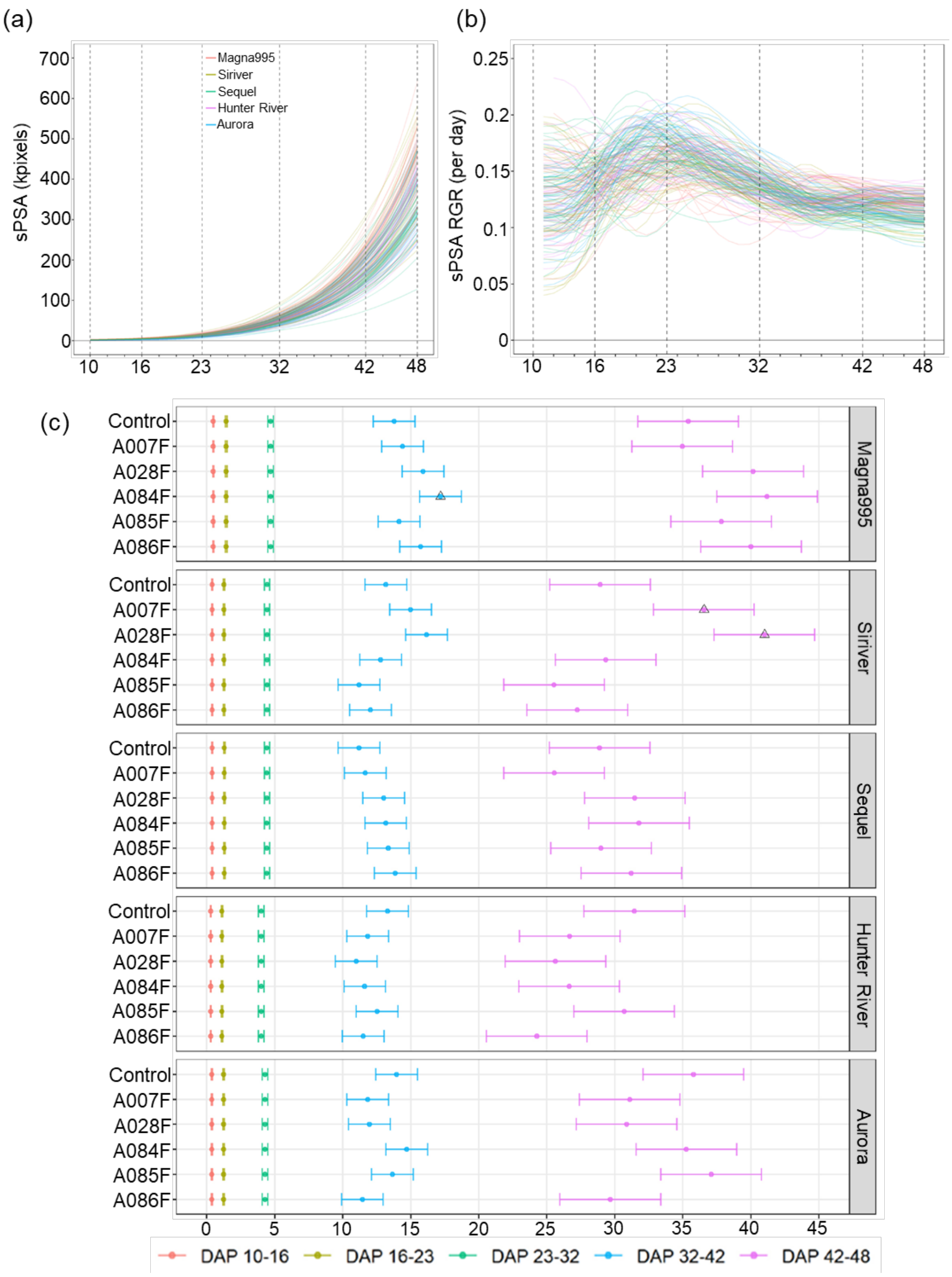

Figure 1. Smoothed Projected Shoot Area (sPSA) from 3D image analysis to indicate the growth of the lucerne cultivars inoculated with PGPB strains, including (a) the cumulative growth, (b) relative growth rate (RGR) and (c) absolute growth rate. Error bars were the LSD (5%). A triangle marker indicates a significant difference from the non-inoculated control for the same cultivar.

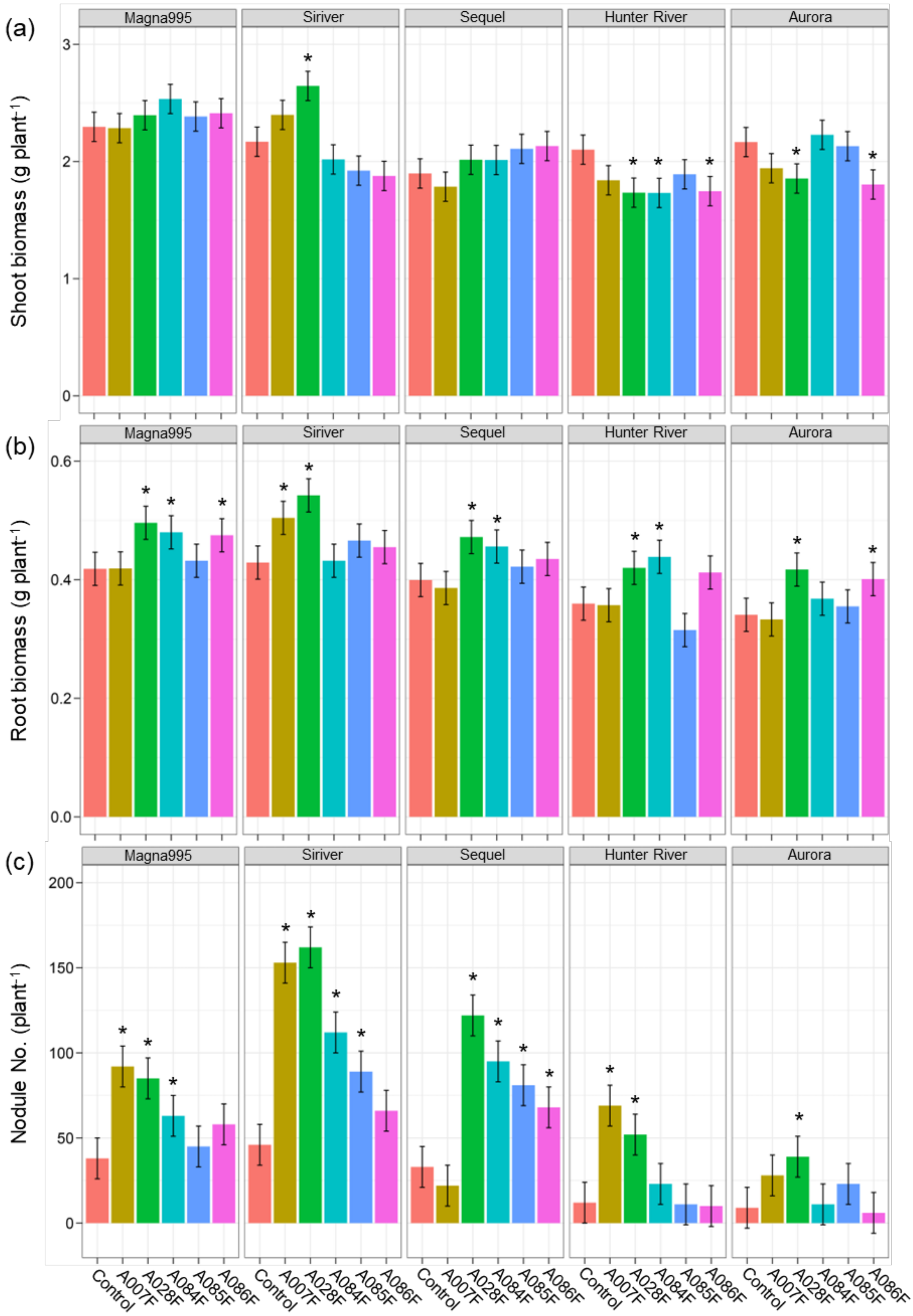

Figure 2. Shoot biomass(a), root biomass(b), and nodule number (c) of the lucerne cultivars inoculated with PGPB strains. Error bars were the LSD (5%). * indicates a significant difference from the non-inoculated control for the same cultivar.

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Notes:

Some Known Unknowns, Unknown Knowns, and Potential Unknown Unknowns Associated with Pasture Legumes for the Dairy Sector: A Northeast US Perspective

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Abstract: *Pasture legumes such as red clover (Trifolium pratense), white clover (Trifolium repens), lucerne (Medicago sativa), and birdsfoot trefoil (Lotus corniculatus) may hold the key to developing truly sustainable systems of agriculture due to their ability to biologically fix N, their high forage value to livestock, and their ability to be integrated with annual grain cropping to reduce external input requirements. Yet, much remains understudied, unknown, and potentially unanticipated with regard to possible tradeoffs associated with increasing use of pasture legumes in agriculture, particularly in support of the dairy industry in the northeast United States. Our group is engaged in research examining multiple aspects of the pasture legume-soil/ecosystem health-animal health continuum to better understand and manage the benefits and potential risks of expanding pasture legume production. An emerging component of this effort, which we are conducting in collaboration with our Australian colleagues, aims to develop a more comprehensive understanding of phytoestrogens associated with pasture legumes common in our region and elsewhere, their environmental and agronomic drivers, and their impacts on cow health and milk quality. This presentation will give an overview of some of our initial findings and provide a conceptual framework that may be useful for prioritizing future research on pasture legumes.*

Notes:

Future directions/priorities for pasture legume research and development – Panel and discussion session.

Notes:

Student competition papers

Diversity for waterlogging tolerance and other agronomic traits in Trifolium subterraneum L. ssp. Yanninicum

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Abstract: *Trifolium subterraneum L. (subclover), the most widespread pasture legume in southern Australia, comprises three subspecies, namely, subterraneum, brachycalycinum and yanninicum. Of these, ssp. yanninicum exhibits high tolerance to waterlogging (WL). However, the genetic base of current ssp. yanninicum cultivars is narrow. The mechanisms of WL tolerance and soil moisture fluctuation (SMF), from WL to water-deficit (WD), have not been studied in subclover. This thesis explored agronomic traits that confer tolerance to both early-season WL and late-season WD in a common garden experiment (Experiment 1) and three glasshouse experiments (Experiments 2-4). In Experiment 1, high diversity in agro-morphological traits was observed within 108 ssp. yanninicum ecotypes. In Experiment 2, the superior WL tolerance of ssp. yanninicum was confirmed, with shoot relative growth rate (RGR) 78-104% of controls, compared to ssp. subterraneum (51-100%) and ssp. brachycalycinum (45-69%). Diversity for WL tolerance within 28 ssp. yanninicum ecotypes was examined in Experiment 3. Shoot RGR ranged from 87-108% of controls and leaf size was maintained (mean 102%); but petiole length (mean 84%) was sensitive to WL. The effect of WL on the oestrogenic formononetin (F), was investigated in Experiments 2 and 3. WL increased F (heritability of 95%), but the magnitude of increase was unrelated to WL tolerance. In Experiment 4, the phenotypic plasticity among three ssp. yanninicum ecotypes was examined under SMF, from WL to WD. Rapid growth post-WL showed enhanced adaptation and fitness to SMF which was not related to WL tolerance. Overall, the study highlights promising genotypes from which to develop 'climate-change-ready' ssp. yanninicum subclover cultivars.*

Key words: soil moisture fluctuation, phenotypic plasticity, water-deficit, formononetin, genotypic diversity.

Introduction

Drought and waterlogging (WL) are critical soil moisture stresses which can adversely affect plant growth and lead to severe growth reductions or even premature death (Suralta et al., 2018). Climate change can increase the incidence of WL and drought stress, particularly in regions with Mediterranean-type climates, such as southern Australia that have already experienced prolonged periods of drought and intensified rainfall. These extreme events are projected to be exacerbated in the future (Grose et al., 2020). Hence, the agricultural challenge today is to improve the persistence and resistance of domesticated plants to a changing climate.

Subterranean clover (subclover, *Trifolium subterraneum* L.), the most widespread annual pasture legume in southern Australia, is highly valued for improving soil fertility and providing high-quality animal feed (Nichols et al., 2013). Subclover comprises three subspecies: *subterraneum*, *brachycalycinum* and *yanninicum*. Of these, ssp. *yanninicum* is regarded as more tolerant to WL than the two other subspecies (Nichols et al., 2013). However, differences in WL tolerance among the subspecies have not been fully characterised and the genetic base of current ssp. *yanninicum* cultivars is narrow. The mechanisms of WL tolerance and soil moisture fluctuation (SMF), from WL to water–deficit (WD), have also not been studied in subclover. To enter this 'climate-changed' era, it is crucial to improve the resilience and stability of subclover pastures.

This thesis aimed to explore agronomic traits associated with adaptation, sheep health and welfare and resilience in the context of climate change, within the world collection of ssp. *yanninicum*. It examined the research question of which shoot and root traits confer both early-season WL and late-season water-deficiency (WD) in ssp. *yanninicum*? The thesis comprised five experimental chapters (Chapters 1-5) which has resulted in four published scientific journal papers (Enkhbat et al. (2021a), (2021b, 2022, 2023), with a fifth paper under review. A common garden experiment (Chapter 1) and three glasshouse experiments (Chapters 2-5) were conducted. The main findings of each chapter are summarised below.

Key findings of this thesis

Chapter 1. It is crucial to explore the potential for genetic improvement of ssp. yanninicum to increase its productivity, as the current cultivars comprise a narrow genetic base from two Greek ecotypes and two naturalised strains. Also, variation within ssp. yanninicum ecotypes for adaptability to southern Australia has not been previously studied. I addressed these knowledge gaps by evaluating diversity for morphological traits, flowering time, and leaf isoflavone content among 118 genotypes (108 ecotypes collected from the Mediterranean basin and 10 cultivars) of ssp. yanninicum in a common garden experiment at the University of Western Australia (UWA) Shenton Park Field Station (31°57´S, 115°5´E). Wide variation among ssp. yanninicum ecotypes was observed and it was concluded that there is high potential to broaden the genetic base in southern Australian pastures, particularly for traits such as larger leaves, longer petioles and longer internodes that can improve plant competitive ability (Nichols et al., 2013). Likewise, ecotypes with a wide range of flowering times (94-149 days after sowing) were identified, with 18 ecotypes having low levels (0.05-0.3% of dry weight (DW)) of the oestrogenic isoflavone, formononetin (F). Details of this work are presented in Enkhbat et al. (2021a).

Chapter 2. There are conflicting results regarding WL tolerance among subclover subspecies and the mechanisms of WL tolerance in subclover have been little studied (Striker and Colmer, 2017). Variation in WL (prolonged for 35 days) tolerance among subclover subspecies, represented by three cultivars each, was examined in a naturally-lit glasshouse at UWA set at 20/15°C day/night. Superior WL tolerance to the other two subspecies was found in ssp. yanninicum (Fig. 1) when assessed by shoot relative growth rate (RGR) under WL, relative to a non-WL control maintained at 80% of field capacity (FC). Shoot RGR of ssp. yanninicum was least affected (78-104% of control), compared to subterraneum (51-100%) and brachycalycinum (45-69%). Low reduction in leaf size and high stomatal conductance are priority traits that can be utilised in a rapid screening breeding method as proxies for WL tolerance in subclover. Details of this research work can be found in Enkhbat et al. (2021b).

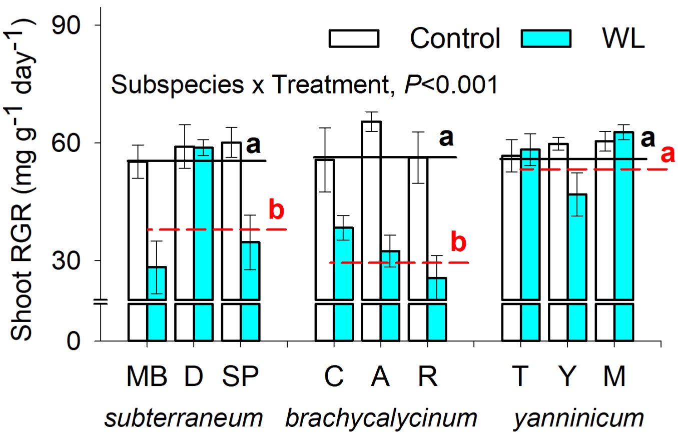

Figure 1. Relative growth rate (RGR) of shoots for nine cultivars from three subspecies of *Trifolium subterraneum* after 35 days of treatment (mean ± s.e.; *n*=4). Treatments imposed after 28 days of growth were: control (free-draining) and waterlogged (WL, water level kept 10 mm above the soil surface). Estimated means of each subspecies in control (black solid lines) and WL (red dashed lines) treatments are shown, with different letters indicating significant differences (l.s.d. *P*=0.05). A two-way ANOVA result is presented. Cultivars: MB, Mt. Barker; D, Denmark; SP, Seaton Park; C, Clare; A, Antas; R, Rosedale; T, Trikkala; Y, Yarloop and M, Meteora.

Chapter 3. Variation for WL tolerance within ssp. yanninicum ecotypes has not been previously studied (Striker and Colmer, 2017). Neither has WL tolerance been related to corresponding eco-geographic variables at their collection sites. This glasshouse study (using the same conditions as Chapter 2) investigated diversity for WL tolerance after 28 days of WL within 32 ssp. yanninicum genotypes evaluated in Chapter 1 (28 ecotypes and four cultivars). Small variance for growth under WL was observed (shoot RGR 87-108% of controls) (Fig. 2). Under WL ssp. yanninicum demonstrated stable shoot growth by maintaining leaf size (mean 102% of controls), but petiole length was reduced (mean 84%). Reduction in petiole length could be used as a preliminary selection trait for WL tolerance in ssp. yanninicum. Variation in WL tolerance of ssp. yanninicum ecotypes was unrelated to eco-geographic variables at their site of origin. Seedling biomass, as a reflection of bigger seeds and larger cotyledons, was also unrelated to WL tolerance of ssp. yanninicum. Further details can be found in Enkhbat et al. (2022).

Chapter 4. Subclover contains three isoflavones, formononetin (F), genistein (G) and biochanin A (BA), which can impact livestock fertility due to their oestrogenic effects (Braden et al., 1967). Of these, F is the most potent. Subclover leaf isoflavone response to WL and its relationship with WL tolerance has been little researched. The critical concern is whether WL stress exacerbates F content to levels >0.3% of DW, considered ‘unsafe’ for grazing. The effect of WL on F content was investigated among 22 subclover genotypes (10 cultivars and 12 ecotypes) using data from Chapters 2 and 3. WL increased mean F levels from 0.19% (control) to 0.31% (WL) in Chapter 2 and from 0.61% to 0.97% in Chapter 3. Isoflavones under WL were highly heritable, particularly F (H2=95%), and the relative proportions of F, G and BA were stable under both WL and well-drained conditions. Subclover with F levels considered ‘safe’ for grazing under well-drained conditions could be ‘unsafe’ under WL. Further details can be found in Enkhbat et al. (2023).

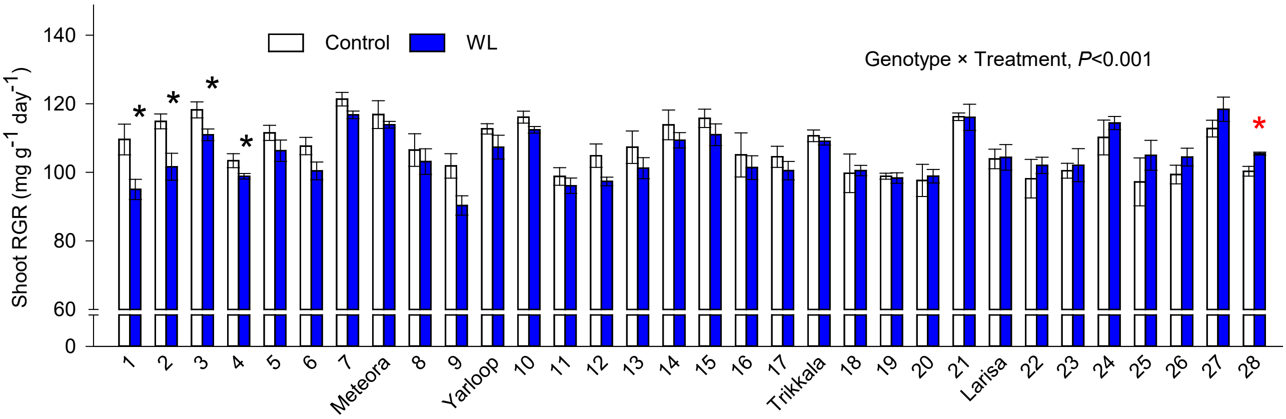

Figure 2. Relative growth rates (RGR) of shoots for 32 ssp. *yanninicum* genotypes, consisting of four cultivars and 28 ecotypes (numbered 1 to 28) after 28 days of treatment (mean ± s.e.; *n*=4). Treatments imposed 21 days after sowing were: control (free-draining) and waterlogged (WL; water level kept 10 mm above the soil surface). A two-way ANOVA result is presented. Significant differences between control and WL treatments of each genotype are shown as *P<0.05 (black indicates that the control treatment was higher and red indicates that the WL treatment was higher). Genotypes are ordered from negative to positive impact of WL on shoot RGR.

Chapter 5. The interactive effects of WL and WD (leading to SMF), commonly encountered in Mediterranean environments, results in a more severe stress impact, which demands both contrasting and immediate responses (Suralta et al., 2018). The growth responses under SMF (from WL to WD) of three diverse ssp. yanninicum ecotypes (A, B and C) was explored, relative to well-watered (80% FC) controls during both phases (WW-WW). WL caused reductions in shoot RGR overall (86% mean of WW control), but Ecotype A (80% of WW) had a significantly greater reduction than Ecotypes B (92%) and C (87%). However, under WL to WD (estimated mean 65% of WW-WW), Ecotype A was less affected (75% of WW-WW control) than Ecotypes B (57%) and C (63%) (Fig. 3a). For root RGR, WL resulted in a greater reduction for Ecotype A (52% of WW control) than Ecotypes B (77%) and C (74%) but under WL-WD, Ecotype A showed a large increase (117% of WW-WW control) than Ecotypes B (94%) and C (87%) (Fig. 3b). Hence, ssp. yanninicum ecotypes demonstrated contrasting adaptation responses to SMF, which were not related to their WL tolerance. This shows rapid growth post-WL in a drying soil profile can enhance adaptation and fitness of ssp. yanninicum.

Conclusions

Annual pasture legumes continue to play a crucial role in sustainability of farming systems globally through a significant contribution to animal feed and soil fertility (Porqueddu et al., 2016). Climatic challenges, including a predicted increase in rainfall variability, require the development of more resilient pasture legumes to overcome combined stresses, such as a sequence of wet and dry conditions (Suralta et al., 2018). My research has demonstrated that genotypes of ssp. *yanninicum* can tolerate transient WL but are susceptible to subsequent WD conditions. Finally, I conclude, that the ideal approach to identify genotypes of ssp. *yanninicum* with better adaptability and greater resilience in Mediterranean-type climate regions should not only focus on a higher tolerance to, and better performance under, WL conditions, but should also identify genotypes with rapid growth ability under post-WL stress in a drying soil profile.

Acknowledgments

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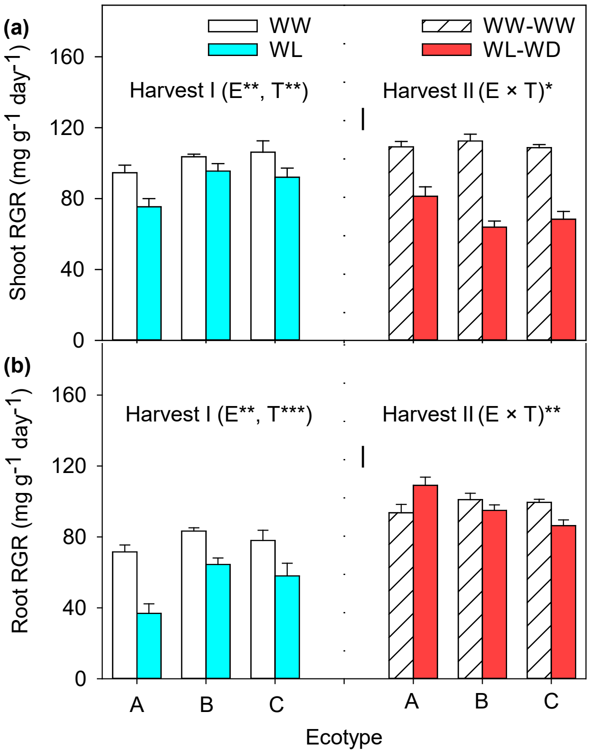

Figure 3. Relative growth rate (RGR) of (a) shoots and (b) roots for three ecotypes (A, B and C) of *Trifolium subterraneum* ssp. *yanninicum* in two treatments at two harvests: well-watered (WW–WW) and waterlogged to water-deficit (WL–WD) (mean + s.e.; *n*=4). Treatments were imposed 21 days after sowing. In the WW–WW treatment, pots were maintained at 80% field capacity (FC). In the WL–WD treatment, pots were subjected to waterlogging (10 mm above the soil surface) for 28 days until Harvest I, with the remaining pots allowed to drain to 40% FC, which was then maintained for 10 days by daily watering (Harvest II). For Harvest I and II, two-way ANOVA results are presented for ecotype (E), treatment (T) and their interaction (E × T): ****P*<0.05; ***P*<0.01; ****P*<0.001. The l.s.d. at *P*=0.05 is provided by vertical bars when E × T is significant.

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Notes:

The influence of CO_2 concentration and soil phosphorus supply on the growth and nodulation of two *Desmanthus* cultivars

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Abstract: *Tropical pasture legumes such as Desmanthus are expected to improve the productivity of the extensive grazing systems of northern Australia. This is because legumes increase forage quality and fix atmospheric nitrogen. However, little is known about the influence of soil phosphorus (P) supply on legume growth and nodulation in mixed pasture swards that include highly-competitive C_4 grasses. Furthermore, predicted increases in CO_2 concentration may influence the relative competitiveness of tropical pasture legumes. A controlled-environment pot trial was conducted to investigate the influence of CO_2 concentration (250, 500 and 750 ppm) and soil P supply (3, 10 and 40 mg P kg^{-1}) on the growth of two Desmanthus spp. cultivars (cvv. JCU 7 and JCU 9). The legumes were grown both as monocultures and as mixed swards with Premier Digit (Digitaria eriantha). Legume shoot yields and tissue P concentrations increased in response to soil P supply. On average across the treatments, the proportion of cv. JCU 9 in the mixed grass/legume swards was 1.9-fold higher than that of cv. JCU 7. In general, legume competitiveness decreased at higher CO_2 concentrations. Under monoculture conditions, legume nodule size increased in response to soil P supply but not CO_2 concentration, and the nodules of cv. JCU 9 were generally larger than that of cv. JCU 7. These results demonstrate that there are differences in competitiveness and nodulation among cultivars of Desmanthus which are likely to influence forage quality and atmospheric nitrogen-fixation. Consideration should be given to both shoot yield and potential nitrogen-fixation when applying P fertiliser for optimum pasture production.*

Keywords: *Desmanthus leptophyllus*, *Desmanthus pernambucanus*, *Digitaria eriantha*, tropical pasture legumes

Introduction

Tropical pasture legumes such as *Desmanthus* are expected to improve the grazing feedbase of northern Australia (Jones and Rees, 1997). This is because legumes are known to increase forage quality and fix atmospheric nitrogen. However, tropical pasture legumes often lack persistence when grown with highly-competitive C_4 grasses (Peck et al., 2012). In part, this may be due to varietal differences in nutrient acquisition efficiency because grasses generally forage more efficiently for available nutrients than legumes (Evans, 1977). Indeed, previous work has demonstrated that *Desmanthus* spp. genotypes produce short, thick roots with short root hairs (McLachlan et al., 2021). Nevertheless, Premier Digit (a highly-productive C_4 grass) and Progardes Desmanthus (a blend of several *Desmanthus* spp. genotypes) have been found to compete effectively for applied P under mixed sward conditions (McLachlan et al., 2022). Further research into the growth of these *Desmanthus* spp. genotypes is therefore warranted.

Improved plant growth due to P fertiliser application is likely to increase the amount of nitrogen fixed by legumes such as *Desmanthus*. However, little is known about the influence of soil P supply on legume nodulation and subsequent nitrogen fixation. Furthermore, increases in CO_2 concentration may influence the relative competitiveness of *Desmanthus* when grown with highly-competitive C_4 grasses. This is because increases in CO_2 concentration are suggested to increase the growth of C_3 species (which include tropical pasture legumes), although there can be large differences in how plants respond to CO_2 (Miri et al., 2012). Nevertheless, CO_2 could influence the forage production and nitrogen fixation of legumes such as *Desmanthus*. The objective of the current study was to therefore investigate the influence of CO_2 concentration and soil P supply on the growth and nodulation of two *Desmanthus* cultivars when grown with and without Premier Digit.

Methods

Plant growth conditions

JCU 7 *Desmanthus* (*Desmanthus leptophyllus* cv. JCU 7) and JCU 9 *Desmanthus* (*Desmanthus pernambucanus* cv. JCU 9) were grown to investigate shoot yield, tissue P concentrations, and nodule presence and diameter in response to CO_2 concentration and soil P supply. The legumes were grown as monocultures and as mixed swards with Premier Digit (*Digitaria eriantha* cv. Premier) in a clay loam soil that was collected from the upper 0–30 cm soil layer of a field at Burketown, QLD, Australia. The soil had a Colwell extractable P concentration of 3 mg P kg^{-1}, a Phosphorus Buffering Index (PBI) of 70, and a pH ($CaCl_2$) of 5.9. The soil was crushed by hand and homogenised before being amended with a basal nutrient solution that included 29.3 mg N kg^{-1}, 54.5 mg K kg^{-1}, 16.7 mg S kg^{-1}, 3.4 mg Mg kg^{-1}, 8.4 mg Ca kg^{-1}, and micronutrients (B, Mn, Zn, Cu, Mo, Co and Fe). Three P-amended soils (3, 10 and 40 mg P kg^{-1}) were prepared by adding KH_2PO_4 to the nutrient solution before it was applied to the soil. KCl was applied to the P3 and P10 treatments to balance the potassium so that it was equivalent to that of the P40 treatment. Cylindrical PVC pots (87 mm internal diameter; 200 mm height) were filled with 1.3 kg (oven-dry basis) of the P-amended soils. The total depth of soil was ~190 mm and the bulk density was ~1.15 g cm^{-3}.

Micro-swards of each species were established by sowing seed to achieve a population of 8 plants pot^{-1}. The species were established both as monocultures (8 grass or legume plants pot^{-1}) and mixed swards (4 grass and 4 legume plants pot^{-1}). After planting, the pots were watered and moved to three glasshouse bays (natural daylight, ~1800 μmol m^{-2} s^{-1} peak intensity; 28/18ºC, day/night) in Armidale, NSW, Australia. The concentration of CO_2 in each bay was regulated during daylight hours to achieve three contrasting treatments of 250, 500 and 750 ppm (overnight concentrations in each bay were ~250 ppm). There were three replicate pots of each species in each P treatment and at each CO_2 concentration. In the bays, the pots were arranged in a randomised complete block design (blocks comprised the different replicates). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight. Four days after planting, the pots were inoculated with a peat slurry of rhizobium inoculant that was sourced from the University of Sydney. An additional 30 mg N kg soil^{-1} was applied to the surface of each pot five weeks after planting due to early signs of nitrogen deficiency.

Harvest and analysis

Plants were harvested after eight weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving & McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. The soil from the legume-only pots was removed as an intact core. The roots were then washed from the soil over 2 mm sieves to assess nodulation. Root systems were scored for the presence/absence of nodules, and the diameter of five random nodules was measured per root sample. Measured parameters were analysed using R (R Core Team, 2020).

Results

The shoot yields of the three tropical pasture species increased in response to CO_2 concentration (P < 0.001) and soil P supply (P < 0.001) (Fig. 1). On average, cv. JCU 9 was more productive than cv. JCU 7. Consequently, the proportion of cv. JCU 9 in the mixed grass/legume swards was, on average, 1.9-fold higher than that of cv. JCU 7. Regardless of these differences, the shoot yields produced by Digit were equivalent or higher than that of the *Desmanthus* spp. cultivars which meant that the proportion of legume in the mixed swards ranged between 5–51% (Table 1).

Table 1. The proportion of JCU 7 and JCU 9 *Desmanthus* in mixed swards with Premier Digit at three CO_2 concentrations (250, 500 and 750 ppm) and three P application rates (3, 10 and 40 mg P kg^{-1}). Values show the mean ± se (n = 3). ANOVA results were: CO_2 concentration P = 0.014, P supply P < 0.001, species P < 0.001.

Species & CO_2 concentration	Legume proportion (%) 3 mg P kg^{-1}	10 mg P kg^{-1}	40 mg P kg^{-1}
JCU 7 – 250 ppm	17 ± 4	7 ± 1	23 ± 2
JCU 7 – 500 ppm	21 ± 3	7 ± 1	22 ± 1
JCU 7 – 750 ppm	13 ± 1	5 ± 1	15 ± 2
JCU 9 – 250 ppm	51 ± 1	13 ± 4	23 ± 1
JCU 9 – 500 ppm	42 ± 6	23 ± 3	13 ± 1
JCU 9 – 750 ppm	35 ± 2	13 ± 3	27 ± 3

Legume tissue P concentrations increased in response to soil P supply by 1.8-fold (P < 0.001) and decreased in response to CO_2 concentration by 1.3-fold (P < 0.001). In contrast, grass tissue P concentrations were only influenced by soil P supply (P < 0.001) (data not shown). When grown as monocultures, the average tissue P concentrations of the species across the treatments were: Digit = 0.10%, cv. JCU 7 = 0.11% and cv. JCU 9 = 0.13%.

Nodule presence and diameter were only assessed on the roots of the *Desmanthus* cultivars grown under monoculture conditions (Table 2). Nodule presence and diameter generally increased in response to soil P supply. *Desmanthus* cv. JCU 9 produced nodules at lower levels of soil P supply than cv. JCU 7. In the P40 treatment, when both *Desmanthus* cultivars produced nodules, the average nodule diameter of cv. JCU 9 across the three CO_2 treatments was 1.7-fold larger than that of cv. JCU 7.

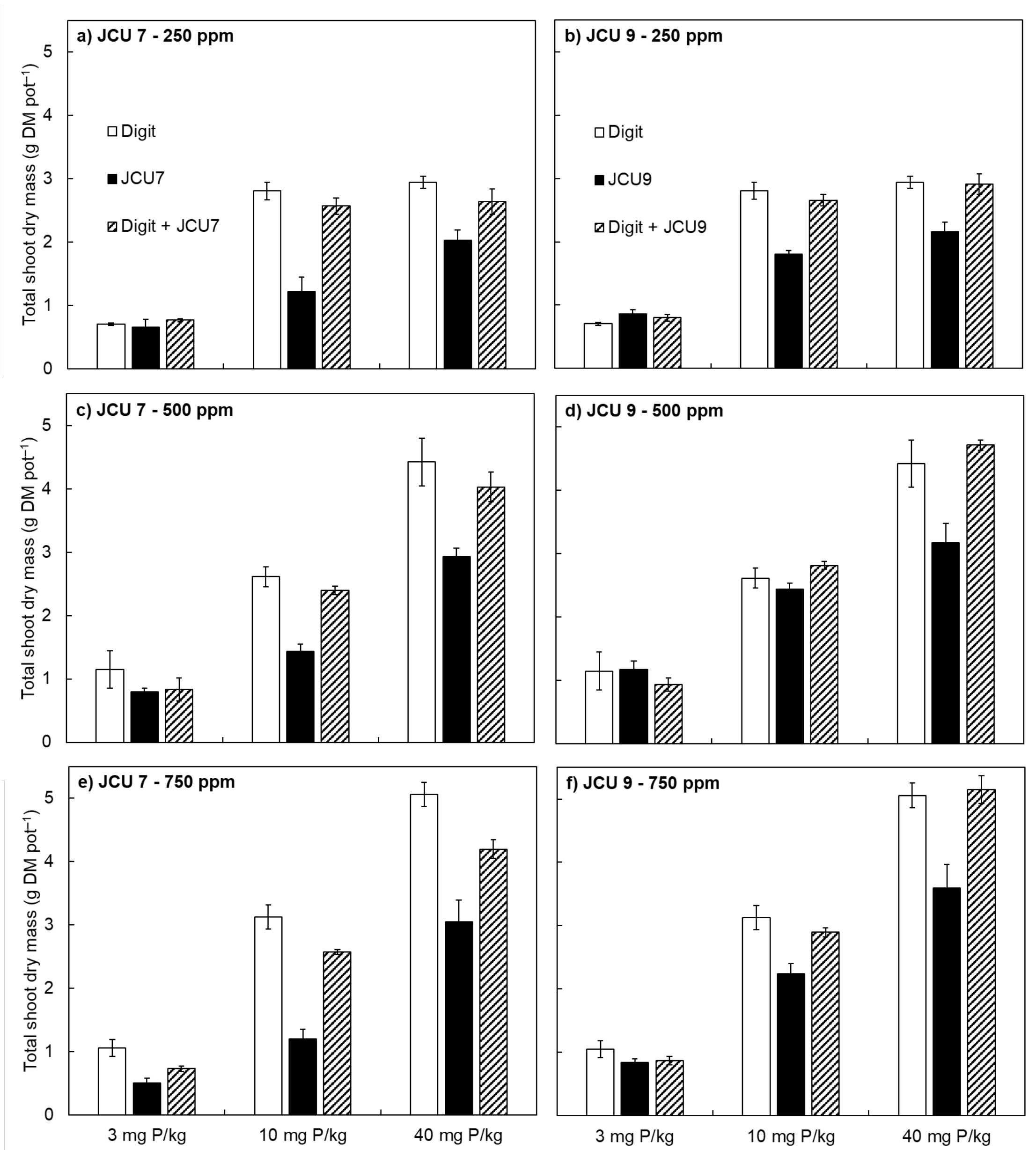

Figure 1. The total shoot dry mass of JCU 7 *Desmanthus* (a, c, e) and JCU 9 *Desmanthus* (b, d, f) when grown in response to three CO_2 concentrations (250, 500 and 750 ppm) and three P application rates (3, 10 and 40 mg P kg^{-1}), as monocultures and in mixed swards with Premier Digit. Values show the mean ± se (n = 3).

Table 2. Nodule presence and diameter of JCU 7 *Desmanthus* and JCU 9 *Desmanthus* when grown in response to three CO_2 concentrations (250, 500 and 750 ppm) and three P application rates (3, 10 and 40 mg P kg^{-1}) under monoculture conditions. Values show the mean ± se (n = 3), except where only one of the replicates had nodules.

Species & CO_2 concentration	Nodule presence (Y/N) and avg. diameter (mm)		
	3 mg P kg^{-1}	10 mg P kg^{-1}	40 mg P kg^{-1}
JCU 7 – 250 ppm	N	N	**Y – 1.2 ± 0.2**
JCU 7 – 500 ppm	N	N	**Y – 3.1 ± 0.3**
JCU 7 – 750 ppm	N	N	**Y – 2.0 ± 0.4**
JCU 9 – 250 ppm	N	**Y – 1.4 ± 0.3**	**Y – 3.5 ± 0.8**
JCU 9 – 500 ppm	N	**Y – 1.0 ± 0.2**	**Y – 3.5 ± 0.4**
JCU 9 – 750 ppm	**Y – 1.5**	**Y – 2.2 ± 0.3**	**Y – 3.9 ± 0.3**

Discussion

There were differences in the growth and competitiveness of the two *Desmanthus* cultivars when grown in response to soil P supply. In particular, cv. JCU 9 competed more effectively with Digit than cv. JCU 7 in the P3 and P10 treatments. Previous work has shown that *Desmanthus* cv. JCU 9 is relatively efficient at utilising acquired P (McLachlan et al., 2021) which likely contributed to the competitive advantage of this cultivar in the present experiment. Not only did cv. JCU 9 produce more biomass, it also produced nodules at lower levels of soil P supply than cv. JCU 7. This result indicates that the relative P-efficiency of cv. JCU 9 is likely to not only benefit forage production but also the atmospheric nitrogen-fixation of this legume. Regardless of these varietal differences, the nodule size of cv. JCU 9 benefited from higher levels of soil P supply. Consideration should therefore be given to both shoot yield and potential nitrogen-fixation when applying P fertiliser for optimum pasture production.

Shoot yields increased in response to CO_2 concentration, particularly between the 250 and 500 ppm treatments. This led to a greater yield response to the P40 treatment, whereby the species could capitalise on the higher soil fertility. However, the yields of the grass and legumes both increased which meant that the benefit to legume competitiveness was minimal. Furthermore, CO_2 concentration had a limited effect on nodule presence and diameter. It is therefore likely that soil P supply will remain an important factor for grass/legume competition in the extensive grazing systems of northern Australia, even as CO_2 concentrations increase.

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Notes:

Enhancing Pasture Legume Performance in a Changing Climate: The Case for Super-Nodulating Varieties

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Abstract: *Pasture legumes play a vital role in sustainable and productive agricultural systems by providing grazing forage and facilitating nitrogen cycling within the pasture sward. Through the process of symbiotic nitrogen fixation (SNF), legumes increase nitrogen availability by establishing a mutualistic association with rhizobia bacteria housed within root nodules, which fix atmospheric nitrogen in exchange for plant-derived carbohydrates. In response to this high carbohydrate demand from nodules, legumes display autoregulation of nodulation (AON) to restrict nodules number to the minimum required to sustain nitrogen supply under current photosynthetic levels. Mutations to the AON pathway can result in super-nodulating legumes, which typically grow smaller than wild-type plants, due to the high carbohydrate cost of producing and maintaining excessive nodule numbers. Recent research has demonstrated that these altered AON super-nodulating mutants are more responsive to elevated CO_2 (eCO_2) conditions, outperforming their non-mutant counterparts in biomass production and nutritional content (Zhang et al., 2023). It is thought that super-nodulating mutants are carbon-limited and can perform better at eCO_2 through improved photosynthesis, facilitated by the re-investment of nitrogen assimilates into photosynthetic machinery. As eCO_2 conditions are predicted to increase in future climates, harnessing the benefits of symbiotic nitrogen fixation through AON mutants has the potential to improve the CO_2 fertilisation effect, without protein yield penalties. This paper critically examines our current understanding of super-nodulating legumes and identifies the necessary steps for translating this research into practical applications within pasture systems.*

Keywords: pasture, legume, nitrogen, photosynthesis, elevated CO2

Introduction

As the impacts of climate change become more evident, the need to sustain and enhance pasture production is essential to maintain sustainable and productive agricultural systems. A major contributor to changing climates is the continued rise of atmospheric carbon dioxide (CO_2) levels (IPCC, 2022). Atmospheric CO_2 levels have risen from 365 ppm in 1998, to 412 ppm in 2021 and are predicted to rise to 985 ± 97 ppm by 2100 (IPCC, 2022). Elevated CO_2 (eCO_2) conditions will directly affect pasture plant productivity, as CO_2 is the primary substrate of photosynthesis. While not directly discussed here, climate change is also predicted to increase global temperatures and water availability (IPCC, 2022) which will also impact on plant productivity. eCO_2 conditions can enhance photosynthetic rate, with free-air-CO_2-enrichment (FACE) studies reporting an average 20% increase in above-ground biomass in C_3 plants (Ainsworth and Long, 2005). This can translate to yield increases in crop species, but these yield increases are offset by significant reductions in protein content (Kant *et al.*, 2012). This occurs because the additional carbon (C) obtained from enhanced photosynthesis effectively dilutes the nitrogen (N) in the plant, leading to an imbalance of C:N ratios and reduced nutritional quality of the crop or pasture (Díaz *et al.*, 1993; Leakey *et al.*, 2009). Longer-term eCO_2 conditions also lead to a photosynthetic acclimation response, where photosynthesis is downregulated and the benefit of eCO_2 on yields reduces (Leakey *et al.*, 2009). Plant productivity under eCO_2 conditions is therefore largely dependent on if plant N uptake can match eCO_2 stimulated carbohydrate production.

Legumes are a valuable tool for increasing nitrogen availability in agricultural systems, due to their ability to access additional nitrogen via symbiotic nitrogen fixation (SNF; Peoples *et al.*, 2012). Nitrogen is an essential nutrient for grazing forage, providing valuable protein in the diet. Within the plant, the majority of N is utilised in photosynthetic machinery, with Rubisco alone accounting for 20% of leaf N within a C_3 leaf (Evans and Clarke, 2019). Crop production reflects photosynthesis integrated over the life of the crop, so adequate N in the pasture translates to increased yields. Nitrogenous fertiliser can be applied to crops (at significant financial and environmental cost), but legumes offer an endogenous N source via SNF.

Legumes access SNF via a symbiotic association with nitrogen-fixing soil bacteria of the *Rhizobiaceae* family (rhizobia), resulting in the formation of a new root organ: the nodule. Within nodules, atmospheric nitrogen (N_2) is fixed by the rhiozbia into a biologically available form, and exchanged for photosynthesis-derived organic acids and other nutrients with the plant host (Clarke *et al.*, 2014). SNF acts as a sink for carbohydrates assimilated in the leaves by photosynthesis; for every gram of N fixed, the host legume supplies 5 to 10 grams of C (Phillips, 1980). To control this high carbohydrate demand from nodules, legumes display autoregulation of nodulation (AON) to restrict the number of nodules to the minimum needed to sustain N supply at current photosynthesis levels (Ferguson *et al.*, 2019). The AON pathway is mediated through signals that move between root and shoot tissues. In the model legume *Medicago truncatula*, upon infection by rhizobia, roots produce CLE peptides which travel up to the shoot within the xylem vasculature. When they reach the shoot they bind and activate the SUNN receptor, triggering production of an inhibitor molecule which travels back to roots via phloem and inhibits nodule meristem growth and further nodule formation (Novák, 2010). Mutations in the AON pathway can result in plants with unchecked nodule formation, known as super-nodulators (Schnabel *et al.*, 2005). Recent research has shown that these super-nodulating legumes may offer a pathway towards matching N and C assimilation in plants grown under eCO_2 conditions (Zhang *et al.*, 2023). With the goal of translating this research into pasture species, this paper reviews our current understanding of super-nodulating legumes and their growth potential under eCO_2, and highlights the necessary steps for incorporating AON mutants into productive pasture systems in future eCO_2 climates.

How will pasture legumes respond to eCO_2?

When grown in eCO_2 conditions, legumes display greater stimulation of photosynthesis compared to non-legumes (Lam *et al.*, 2012; Lee *et al.*, 2003; Rogers *et al.*, 2009). This is particularly true in managed systems (Ainsworth and Long, 2005), but in natural systems, legumes ability to respond to eCO_2 may be limited by nutrient availability (Hungate *et al.*, 2004; van Groenigen *et al.*, 2006). With no other limiting nutrition, legumes should be able to capitalise on eCO_2 through increasing N_2 fixation with additional photosynthates, alongside reduced drought impacts, as eCO_2 conditions also reduce stomatal conductance (Rogers *et al.*, 2009). Legumes in managed pasture systems should therefore be able to maintain their C:N balance, whereas non-legumes grown at eCO_2 have reductions in leaf and grain N content (Ainsworth and Long, 2005). The proposed mechanisms for this is that greater photosynthate availability from eCO_2 (the CO_2 fertilisation effect) supports increased N_2 fixation (Rogers *et al.*, 2009). Maintaining N content in pastures is essential as legumes are important components of animal forage due to their increased protein content (Angus and Peoples, 2012).

Studies on grassland performance under eCO_2 conditions offer an insight into pasture species responses to eCO_2 (Andresen *et al.*, 2015; Fitzgerald *et al.*, 2022; Lam *et al.*, 2012; Morgan *et al.*, 2001). A large, multiyear FACE study on white clover (*Trifolium repens*) reported an increase in photosynthetic C uptake and increased leaf carbon content at eCO_2 (Ainsworth *et al.*, 2003). Grassland field studies have also found that under eCO_2 conditions, SNF increased N content in white clover, allowing the C:N ratio to be maintained in ecosystem as a whole (Zanetti *et al.*, 1996), and in mixed semi-grassland managed as a forage crop, legume leaf N and C:N ratio weren't effected by eCO_2, but non-legume species did have reduced leaf N and increased C:N ratio (Winkler and Herbst, 2004). Studies have also shown that grain legumes, such as soybean and pea, can increase above-ground biomass with no reduction in leaf N at eCO_2, but biomass gains were not as large as predicted (Cabrerizo *et al.*, 2001; Morgan *et al.*, 2005; Rogers *et al.*, 2006). The literature overall reports little to no changes to rhizobial nitrogenase activity under eCO_2 (Cen and Layzell, 2004), suggesting either nodule number or size may be driving increased SNF under eCO_2. However, a study in soybean reported that while N-fixing capacity was increased under eCO_2, the increase in symbiotically fixed-N content in shoot did not correlate with nodule number, fresh weight, and density changes (Li *et al.*, 2017). This suggests that different species and cultivars may have differential responses.

Acclimatisation to eCO_2, which reduces photosynthetic gains, does still occur in legumes, but not to the same extent as in non-legumes (Ainsworth *et al.*, 2003). Legumes reduce photosynthetic acclimatisation by redirecting extra C to N fixation, rather than by reducing rubisco content and photosynthetic activity (Leakey *et al.*, 2009), and modelling has suggested that promoting physiological adaptations would provide further benefits (Soussana and Hartwig, 1995; Zanetti *et al.*, 1996). This suggests that there is still yield potential available if photosynthetic acclimatisation could be overcome completely, and in legumes this could then increase SNF and N content to boost yields and nutritional value further.

The role of nodule autoregulation in optimising symbiotic nitrogen fixation

The regulation of nodule number and plant carbon investment in SNF is controlled by the autoregulation of nodulation (AON) pathway (Schulze, 2004). Several mutants have been identified with disordered AON, causing either no nodules to form, or excessive nodule numbers (termed super-nodulators). Super-nodulating mutants either don't produce, or don't integrate, the AON signals due to mutations of key proteins in the AON pathway, resulting in unchecked nodule formation (Ferguson *et al.*, 2019). Super-nodulating mutants have been detected in a number of legume species including medic, soybean, lotus and pea (Novák, 2010). When grown at ambient CO_2, super-nodulating plants perform poorly due to the high C cost of their excessive nodulation, with small above-ground biomass and numerous small nodules (Novák, 2010), and they do not have enhanced uptake of N_2 from the atmosphere compared to wild-type plants (Cabeza *et al.*, 2014). This is likely due to their inability to support the increased nodule number due to limited photosynthates.

When grown under eCO_2 conditions however, super-nodulating plants outperform wild-type plants, with increased above-ground biomass and no change in C:N ratios (Qiao *et al.*, 2021; Zhang *et al.*, 2023). An earlier study has also reported that specific nitrogen fixation activity was not increased under short or long term increased CO_2 assimilation in super-nodulating *Medicago truncatula* mutants (Cabeza *et al.*, 2014). The responsiveness and sensitivity of nodulation to eCO_2 suggests that photosynthesis and nodulation are interdependent. Zhang *et al* (2023) hypothesise that super-nodulating plants are carbon limited and under eCO_2 they are more productive due to increased N investment in photosynthetic machinery; super-nodulating plants have increased photosynthetic activity and investment beyond what non-mutants have at eCO_2. In super-nodulating mutants additional C derived from photosynthesis at eCO_2 supports N_2 fixation, allowing further N investment back into photosynthesis machinery, which in turn further supports additional N_2 fixation in a cyclic manner. There is compelling evidence then for adapting super-nodulating mutants into pasture species to secure sustainable pasture productivity as atmospheric CO_2 levels increase.

Translating super-nodulation to pasture

While proof-of-concept studies have demonstrated the potential of super-nodulating legumes in future climates, robust field-testing will be essential to translate into pasture-ready cultivars. Scaling up from these initial studies will benefit from careful modelling of super-nodulating responses to environmental conditions in the field, such as has been achieved for predicted photosynthesis enhancements (Wu *et al.*, 2023). Under dynamic field conditions predicted gains may not be fully realised, as observed previously in the in soybean FACE experiments (Morgan *et al.*, 2005). There is also evidence that if other nutrients or conditions become limiting, the ability of legumes to respond favourably to eCO_2 conditions may be impacted. Rogers *et al* (2009) hypothesise that if an environmental factor prevents stimulation of N_2 fixation at eCO_2, photosynthate will accumulate and lead to photosynthetic acclimation. Recently, a study in fava beans reported that decreased C assimilation and nodule sink strength from drought conditions resulted in decreased N_2 fixation and accumulation of photosynthates in leaves at eCO_2 (Parvin *et al.*, 2020). In the case of phosphorus (P), legumes with SNF don't seem to require more P than if they acquire N from a mineral source (Vitousek *et al.*, 2002). Therefore, it not thought that SNF is limited by P availability, but other systems can be impacted by P-deficiency and could reduce yield. Conversely, additional C and N fixed at eCO_2 by legumes may allow the plant to access more P through additional root growth and secretion of chemicals to mobilise P in soil (Houlton *et al.*, 2008; Vitousek *et al.*, 2002).

Complete disruption of the AON pathway may risk undesirable off-target effects. The AON pathway has evolved to ensure that valuable carbon resources, in the form of photosynthates, are not depleted to maintain unneeded or inefficient nodules (Ferguson *et al.*, 2019). Within soil, there is a pool of rhizobia that can infect legumes, leading to various levels of N_2 fixation efficiency. If a low efficiency strain infects the host it becomes a sink for C with little N benefit (Libault, 2014). Plant hosts therefore tightly control the infection process and nodule development to ensure maximum efficiency. If the plant host loses control of nodulation, there a risk of un-productive rhizobia colonising. An additional consideration is that under eCO_2, there may be more nodule exudates (derived from photosynthates) which could alter the rhizobial community and influence nodulation efficiency. Management practices, such as seed inoculation, will therefore be critical to maintain productive SNF. Application of fertiliser to super-nodulating pasture may also decrease efficiency in the system if the host cannot adjust SNF.

One approach to managing this risk is to optimise the number of nodules to fall between wild-type and super-nodulating plant nodule numbers. Super-nodulators typically have 6-10 fold more nodules than wild-type plants (Novák, 2010), so modifications to AON mutants could produce an optimised number of nodules for a given eCO_2 concentration. Any breeding program should also select for increased photosynthetic capacity to maximise on N investment from enhanced SNF. Forage legumes are an ideal target for super-nodulator incorporation as they represent a high reserve of photosynthetic and shoot growth capacity, as they have been selected for high green biomass production.

Breeding the super-nodulation phenotype into pasture species will require careful consideration of cultivar genetic background and phenotype. Natural variation in photosynthetic performance in pasture cultivars can be exploited to identify the most-responsive background for super-nodulating incorporation through studying eCO_2 responses of cultivars as has been done in ryegrass (Yiotis *et al.*, 2020). Collaboration with legume pasture breeding programs, such as the Consortium for Alfalfa Improvement, will ensure the super-nodulating phenotype is incorporated into cultivars with desirable traits such as drought hardiness, nutritional value and pest resistance among other complex traits (Bouton, 2012). N and C partitioning under eCO_2 will also be an important consideration and may vary by species and cultivar (phenotypic plasticity). Alterations to source and sink strength alone can impact yields (Qin *et al.*, 2023). Photosynthesis-derived carbohydrates are also involved in sugar sensing and signalling pathways, so additional C fixation at eCO_2 can alter signalling pathways, including in the roots where sugars cross-talk with hormones to regulate growth (Thompson *et al.*, 2017). No study has yet quantitively assessed the movement of C to nodules at aCO_2 and

eCO_2 in super-nodulating mutants, so further basic research into C and N partitioning in super-nodulating legumes grown under eCO_2 conditions is required.

The incorporation of super-nodulating legumes into mixed pasture swards should also be considered (Humphries, 2012). For example, in ryegrass pasture, adding N under eCO_2 increased yield (Schneider *et al.*, 2004), while eCO_2 conditions also increased N uptake in wheat (Butterly *et al.*, 2016). Supplementation of the pasture with additional N generated by SNF in super-nodulating legumes should improve photosynthesis and yield quantity and quality in non-legumes grown in the pasture. An important consideration will also be the susceptibility of the pasture to disease and pest herbivory. For non-legumes, eCO_2 can reduced leaf N content and increase C:N ratio (Ainsworth and Long, 2005), which reduces forage quality and can increase insect pest damage as the insects eat more to compensate for reduced N (Whittaker, 1999). For super-nodulators, the quality (N content) of legume forage crop will not be reduced, and increased pest feeding should not occur (Karowe, 2007; Lau *et al.*, 2008). There are conflicting reports however that herbivory and disease can increase in legumes under eCO_2 conditions, due to increased leaf sugar content and compromised defence systems (Hamilton *et al.*, 2005). Pathogenic disease risks may also be increased due to increased leaf area and biomass (Lam *et al.*, 2012). Further study into pest and disease impacts will be essential to ensure super-nodulating varieties can be incorporated into healthy, productive pastures.

In summary, the adaptation of super-nodulating legumes into pasture systems presents an opportunity to improve pasture productivity under future climates with elevated atmospheric CO_2 concentrations. Pasture legumes are ideally suited to maximise the benefits of super-nodulation as they are bred for green biomass and protein quality, two attributes that super-nodulating legumes increase beyond non-mutants in eCO_2 conditions. Enhancing SNF will also provide economic and environmental benefits, as the need for additional exogenous fertilisation will be reduced as super-nodulating legumes import nitrogen into grassland ecosystems through release of SNF-derived N. Increased SNF in pasture systems will also support increased grassland productivity without additional nitrous oxide emission (Barneze *et al.*, 2020), and may improve carbon sequestration in the soil (Soussana and Hartwig, 1995). Optimising the health and productivity of super-nodulating legumes will require additional research of super-nodulators responses to eCO_2 in field conditions, and better understanding of their disease susceptibility and any impacts from other environmental conditions, including drought and temperature.

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