Australian Grasslands Association

A partnership between the Grassland Society of Southern Australia and the Grassland Society of NSW

Australian Legume Symposium

William Angliss Conference Centre, Melbourne

February 8 and 9, 2012

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Australian Legume Symposium

Proceedings of an Australian Grasslands Association Symposium

Melbourne Australia

February 8 and 9, 2012

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The Grassland Society of Southern Australia Inc.

The Grassland Society of Southern Australia Inc. is the peak farmer organisation in southern Australia dedicated to the transfer of information and technology relating to temperate grasslands of clover and grass.

Formed in 1959, the Grassland Society of Southern Australia Inc. has branches in Victoria, South Australia, Tasmania and southern New South Wales. The Society welcomes new members (student, ordinary and corporate) with an interest in grassland farming. Pasture establishment, maintenance, utilisation, persistence and research are all key areas of interest for the 850 members of the Society, 50 per cent of whom are directly involved in livestock enterprises. The Society has a travel grant program, awards two student bursaries annually, runs an annual bus tour, publishes a bimonthly newsletter and holds and annual conference. Branches hold seminars and field days throughout the year.

The Grassland Society of New South Wales Inc.

The Grassland Society of NSW is the premier non-government organisation for transfer of information and technology relevant to pasture, grazing and land management in NSW. The Grassland Society of NSW was formed in March 1985 at a meeting of 28 interested people. The Society has 500 members and associates, 75 per cent of whom are farmers and graziers. The balance are agricultural scientists, advisers, consultants and executives or representatives of organizations concerned with fertilizers, seeds, chemicals and machinery.

The aims of the Society are to advance the investigation of problems affecting grassland husbandry and to encourage the adoption into practice of results of research and practical experience. The Grassland Society of NSW holds an annual conference to promote grassland farming and research publishes a quarterly newsletter and runs a number of field days, seminars and other activities through the regional branch network.

Australian Legume Symposium - Organising Committee

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Carol Harris - *Editor, NSW Department of Primary Industries, Glen Innes NSW*
Clare de Kok - *Adminstration, Grassland Society of Southern Australia,*
Stuart Kemp - *Scientific Advisor, Pasture Wise Pty Ltd*
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Introduction

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On behalf of the organising committee, I am pleased to present the proceedings of the 2012 Australian Legume Symposium, the inaugural venture of the Australian Grasslands Association. The collective power of the scientific, farming and extension community has been invited to participate in this forum to review the legumes sector of the pastures industry. This two day symposium aims to draw on that power to contribute to the pasture legume research and development of directions that should be pursued in the medium to long term.

The Australian Grasslands Association is a joint initiative of the Grasslands Society of Southern Australia and the Grasslands Society of NSW, with a view to increasing the involvement of the societies in the scientific world. This has been designed to ensure the societies are not distracted from their core activities providing para-scientific forums to transfer research and knowledge among the industry to support farm management.

The primary activity of the Association will be to facilitate a number of pasture research reviews over the next five years. These reviews will be on a different topic each year and will result in a series of technical symposia that will have a common set of objectives:

To provide a forum for researchers to: interact, exchange ideas and have meaningful discussion, present and publish their research and participate in planning the role of pastures in Australia's future

To set the agenda for research for the medium term: the next 10 years (i.e. develop industry wide investment priorities for use by funding bodies)

To consider the long-term "Blue sky" ideas that will push the boundaries – what and how do we take the next quantum leap?

The output of the symposium will be a white paper, written by the facilitators and presenters of the focus sessions within the symposium, capturing and characterising the topics addressed in the presentations and ensuing discussion. The credibility of all presented papers, the discussion and the summary paper will be leveraged by publishing in the peer-reviewed journal Crop and Pasture Science.

Why is this important?

Legumes were chosen as the starting point for this research series as the contribution of legumes to grasslands farming, animal production, to broader agriculture and to the Australian economy is sometimes overlooked.

As background, the benefits legumes confer to pasture growth through direct contribution and the nitrogen cycle (feeding pasture grasses), plus feed quality improvement and subsequent improvements in animal production are easily and commonly attributed to legumes.

Less often attributed to legumes are the benefits to subsequent grain and other crops, flowing from nitrogen fixation as well as other benefits derived from preceding pasture or ley phases and the business risk management benefits observed in recent years.

These benefits were identified very early in Australian agriculture, and plant collection and breeding programs were set up to provide continuous improvement to plant types and characteristics, particularly in the Mediterranean legumes (and to some extent tropical legumes) while our neighbours in New Zealand created a niche in the temperate legumes. At the same time commercial seed production capabilities have developed, which along with a cost of goods story have enabled Australasia to become a major supplier of legume genetics, via seed, to the world.

Despite the broad range of well-adapted species and varieties of legumes used in Australia, a series of droughts have multiplied the agents of pasture deterioration (including undergrazing or overgrazing at critical times, low fertility/pH, soil structure issues such as compaction, weeds, plant diseases and insect pests). This pressure has resulted in the contribution of legumes to animal and cropping businesses being scrutinized.

Given the recent pressures, and the gravity of the contribution of legumes to Australia's productive capacity, it is important that the industry maintains a focussed forum for discussion of specialised themes such as legumes, outside of the more broad agronomy conferences, where participants can share their knowledge and enthusiasm, present and publish their research, and perhaps more importantly to gain feedback from their peers and from end-users of legumes. Such an opportunity will support the maintenance of technical currency and competency in such specialised themes.

Reductions in public and levy funded pasture R&D, a dwindling supply of graduate and post graduate students and an aging (and declining) population of pasture scientists have all contributed to the isolation of pasture researchers. There are few opportunities for researchers to interact with others from similar or related fields in a formal environment and often funding and/or time constraints inhibit this activity at an informal level. At times there is also a tension between the need of researchers to publish and their need to complete projects and apply for the next source of funding that, increasingly, is resulting in less science being published.

Further, with the limited pools of funding being competitively sought after and the increasing prevalence of commercial priorities, there is little incentive for scientists to openly discuss their research and ideas for future research.

As a result pasture research is increasingly being conducted in isolated pockets with minimal exchange of ideas. This makes it difficult for the pasture industry to present to funding organisations a clear, well-grounded and broadly supported message about what the funding priorities should be.

To progress the pasture industry forward at a higher rate of improvement and in a more efficient manner there needs to be regular, structured, wide reaching reviews of the pasture industry. Too often a review of a particular part of the industry is only conducted in response to a strategic shift in the industry, occurrence of a market or research failure or a large project proposal being submitted. In many ways this is too late and a highly inefficient way of operating. If we are to keep developing and improving our pasture industries at a pace that is likely to match the demand for food and changing social and climatic conditions, then we need to conduct regular, critical reviews of its past, its current status, the successes and failures and where we need to go next.

Previous times the industry has met in a devoted forum to discuss legumes include the 1993 alternate pasture legumes conference. The outcomes of that forum have contributed to several of the papers being delivered during the symposium.

The mission of the Australian Grasslands Association is therefore to provide a regular series of reviews that will enable the pasture industry to promptly recognize the issues it faces and respond to them through either changes in existing research priorities or the creation of new priorities. To do this we intend to harness the collective power of the relevant scientific community in a forum that enables them to contribute directly to the review of the industry, the development of industry wide investment priorities and the setting of the research agenda for use by funding organisations.

Session 1 - Role of legumes in Australian farming systems

Temperate pasture legumes in Australia – their history, current use and future prospects

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Dr Phil Nichols has been a Senior Research Officer in pasture science with the Department of Agriculture and Food Western Australia since 1986. Prior to this, he spent two years with the NSW Department of Primary Industries at Yanco. Phil has active research interests in pasture legume breeding and selection, ecology and agronomy and has been involved in the release of 14 subterranean clover and 2 lucerne cultivars and the development of messina, a new pasture legume for saline land.

Abstract

Australian farmers and scientists have embraced the use of exotic pasture legume species more than any other country, with 36 annual and 13 perennial legumes with cultivars registered for use. Lucerne (*Medicago sativa*) and white clover (*Trifolium repens*) were introduced by the early European settlers and are still important perennial species in Australia. But several other species, notably annual legumes, have been developed specifically for Australian environments, leading to the evolution of unique farming systems. Among these the most successful species have been subterranean clover (*T. subterraneum*) and annual medics (*Medicago* spp.), while a suite of new species, including serradellas (*Ornithopus compressus* and *O. sativus*), biserrula (*Biserrula pelecinus*) and other *Trifolium* species, has expanded their range of adaptation. New perennial legumes have also been developed, initially to expand the range of legume adaptation in high rainfall pastures, and more recently to reduce groundwater recharge and the onset of dryland salinity. This paper reviews the origins and development of Australia's pasture legumes, their current use and the future prospects for new pasture legume cultivars.

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Nitrogen from Australian pastures

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John Angus worked as a research scientist in CSIRO Canberra from 1973 to 2010 and is now a CSIRO research fellow and an adjunct professor at Charles Sturt University in Wagga Wagga. With his wife Patricia, he also runs a dryland farm near Stockinbingal in southern NSW, producing cereals and canola on two-thirds of the area, and lamb and wool on the other one-third. His research interests are in the management of water, nitrogen and soil-borne diseases in dryland crop and pasture sequences. Originally from Victoria, he has a B.Agr.Sc and PhD from the University of Melbourne.

Abstract

Legume-based pastures, particularly those containing a large proportion of lucerne, have a prodigious capacity to fix N. Budgets of N show that permanent pastures, when growing with no management limitations, can supply more N than is removed in animal products and can eventually lead to excess soil N. For a mixed crop-livestock farm, a pasture phase occupying about 40% of the land area can maintain a stable N balance.

The actual performance of pastures on farms normally falls below the potential. The area or pasture continues to decrease in the wheat-sheep zone and, to a lesser extent, in the high-rainfall zone. The quality of pastures, as indicated by the area topdressed, the mean stocking rate and the input of superphosphate has decreased in the period 1990 to 2010. It is therefore likely that N fixation by pastures has decreased in the wheat-sheep zone and is static or falling slightly in the high-rainfall zone.

Evaluating the financial N contribution may help to reverse the decrease. In the case of permanent pastures, N fixation up to the demand for animal production gives is justified by 2012 prices and costs. A larger contribution comes when additional N is used by crops grown in sequence with the pasture. At 2012 prices and costs the estimated value of this contribution is 10-20% of the gross margin of a pasture.

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Session 1

Policy approaches and difficult choices to reduce nutrient losses from grazing systems in Australia

C.J.P. Gourley[A] and D.M. Weaver[B]

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Cameron has been working for 29 years in the area of soil, pasture and grazing animal research. He started his research career in the area of soil fertility and soil test interpretation in 1982. Cameron completed his PhD with the Department of Soil, Water and Climate at the University of Minnesota, USA in 1991. Cameron currently leads a state-wide group of Soil Science research and technical staff and is based at DPIV Ellinbank.

Abstract

The continued and growing reliance on external nutrient inputs to Australian grazing systems is likely to result in greater nutrient surpluses, inefficiencies in nutrient use and inevitably, greater leakage of nutrients from farms, which will in turn put more pressure on Australian inland and coastal water resources. The mix of land management regulations and government funded incentive schemes strongly implemented in the EU, USA and to a lesser extent New Zealand, which aim to reduce nutrient emissions from animal production systems and improve water quality, seem unlikely to be implemented in Australia in the near future. While there are some examples of regulatory policy approaches in Australia around important and impaired coastal and inland waters, most policy options involve voluntary schemes or self-regulation by industry, often including financial incentives to both industry organisations and farmers to off-set the costs of implementing BMPs. We conclude that diffuse pollution from grazing systems is not currently a major policy concern for government or industry at the national level, and other issues such as GHG emissions and irrigation water allocations have been given a much higher policy priority. Nevertheless, as our international markets become more aware of environmental impacts and purchased nutrients continue to increase in cost, governments and industry will need to ensure that our animal production systems strive to reduce nutrient emissions and increase nutrient use efficiency. Meeting these greater societal expectations for water quality and other services in the future will require somewhat varied and difficult choices to balance agronomic and environmental goals. Responses may vary from potential caps on nutrient inputs and productivity per hectare, re-positioning of higher input farms to more resilient parts of the national landscape, or accepting that there are unavoidable water quality consequences from livestock production.

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Lucerne's role in pastoral agriculture

J. H. Bouton

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Session 1

Professor Joe Bouton is a senior professor with the Samuel Roberts Noble Foundation in Oklahoma. The Noble Foundation is recognised as one of the USA's leading research facilities conducting plant science research and agricultural programs to enhance agricultural productivity regionally, nationally and internationally. Joe conducts breeding and genetics research on temperate forage species for use in pasture and livestock systems throughout southern USA. He has commercialised 17 cultivars, but is best know for releasing Alfagraze alfalfa, MaxQ tall fescue and Durana and Patriot white clover.

Abstract:

Pastoral agriculture is unique among the world's agricultural production systems. Lucerne's (a.k.a. alfalfa), *Medicago sativa* L. subsp. *sativa*, has a long history of playing a very important role in current pastoral agriculture that is now expanding to include new roles outside of traditional hay and grazing production systems such as sprouts for salads, nutritional supplements, and bioenergy feedstock, as well as being the forage legume of choice for delivery of new traits via biotechnologies. The use of biotechnologies in lucerne improvement will cause re-examination of research methods and will require unique collaborations that are both interdisciplinary and even cross-institutional. The Consortium for Alfalfa Improvement (CAI) is discussed as a model for this type of collaboration. Breeding programs will continue development of cultivars with the proper fall dormancy, a broad genetic base for pest resistance, increased local adaptation, persistence, and yield, but also adding new complex traits to these base traits. Increasing nutritional quality via down regulation of lignin genes, and increasing persistence via grazing tolerance, drought tolerance, and tolerance to acid, aluminum toxic soils are discussed as examples of the potential impact and challenges surrounding incorporation of complex traits. However, it is the future potential for lucerne to become a major part of tropical or sub-tropical production systems, or even an important adjunct to overcome deficiencies in the widely used perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) temperate systems, that begs further attention.

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Future trends in lucerne production and development for efficient livestock production in southern Australia

A.W. Humphries

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Dr Alan Humphries is the leader of the SARDI Pasture Science Program Area. Alan is SARDI's senior lucerne breeder and plays an active role in breeding lucerne with improved tolerance to acidity and grazing. Alan has a key interest in exploring new opportunities for lucerne in emerging farming systems, including the use of the wild relatives of lucerne to increase performance in drought conditions, intensive grazing systems, and in mixtures with other annual and perennial pasture species. Alan has released 5 cultivars.

Abstract

Lucerne is a deep-rooted herbaceous perennial legume with high levels of summer production and adaptation to a broad range of agro-ecological environments in southern Australia. The ability of lucerne to extend the growing season of winter-based pasture and respond quickly to rainfall after periods of drought makes it one of the most valuable plants in our feed base. However, for all the advantages of lucerne, it remains underutilised. Lucerne is often considered to be a specialty fodder crop, requiring careful management to achieve high levels of production and persistence. This paper investigates the opportunity of whole farm integration of lucerne; from specialty fodder crop to traditional pasture. The future trends of lucerne production in temperate grazing and intensive dairy systems are discussed in relation to breeding objectives identified to meet these demands. If lucerne is to be used more commonly as a pasture, the plant and systems must adapt. This paper investigates the plant traits and management principles that are important for growing lucerne in mixtures with other plants and improving the integration of lucerne into the whole farm plan.

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Session 2 - Current research to address gaps

a) Cropping zones

Challenges for annual pasture legumes in crop rotations.

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Dr Brad Nutt is a Senior Research Officer with the Department of Agriculture and Food Western Australia (DAFWA) based in South Perth. Brad achieved his Agricultural Science Degree at University of Western Australia from 1983-86 and completed his PhD at Murdoch University in 2008. He has 25 years experience pasture breeding, selection and agronomy in the mixed farming systems in WA. He started with Department of Agriculture in 1987 as a research officer based at Geraldton and from 1993 to 2000 worked as a pasture researcher for Centre for Legumes in Mediterranean Agriculture at the University of WA. Since 2000 Brad has been with DAFWA at research officer working on wheatbelt pasture systems, including work on a Libyan rangeland project from 2008 to 2011. He was the principle breeder behind the development of Cadiz, Eliza, Erica and Margurita French serradella, Santorini, Charano and Yelbini Yellow serradella and Prima gland clover.

Abstract

Fallows rich in pasture legume are important tools in the management of crop sequences, providing the multiple benefits of improved soil fertility, opportunity for the control of intractable crop weeds and high quality livestock fodder. Traditionally they have been based on self-regenerating annual legumes, subterranean clover and to a lesser extent annual medic, and its success is dependant on the legumes being able to maintain a viable soil seed reserve. However, the incentive to introduce and maintain legume based fallows within cropping rotations varies according to relative commodity prices and the cost and ease of applying alternative strategies to achieve the same benefit. The availability of relatively cheap nitrogen, herbicides and fungicides can substitute for the role of legumes in a fallow cycle. Also the two decades (1989 to 2009) of low returns from wool and sheep relative to cropping has shifted the priority for management to the control of weeds within a crop rotation at the expense of legume content in a rotational pasture. A new drive to increase legume content in ley pastures could be expected with the new phase of improved returns from livestock components of a mixed farming system. In some situations this will require the adoption of "legume friendly" rotational management and re-sowing where the legumes have failed to persist. A challenge for pasture legumes particularly where they need reintroduction is the need to keep costs of establishment and subsequent management within a crop rotation as low as possible.

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Re-exploring forage legumes in sub-tropical grain systems

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Abstract

The use of legume-based pastures in crop rotations has been common place in southern Australia, however similar systems have rarely been practised in Australia's subtropical regions. Cropping systems in this region are now being challenged by declining soil fertility, high fertiliser requirements and disease and pest pressures. Over 9 summer and 7 winter seasons we compared the productivity, water use and water-use-efficiency (WUE) and N-balance of both new and existing forage legumes suited to short rotations in subtropical grain systems with common annual grass forages (oats and forage sorghum). Production of winter-legumes was highly variable, but sulla (*Sulla coronarium*) produced more than oats in one season at 3 sites. Summer legumes, burgundy bean (*Macroptillim bracteatum*) and lablab (*Lablab purpureus*) performed consistently, though always producing less than forage sorghum. Lucerne extracted more water and had lower WUE than burgundy bean or lablab. . Based on soil N balance, lablab fixed the most atmospheric N (50-120 kg N/ha), significantly more than other species, which varied greatly as a result of existing soil N availability. Of the legumes tested we found that burgundy bean and lablab seem the most likely to be successfully integrated into cropping systems in the region. Further evidence of the rotational benefits provided by these legumes is required before farmers would be willing to accept the reduced productivity compared to grass forages currently used.

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Development of an early season barrel medic (*Medicago truncatula* Gaertn.) with tolerance to sulfonylurea herbicide residues

D.M. Peck and J.H. Howie

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Abstract

Sulfonylurea (SU) herbicides are extensively applied to crops in the cereal/livestock zones of southern Australia. In low rainfall areas with alkaline soils, SU residues can persist over summer and can severely affect sown or regenerating medic pastures. A cohort of early season barrel medics (*Medicago truncatula*) bred and selected for tolerance to SU herbicide residues, were evaluated at multiple field sites over three years (year of establishment and subsequent regeneration). Two lines (Z2438 and Z2415) were identified with dry matter production and seed yield in the establishment year equivalent to their recurrent parent, Caliph, an early maturing, aphid tolerant, barrel medic cultivar. They also had lower levels of hardseededness than Caliph, enabling them to regenerate in greater numbers in the following year and thus produce more dry matter. The two lines demonstrated good tolerance to simulated SU herbicide residues, producing up to ten times the dry matter of the SU intolerant parent Caliph. We anticipate that one or both of the two lines will be commercialised soon, enabling farmers in low rainfall areas with neutral-to-alkaline soils to successfully grow barrel medic pastures in the presence of SU herbicide soil residues resulting from applications to a prior crops..

Notes

How will pasture cope with increasing climate variability in the south-west of Western Australia

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Abstract

The south-west of Western Australia has already experienced a declining trend in annual rainfall and gradual warming. The distribution of rainfall has also changed with relatively lower autumn rainfall, patchy breaks to the season and shorter springs. This has important implications for the productivity of legume pastures in this region, dominated so strongly by annual species, particularly subterranean clover (*Trifolium subterraneum*), French serradella (*Ornithopus sativus*) and annual medics (*Medicago* spp.). For annual pasture legumes, appropriate patterns of seed softening and germination behaviour, efficiency of phosphorus and potassium uptake and drought resistance of seedlings and mature plants are regarded as important characteristics. While these traits can be targeted in pasture breeding programs, it will also be important to exploit system opportunities to optimise the annual legume component of the feed base. This may take the form of incorporating strategic shrub reserves and grazing crops to allow for pasture deferment in autumn/winter. The perennial growth form may become more important in this context and this is discussed in terms of the development of the drought tolerant perennial legume tedera.

Notes

Session 2 - Current research to address gaps

b) Permanent pasture zones

Challenges for legumes in permanent pastures of the high rainfall zone

G. A. Sandral

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Graeme Sandral is a Pasture Ecologist with the NSW Department of Primary Industries based at Wagga Wagga. Graeme has led several research projects including plant breeding and selection efforts in the acid soils component of the National Annual Pasture Legume Improvement Program. Graeme has also been an active project leader in the Salinity CRC and now Future Farming Industries CRC. In these roles Graeme initiated and led plant breeding efforts in the Lotus genus. Graeme is currently completing a PhD through the University of Western Australia. His PhD research focused on rare and endangered perennial Lotus species endemic to the Macronesian Islands (Cape Verde, Canary Islands, Maderia's and Azores).

Abstract

The three most commonly used legumes (subterranean clover, white clover and Lucerne) in perennial grass based pastures of the high rainfall zone in Australia have significant limitation when it comes to the stable production of fodder and nitrogen. Some of these are intrinsic to the plant such as the variable nature of subterranean clover production which is governed by rainfall events particularly the break of season. Other limitations relate to plants weaknesses such as lack of drought tolerance in the case of white clover or a lack of acid soil tolerance in the case of Lucerne. These factors limit either the placement of these legumes in the landscape or the reliability with which they supply fodder and nitrogen in grass based pastures. Consequently many grass based systems in the high rainfall zone are limited by the amount and reliability of nitrogen supply.

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Legumes are the best option for improving returns from sown pastures in northern Australia.

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Abstract

Productivity decline in sown grass pastures is widespread in northern Australia and reduces production by approximately 50%. The economic impact of the decline is estimated at over $17B at the farm gate over the next 30 years. Buffel grass (*Pennisetum ciliare*) is the most widely established sown species in northern Australia (>75% of plantings) and has been estimated to be "dominant" on 5.8 M hectares and "common" on a further 25.9 M hectares of Queensland. The decline in pasture productivity with age is directly attributable to a lack of available nitrogen in the soil as the nitrogen and other nutrients become 'tied-up' in soil organic matter, roots and crowns of old grass plants. This lack of available nitrogen limits dry matter production and may be exacerbated by overgrazing that leads to reduced pasture condition and land degradation. Whole farm economic analysis compared twelve strategies that industry has used to increase productivity on ageing buffel grass pastures across four districts of inland Queensland. Legumes are the most cost effective mitigation option and can reclaim 30-50% of lost production providing whole farm returns of up to $1,300/ha over 30 years, and benefit:cost ratios of 4 -10. However, commercial use of legumes has achieved mixed results with notable successes but many failures. There is significant opportunity to improve commercial results from legumes using existing technologies, however there is a need for targeted research to improve the reliability of establishment and productivity of legumes.

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Improving white clover for Australasia

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Abstract

Improving the genetic potential of temperate forage legumes ensures Australasian pastoral industries can increase production, profitability and sustainability. Lifting the rate of genetic gain by integration of molecular tools, innovative conventional breeding strategies, and genetic resources is the major objective of our white clover breeding programme. Our forage plant breeders are supported by a range of underpinning research activities including; germplasm exploration and enhancement, plant physiology, plant health, forage quality, agronomy, quantitative genetics and biotechnology; and have collaborative interfaces with animal performance and farm systems science. This paper focuses on some of the key achievements of our white clover breeding research and development, and the success and future of the New Zealand/Australia trans-Tasman collaboration to generate new cultivars for Australian environments.

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Breeding of an early flowering and drought tolerant *Lotus corniculatus* variety for the high rainfall zone of southern Australia

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Abstract

In the high rainfall zone of Australia (HRZ > 600 mm) most pasture systems are dominated by perennial grasses which have low levels of inter-dispersed legume. Numerous authors have shown that legume content of between 20 and 50% is required to maximise livestock production. Consequently legume content of these systems needs to be increased if livestock production is to be improved. Perennial legume options such as lucerne and white clover are limited in their application in this zone due to lucerne's sensitivity to acid soils (<4.8 $CaCl_2$) and waterlogging and white clovers inability to survive most of the annual summer droughts. To address this problem a breeding program was undertaken using the species *Lotus corniculatus* to develop varieties suitable for the HRZ of southern Australia. In the first cycle, 365 populations of *L. corniculatus* were screened in nurseries to select the best 62 plants from the best populations at Bakers Hill and Medina in Western Australia. These selections were subsequently grown as half-sib families in spaced-plant nurseries at Waroona and Bakers Hill. In the second cycle 61 individuals were selected from the Bakers Hill and Waroona sites and hand crossed to produced 3160 plants from 202 pair-crosses which were gown as spaced plants at the University of Western Australia Field Station in Shenton Park. In the third cycle the polycross for three populations (YF, T and F) was made up of plants selected from 3160 plants and two plants that had survived four years on a non-wetting sand at Bakers Hill, which included a significant drought year in 2006.

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An overview of dryland legume research in New Zealand

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Abstract

With limited funds, and the relatively low importance of dryland pastures in New Zealand, research has been targeted at the species most likely to induce transformational change on-farm. Lucerne research into biophysical influences on plant growth and development has added flexibility to spring grazing management. Coupled with additional agronomic research and extension, farmers now have the confidence to use lucerne as a direct feed source for sheep, beef and deer. Research on Caucasian clover identified the long duration to secondary leaf production as the physiological basis for poor clover performance in mixed swards. Despite agronomic strategies to overcome this, its use is now limited by commercial constraints. A 10 year 'Maxclover' grazing experiment at Lincoln University demonstrated the superiority of subterranean clover with cocksfoot over perennial ryegrass and white clover for pasture persistence, quality and animal performance. Pastures with high legume content had higher water use efficiency and produced greater animal and pasture production. Balansa and gland clovers both show a strong influence of photoperiod on time of flowering, which suggests they may be suitable for oversowing into areas of winter wet and summer dry hill and high country. Further research into their ecological niche and ability to regenerate each autumn is required. For all legumes, the role of inoculation requires further research with recent results suggesting indigenous, rather than commercially introduced, bacterial populations are dominant in root nodules. Uptake of dryland pasture species for on-farm use has only been successful when research, extension and agribusiness interests have been aligned.

Notes

Session 3 - Research solutions for specific problems

Factors affecting N_2 fixation by pasture legumes

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Mark Peoples is Deputy Chief of CSIRO Plant Industry in Canberra. For over 25 years Dr Peoples has undertaken farming systems research in cropping and forage systems that has aimed to either identify the key factors regulating inputs of biological N-fixation by legumes, or determine the relative impact that broadleaf break crops, pastures and agronomic practices can have on subsequent root growth and plant yield through affects on soil N or water availability, soil microbial composition and soil structural attributes. This work has resulted in over 200 peer-reviewed publications.

Abstract

The amounts of foliage nitrogen (N) fixed by various annual and perennial legumes growing in Australian pastures can range from <10 to >300 kg N/ha per year. Differences in N_2 fixation result from variations in the proportion of the legume N derived from atmospheric N2 (%Ndfa) and/or the amount of legume N accumulated during growth. The levels of %Ndfa are most heavily influenced by (i) the number of rhizobia in the soil and relative effectiveness of those strains, and (ii) the concentrations of soil mineral N or applications of N fertiliser (in dairy pastures), but can also be dependent upon (iii) the legume species or cultivar present. The accumulation of legume N relates primarily to the legume content and net productivity of the pasture. On average 20 kg of shoot N can be expected to be fixed for every tonne of legume herbage dry matter produced. Strategies with the potential to improve N2 fixation inputs include (i) sowing new legume species and host-rhizobial strain combinations that either have agronomic advantages over subclover and annual medics, or are more tolerant of hostile soil environments, (ii) the amelioration of nutritional constraints to legume growth, and (iii) the use of lucerne to offset the year-to-year variability in productivity and N2 fixation commonly experienced by annual legumes.

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Factors that contribute to poor survival of rhizobia on preinoculated legume seed

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Abstract

The increase of sown legumes in Australia provides an opportunity to exploit the beneficial role of rhizobial inoculants maximising the productivity and increasing nitrogen input in farming systems. Rhizobial strains are continually selected for newly introduced and existing legumes ensuring that farmers have access to highly effective N2-fixing rhizobia when purchasing legume inoculants. It is essential that the process of inoculating seed is not detrimental to rhizobial viability prior to sowing.

Preinoculation of seed is a convenient alternative method to inoculating seed on-farm. With preinoculation, a range of plant-growth and protection agents, polymer adhesives, colour pigments or dyes and powder materials are incorporated into an inoculant slurry prior to seed coating.

However, our recent point-of-sale surveys support findings of previous studies that survival of rhizobia on preinoculated seed is variable and can be generally poor.

We focused our research on some of the factors that may contribute to poor survival of rhizobia on commercially produced preinoculated seed. Experiments at commercial facilities and in the laboratory examined factors affecting survival. We found that rhizobial survival was affected by water quality, polymer, rate of change in water activity, rhizobial strain and inoculant maturity.

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Session 3 - Breeding for specific problems and traits

a) Breeding for specific problems and traits

Boron tolerance in annual medics (*Medicago* spp.)

J. H. Howie

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Abstract

Boron is present at toxic levels in the subsoils of much of the semi-arid south-eastern Australian cereal-livestock zone. Boron toxicity is typically associated with alkaline soils, where annual medics (*Medicago* spp.) are generally the best adapted pasture legume. New medic cultivars have been developed for which there is no published boron tolerance information. Five species of annual medic represented by 13 cultivars were grown in soil amended with boron and evaluated for boron tolerance. A rating system based on expression of symptoms was modified from earlier research. There was a wide range of response to boron, both between and within species. Cultivars varied widely in their expression of symptoms: from showing no or few leaf symptoms (tolerant) to significant leaf necrosis (very sensitive). Earlier research findings for these and other medics are also presented to provide an integrated summary of both published and unpublished data and thus provide a comprehensive and up-to-date comparison between different species and most commercialised cultivars. This information will be useful for plant breeders, agronomists and farmers who manage soils with high boron levels.

Notes

Session 3

Spatial and temporal variation in plant-available manganese (Mn) concentrations and the impact of Mn toxicity on seedling lucerne

R.C. Hayes, M. K. Conyers, G. D. Li, G. J. Poile, A. Price, B. J. McVittie, M. J. Gardner, G. A. Sandral and J. I. McCormick

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Abstract

Spatial and temporal variation in plant-available manganese (Mn) was observed at two field sites in southern NSW, Gerogery and Binalong, over a 12-month period. At a given sampling, concentrations of plant-available Mn in the surface 0.2 m varied by up to 261% (2.5-9.7 mg/kg) and 182% (8.7-24.6 mg/kg) across the Gerogery and Binalong sites, respectively. The concentration of Mn in a given plot also varied by up to 175% when different sampling times were compared at both sites. There was little consistency between sites for the peaks and troughs of available Mn. However, in both instances the peaks occurred during months in which newly-sown lucerne swards might be emerging in southern NSW; April-May for an autumn planting or August for a spring planting. A subsequent pot study revealed that high rates of available Mn reduced lucerne seedling survival by 35%, while shoot growth was inhibited by 19% on the plants that did survive. Though recent studies have looked to select lucerne for improved performance under low pH/ high Al conditions, no effort has yet been made to incorporate tolerance of Mn toxicity in the elite lucerne germplasm. This paper discusses the importance of Mn toxicity in the development of acid soil tolerant lucerne germplasm, and the opportunities that exist to address this gap through breeding and selection.

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Bean leafroll virus is widespread in subterranean clover (*Trifolium subterraneum* L.) seed production fields and can be persistently transmitted by bluegreen aphid (*Acyrthosiphon kondoi* Shinji)

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Abstract

In the mid 2000's subterranean clover (*Trifolium subterraneum*) seed producers in South Australia reported symptoms of a red-leaf disease in fields with reduced seed yields. The red-leaf symptoms resembled those caused by several clover infecting viruses. A set of molecular diagnostic tools were developed for the following viruses which are known to infect subterranean clover: Alfalfa mosaic virus (AMV); Bean leafroll virus (BLRV); Beet western yellows virus (BWYV); Bean yellow mosaic virus (BYMV); Cucumber mosaic virus (CMV); Pea seed-borne mosaic virus (PSbMV); Soybean dwarf virus (SbDV) and Subterranean clover stunt virus (SCSV). Surveys of subterranean clover seed production fields in 2008 in the south-east of South Australia and western Victoria identified BLRV, AMV and CMV, with BLRV the most widespread. Surveys of pasture seed production fields and pasture evaluation trials in 2009 confirmed that BLRV was widespread. This result will allow seed producers to determine whether control measures directed against BLRV will overcome their seed losses. Bluegreen aphid (BGA, Acyrthosiphon kondoi) was implicated as a potential vector of BLRV because it was observed to be colonising lucerne plants adjacent to subterranean clover seed production paddocks with BLRV, and in a glasshouse trial it transmitted BLRV from an infected lucerne plant to subterranean clover in a persistent manner.

Notes

Tolerance of pasture legumes to a new virulent bluegreen aphid (*Acyrthosiphon kondoi* Shinji) found in south-eastern Australia.

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Abstract

A new bluegreen aphid biotype (BGA, *Acyrthosiphon kondoi* Shinji) has been found in Australia that causes severe damage and mortality in seedlings of previously tolerant pasture legume cultivars. Populations of BGA collected at Urrbrae and Binnum, SA in 2009 caused 100% mortality in 40 cultivars of annual and perennial *Medicago* spp. and annual *Trifolium* spp.. Delaying inoculation from the trifoliate to the 6–8 leaf stage and removing susceptible genotypes from experiments had no impact on reducing mortality from 100% in previously resistant barrel medics. A half sib family of lucerne from the SARDI breeding program has maintained resistance to the Urrbrae 2009 BGA.

A detailed study of the virulence of BGA populations collected from Toowoomba (Qld), Tamworth, Howlong (NSW), Launceston (Tas.), Colebatch, Kimba, Urrbrae and Vivonne Bay (SA) in 2010–2011 on 33 pasture legumes provides evidence of new virulent BGA being widespread, despite these populations causing less severe damage and mortality than the two populations collected in 2009.

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Performance of annual legumes grown with and without lucerne

G.A. Sandral[A], B. J. Nutt[B], R. C. Hayes[A], G. D. Li[A], and J. M. Virgona[A].

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Abstract

Bioeconomic modelling (using MIDAS -Model of an Integrated Dryland Agricultural Systems) of a typical farm in southern NSW receiving 450 to 550 mm of average annual rainfall has shown that when the pasture phase includes lucerne (*Medicago sativa*) with annual pasture species farm profit was increased by an estimated 30% (Dear *et al.* 2010a) above that achieved when the pasture consisted of annual species alone. This is supported by various studies summarised by FitzGerald *et al.* (1980) that show animal production increases of 9 to 128% when lucerne based pastures were compared with annual-only systems. The inclusion of perennials, however, has been shown to reduce seed reserves of subterranean clover (*Trifolium subterraneum*) (73 to 84%, Dear *et al.* 2003), seedling emergence (77%, Dear *et al.* 1998) and seedlings survival (78% Dear *et al.* 1998). The current study compares a range of annual legumes including subterranean clover for their ability to produce and survive in the presence of lucerne. The findings indicate that when grown with lucerne, annual legume dry matter production and seed yield is reduced and that the descending order of dry matter performance for the top 10 treatments examined was Annual Mixture, *T. hirtum, T. globosum, T. cherleri, T. pauciflorum, T. resupinatum, T. arvense, T. pallidum, T. echinatum,* and *T. subterranean*. Results also indicated that *T. pauciflorum* and *T. globosum* ranked higher in both dry matter production and seed reserves than *T. subterraneum* when grown with lucerne and that *T. subterraeum* was inferior to most alternative species tested in its capacity maintain seed reserves in the presence of lucerne.

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Session 3 - Breeding for specific problems and traits

a) Molecular breeding

Transgenic white clover (*Trifolium repens* L.) plants with modified organic acid biosynthesis for aluminium tolerance

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Abstract

Acidic soils are estimated to occur on more than 40% of the Earth's land area. Solubilization of aluminium ions (Al^{3+}) in acidic soils inhibits root growth in many crop plants and leads to the formation of insoluble Al-P complexes that limit phosphorus availability. Some plants have a natural mechanism of Al tolerance involving the secretion of organic acids (OA), such as citric, oxalic and malic acid, which are able to chelate Al^{3+} in the rhizosphere ameliorating its toxic effects and liberating bound phosphorus from Al-P complexes. We have used a transgenic approach to up-regulate organic acid biosynthesis and their secretion from roots of white clover plants, aiming to enhance Al tolerance and P acquisition efficiency in acidic soils. Transgenic white clover plants expressing chimeric genes encoding white clover nodule-enhanced malate dehydrogenase, phosphoenolpyruvate carboxylase and citrate synthase coding sequences under the control of constitutive (enhanced CaMV35S) and root-specific (white clover phosphate transporter TrPT1) promoters were generated and analysed at the molecular and phenotypic levels. Screening transgenic plants grown hydroponically enabled the identification of transformation events that exhibit a higher level of Al tolerance than corresponding isogenic non-transgenic controls. Selected transformation events will be subjected to detailed, quantitative characterisation of root growth dynamics under different P and Al treatments using a plant phenomics platform.

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Biserrula pelecinus L. - genetic diversity in a promising pasture legume for the future

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Abstract

Biserrula pelecinus L. is a Mediterranean annual pasture legume and performs best on well-drained sandy loams and medium loams with a pH 4.5 – 7. Diversity analysis of germplasm collection of 279 accessions using 18 agro-morphological traits and 22 eco-geographical data of the collection sites and AFLP markers was conducted to develop a core collection of ~10% of the original collection. This core collection well represented the diversity of the whole collection. In a previous comparison among mainly legume pasture species there was significant variation in *in vitro* methane production, with *B. pelecinus* showing particularly low methanogenic potential. We hypothesised that the core collection of *B. pelecinus* has variation in fermentability traits. The biserrula core comprising 30 accessions from seven different countries with the checks subterranean clover and bladder clover were examined for variability in *in vitro* rumen fermentation, including methane production by rumen microbes, and possible links of these traits to plant morphological characters. There was significant variability in fermentability profiles among accessions. Methanogenic potential of the accessions was 90% heritable (broadsense). Although all the accessions tested showed low gas pressure and methanogenic potential, they sustained volatile fatty acid (VFA), which indicates low digestibility and high nutritive value. In conclusion, significant variation exists in the fermentability traits within the core collection of *B. pelecinus*. These traits may be used in selection for an environmentally friendly cultivar of *B. pelecinus*.

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LXR™ transgenic white clover plants (*Trifolium repens* L.) with delayed leaf senescence, increased seed yield and improved stress tolerance

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Abstract

Leaf senescence is a developmental process that allows plants to metabolize macromolecules in leaves that are no longer needed for photosynthesis and to recover metabolites, including amino acids and nucleic acids, for use in actively-growing tissues or storage organs. Cytokinins are a class of plant hormones known to inhibit leaf senescence. Transgenic LXR™ white clover plants expressing the *isopentenyl transferase* (ipt) gene from *Agrobacterium tumefaciens* involved in cytokinin biosynthesis under the control of the AtMYB32 promoter were generated. LXR™ white clover plants exhibited delayed leaf senescence under glasshouse and field conditions, in comparison with leaves from non-transgenic control plants. In contained growth environment and field trials, these LXR™ white clover plants also exhibited enhanced seed yield, improved recovery from drought stress, and enhanced herbage quality in comparison with non-transgenic control plants.

Notes

Biosafety studies for the release of alfalfa mosaic virus resistant transgenic white clover (*Trifolium repens* L.)

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Abstract

Transgenic white clover plants ectopically expressing a chimeric gene enconding the AMV coat protein (*AMVCP*) were shown to be resistant to alfalfa mosaic virus under glasshouse and field conditions. Experimental transgenic white clover varieties with AMV resistance were developed. Biosafety studies for the release of AMV resistant transgenic white clover varieties were undertaken. These included the detailed sequence characterization of the transgene locus, *AMVCP* and *npt2* transgene expression analysis (at RNA and protein level) in different plant organs and development stages of containment glasshouse- and field-grown transgenic plants, comparative assessment of natural toxicants and key nutritional parameters in transgenic plants and non-transgenic control plants, as well as comprehensive metabolic profiling. The comparative assessment of cyanogenic glucosides, phytoestrogens and saponins showed that the levels of these natural toxicants in transgenic white clover plants were within the range detected in non-transgenic plants grown under equivalent conditions. Transgene detection assays based on Real-Time PCR were designed for a range of samples including fresh and dry herbage, hay, pollen, seeds, pollen content in honeybees and honey. Furthermore gene flow in white clover was studied under Australian field conditions. A field trial utilizing a dominant phenotypic marker (red leaf pigmentation) showed that gene flow, primarily via pollination by honeybees, dramatically decreases with increasing distance between flowering plants and conforms to a leptokurtic pattern.

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POSTER PAPERS

Perspective on forage legume systems for the southern Great Plains of USA

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Abstract

Legumes have tremendous potential to fix N to non-legume crops, and to improve forage production, seasonal distribution, nutritive value, and soil structure and fertility in forage systems. Several medics and clovers are compatible and can be established with tall fescue, however, due to limited precipitation, annual legumes have not reliably regenerated in permanent grass swards. Hairy vetch has consistently established in perennial grass swards, although it required annual establishment. Hairy vetch was not as economical as N fertilizer in the perennial grass (bermudagrass and tall fescue) systems, but it was the primary component in the annual (rye-ryegrass) system, which was profitable, similar to 112 kg N ha-1-fertilizer. Alfalfa may have the greatest potential in cool-season perennial grass systems in the southern Great Plains, however, the shortened stand life under grazing will limit its adoption. There is a need for greater germplasm and rhizobia evaluation as well as a need for seed production, legume establishment, and grazing research to improve farmer/rancher adoption of legumes.

Key words: legume species, economics, pastures, dry climates

Introduction

Legumes have tremendous potential to fix N to non-legume crops, and to improve forage production, seasonal distribution, nutritive value, and soil structure and fertility in forage systems (Howieson *et al.* 2000). Legumes are inherently appealing to those seeking agricultural systems that are independent of constant outside inputs (Pearson 2007). In warm climates, however, legumes have not been widely adopted by farmers/ranchers (Thomas and Sumberg 1995). In drier climates subject to temperature extremes, adoption of legumes in forage systems is particularly difficult (Muir *et al.* 2011). In order for legumes to be successful in forage systems, there are several criteria that must be met. First, legumes must be well-adapted to the environment. Second, they must establish easily, be compatible with grasses, and be grazing-tolerant. Finally, they must be economically superior to the alternative or, in most cases, the standard commercial N fertilization system.

Germplasm evaluated

Over a period of several years, a number of legume species have been evaluated for productivity, adaptation, and persistence for the southern Great Plains. These can be divided into several categories. Legumes in the southern Great Plains can grow in either summer or winter but rarely can the same species grow during both seasons. Cool-season perennials usually act as short-lived annuals and typically die out in the hot, dry summer following establishment. A number of cool-season annual legumes have been evaluated at various locations in the extreme southern Great Plains, including Vicia spp., Trifolium spp., and *Medicago* spp. (Muir *et al.* 2005). Systematic evaluation of a wider range of medic germplasm has taken place more recently at The Noble Foundation in southern Oklahoma. All available accessions (1231) from six different species, black medic (*M. lupulina*, 245 accessions), spotted burr medic (*M. arabica*, 80 accessions), Tifton burr medic (*M. rigidula*, 159 accessions), rigid medic (*M. rigiduloides*, 195 accessions), button medic (*M. orbicularis*, 337 accessions), and little burr medic (*M. minama*, 215 accessions), were obtained from GRIN (http://www.ars-grin.gov/) or collected locally and evaluated for adaption (freeze tolerance, winter drought tolerance, forage production, and seed production) for the southern Great Plains (Butler *et al.* 2011a). We concluded that button medic followed by rigid medic had the greatest potential for the region. In addition, large seeded hairy vetch and field pea were the only legumes to be successfully established in existing tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort] swards (unpublished data), therefore evaluations were expanded for common vetch (595 accessions), hairy vetch (*V. villosa*, 87 accessions), field pea (P. sativum, 50 accessions). Based on these observations, it was determined that common vetch is susceptible to freeze damage and is not well adapted. Field pea, although well adapted for forage production, is susceptible to powdery mildew and does not produce seed consistently in this region. Hairy vetch offers the greatest potential for interseeding in existing swards since it appears to have adequate seedling vigor and drought tolerance. Persistent populations of naturalized exotic (introduced) warm-season perennial legumes are basically non-existent in the southern Great Plains. Tropical or sub-tropical annuals produce well in growing seasons with abundant and evenly distributed rainfall or under irrigation but rarely reseed themselves (Muir *et al.* 2008). Several native (trailing wild bean - *Strophostyles helvula*, smooth-seeded wildbean – *S. leiosperma*, and bundlflowers – *Desmanthus illinoensis*) and introduced (lablab – *Lablab purpureus*, cowpea – *Vigna unguiculata*, mungbean – *V. radiata*, burgundy bean - *Macroptilium bracteatum*, Korean lespedeza - *Kummerowia stipulacea*, and soybeans - *Glycine max*) legumes have been evaluated, however, regrowth after defoliation from grazing has been disappointing (T.J. Butler, unpublished data).

Establishment and compatibility with grasses

Guretzky *et al.* (2012) reported that annual medics (button, little burr, rigid, and Tifton burr) and clovers (arrowleaf – *T. vesiculosum*, crimson – *T. incarnatum*, and rose – *T. hirsutum*) could successfully be established along with tall fescue. Hairy vetch and field peas, however, were too competitive to the tall fescue seedlings. Butler and Malinowski (2012) reported that summer legumes did not regenerate in any of the three years or two locations evaluated, and reseeding of the winter annual legumes was negligible in the first two seasons due to limited rainfall. In the third season when autumn moisture was sufficient to allow for reseeding, it was reported that button medic (58%) and little burr medic (55%) had the greatest percentage of stand at the first location and arrowleaf clover (49%) and Tifton burr medic (54%) had the greatest percentage of stand and reseeding potential at the second location. Butler *et al.* (2011c) reported that alfalfa and tall fescue could successfully be established in alternating drill rows and a combination of alternating and perpendicular row orientations (referred to as checkerboard pattern), while binary mixture and perpendicular only orientations resulted in excessive competition. They found the combination of alternating and perpendicular planting orientation (checkerboard pattern) offered the best potential to minimize preferential grazing while maintaining adequate stand density and persistence in the southern Great Plains. Results focusing on inter-seeding native herbaceous perennial legumes into existing grass swards have not been promising (Muir and Pitman 2004).

Economics of grazing systems

Grazing monoculture stands of alfalfa with stocker calves in summer is profitable ($314 ha-1) (Butler *et al.* 2012a). However, cumulative profits from grazing monoculture alfalfa may not be as profitable as alfalfa for hay production since the stand life is often reduced by grazing. In this and other studies (data not shown), alfalfa has persisted 3 years under grazing and 4-5 years when harvested as hay. Biermacher *et al*. (2012) reported summer stocker grazing was more profitable when bermudagrass was fertilized with 112 kg N ha-1 ($212 ha-1) compared to bermudagrass-alfalfa ($86 ha-1) mixture. Biermacher *et al.* (2012) also reported that grazing a bermudagrass-vetch-clover system ($130 ha-1) was less profitable than bermudagrass fertilized with N fertilizer during a three-year study. Interrante et al. (2012) reported that the net returns for tall fescue-vetch-clover-pea system ($93 ha-1) was less profitable than tall fescue fertilized with 112 kg N ha-1 ($224 ha-1). However, despite this low economic benefit of legume incorporated- compared to N fertilized-systems, another study reported that the net returns of rye-vetch-clover-pea ($229 ha-1) was similar to net returns of a rye-ryegrass system ($282 ha-1) fertilized with 112 kg N ha-1 (Butler *et al.* 2012b).

Ongoing and future research

Due to the tremendous economic potential and the severe limitations of legumes in forage systems, there is great opportunity for researchers to develop legume-inclusive regenerative, semi-closed forage systems on which the future of the southern Great Plains ruminant production depends (Pearson 2007). One priority should be to evaluate a larger number of rhizobia and legume germplasm (Howieson *et al*. 2000). Despite the fact that this approach will likely require more collection trips and funding, there exists a potential long-term sustained benefit in the future. Developing viable weed control options for forage legumes is needed since there are such few options (Butler *et al*. 2011b). More emphasis is needed on research targeting improving seed production potential of these species. Under grazed conditions, increased seed production may improve reseeding potential, while if grown for seed production, high seed yields will make seed for forage establishment more readily available and less expensive. Improved establishment techniques are also needed, especially in mixtures with warm-season grasses. Improvement in grazing management (rotational grazing) could improve systems, but combining various systems will likely provide the greatest advantage.

Conclusions

Several medics and clovers are compatible and can be established with tall fescue, however, due to limited precipitation, annual legumes have not reliably regenerated in permanent grass swards. Hairy vetch has consistently established in perennial grass swards, although it required annual establishment. Hairy vetch was not as economical as N fertilizer in the perennial grass (bermudagrass and tall fescue) systems, but it was the primary component in the annual (rye-ryegrass) system, which was profitable and similar to N-fertilized system. Alfalfa may have the greatest potential in cool-season perennial grass systems in the southern Great Plains, however, greater management may be needed. There is a need for greater germplasm and rhizobia evaluation as well as a need for seed production, weed control, and grazing research to improve farmer/rancher adoption of legumes. Future research should improve establishment of legumes and develop management systems that extend the life of legumes in forage systems with appropriate economic analysis for farmer adoption.

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Annual and biennial legume evaluation in northern New South Wales

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Abstract
Spring herbage mass of 48 annual/biennial legumes was evaluated in ungrazed plots at the Tamworth Agricultural Institute on a Red/Brown Chromosol soil from 2003 to 2005. Entries that were not significantly different to those with the highest herbage mass were *Trifolium purpureum* (pur-A and 139465) and *Hedysarum flexuosum* (SA 35361 and SA 35362) in 2003; *H. coronarium* cvv. Necton, Aokau and Sparacia, and *H. carnosum* (SA 35358) in 2004, and *H. coronarium* cvv. Necton and Aokau, *Medicago truncatula* cv. Jester, H. carnosum (SA 35358) and both *H. flexuosum* lines in 2005.

Key words: *Medicago*, *Lotus*, *Ornithopus*, *Biserrula pelecinus*

Introduction
On the North-West Slopes of New South Wales (NSW), subterranean clover (*Trifolium subterraneum*) is traditionally the dominant annual legume sown on Chromosol soils, while annual *Medicago* spp. occur naturally and are best adapted to the heavier textured, alkaline Vertosols, with lucerne (*Medicago sativa*) the most commonly sown perennial legume grown across a range of soil types (Lodge *et al*. 1991). In this region, national plant evaluation programs, funded either through the National Annual Pasture Legume Improvement Program (NAPLIP) or the Co-operative Research Centre for Plant-based Management of Dryland Salinity, have evaluated a range of perennial legumes (Li *et al*. 2008), alternative *Medicago* subspecies (Li *et al*. 2010a) and chicory lines and cultivars (Li *et al*. 2010b), as well as a wide range of annual, biennial and perennial legume material (Boschma *et al*. 2011a, b). However, there remains a range of material from the genera *Trifolium*, *Lotus*, *Medicago*, *Trigonella*, *Ornithopus*, *Biserrula*, and *Hedysarum* that requires further testing in the predominantly summer rainfall environment of northern NSW.

This paper reports the spring herbage mass (HM, kg dry matter (DM)/ha) of 48 entries from the above genera for a three-year period, and compares and contrasts their performance.

Methods
The Tamworth Agricultural Institute (TAI), is located 5 km south-east of Tamworth, NSW (31°09' S; 150°59' E; elevation 434 m; average annual rainfall at Tamworth, 672 mm). Rainfall data were recorded daily at an automatic weather station located 500 m from the experimental area. The site was located on a Red/Brown Chromosol soil (Isbell 1996) in a paddock (~5 ha in area), with a long history (>50 years) of crop and pasture rotations (mainly winter cereals and lucerne). In the six months prior to sowing, the area received five applications of 1–2 L/ha of glyphosate (450 g a.i./L) and four weeks before sowing 1.7 L/ha of trifluralin (400 g a.i./L) was applied and incorporated by harrowing.

Forty eight annual and biennial legume entries were sown in plots (2 x 4 m) on 9 May 2003 in a spatially adjusted randomised complete block design with three replicates. Fifteen of these entries were previously evaluated in regional studies (Boschma *et al*. 2011b) including four lines that were subsequently released as cultivars. Molybdenumised single-superphosphate (8.8% phosphorus (P), 11% sulphur (S), 0.05% molybdenum) was broadcast at sowing (250 kg/ha) and single-superphosphate was applied in the second and third years (150 kg/ha) of assessment. Sowing rates for each entry were adjusted for seed size and germination percentage, with subterranean clovers being sown at a rate of 12.5 kg/ha, other clovers from 5–15 kg/ha, medics 7–10 kg/ha, serradella and *Trigonella* spp., 7–8 kg/ha, *Biserrula* spp., 5 kg/ha, and *Hedysarum* spp., 12.5 kg/ha. Soil cores (25-mm diameter) were collected to a depth of 0.10 m across the site on 10 September 2003 and analysed by Incitec Pivot for pH (in water and $CaCl_2$), organic carbon, nitrate nitrogen, S, P, potassium, cation exchange capacity (CEC), calcium/magnesium ratio and electrical conductivity.

Herbage mass was assessed on 3 occasions during spring 2003 and twice in spring 2004 and 2005. Plots were not defoliated between successive assessments and the legumes were allowed to senesce at the end of spring each year. After senescence, tall, rank plots were first defoliated with a brush-cutter, before all plots were cut to a height of ~80 mm above ground level using a rotary mower with the cut material (which included some seed heads) remaining on the plots. The plots were also mown (~80 mm above ground level) in summer as required. For each plot, legume HM was determined visually using a 0–50 scale (low to high scale). At each assessment 12–15 calibration quadrats (0.5 x 0.5 m) were also visually assessed, cut to a height of ~10 mm above ground level and the harvested material dried at 80°C for 48 h. Calibration quadrat scores and dry weights were regressed (linear or quadratic, R^2>0.80) and plot scores converted to kg DM/ha.

Herbage mass data for October 2003 and September 2004 and 2005 were analysed and are reported in this paper (Table 1). Examination of residuals indicated that these data did not require transformation. Data were analysed using the spatial approach of Gilmour *et al*. (1997) which accounts for field trend, and row and column effects in a linear mixed model framework using ASREML (Gilmour et al. 2006), with linear row and column terms fitted as fixed terms and entry and replicate effects fitted as random terms. A confidence level was calculated to indicate the probability (denoted by C) of one legume having superior herbage mass to another and one legume was considered superior to another if $C \geq 0.95$, inferior if $C \leq 0.05$ and similar for all other values of C.

Results and discussion

Rainfall at TAI was below average from May 2003 (sowing) to July, and again in September 2003 (Fig. 1), but above average in spring and summer (October 2003–February 2004). In the second year, rainfall in autumn-spring (March–November 2004) was below average (77% of the long-term average, LTA), while in 2005, rainfall from January–May was also well below average (34% of LTA), but above average for most months after June (Fig. 1). The soil at the TAI site did not appear to have any chemical constraint likely to limited legume growth.

In 2003, purple clover (pur-A) had the highest HM (7549 kg DM/ha, Table 1) and was similar to both of the *H. flexuosum* lines and purple clover (139465). Hykon rose clover had the lowest HM (1634 kg DM/ha) and was similar to Nungarin and Izmir subterranean clovers and Bindaroo button medic. All *Hedysarum* entries were ranked <9th in spring 2003, <12th in spring 2004 and <8th in spring 2005, except for *H. carnosum* (SA 34401) which was ranked 32nd and 39th in spring 2003 and 2005, respectively and Sparacia sulla (ranked 18th in spring 2005).

Herbage mass was highest in 2004, ranging from 1192 (Nungarin subterranean clover) to 11234 kg DM/ha (Necton sulla). Necton sulla HM was similar to Aokau and Sparacia sulla and H. carnosum (SA 35358). There were 11 entries (Table 1) with low HM that were similar to Nungarin subterranean clover. In 2005, Necton sulla had the highest HM (5949 kg DM/ha), and was similar to Jester barrel medic, Aokau sulla, *H. carnosum* (SA 35358), and both *H. flexuosum* lines. Cadiz pink serradella had the lowest HM (779 kg DM/ha) and was similar to four other entries (Table 1).

A regional evaluation study at three sites [Curban, Terry Hie Hie and Moree, Boschma *et al*. (2011b)] reported that the best performing entries over two years were *T. resupinatum* cvv. Nitro and Prolific, *T. vesiculosum* cv. Cefalu, *T. isthmocarpum*, *T. glanduliferum* and *T. michelianum*, but in the current study these entries were not ranked highly. However, similar to the results of Boschma *et al*. (2011b) at the Moree site, the sulla entries tested in the current study were among the best performing entries at the TAI site which also had high levels of calcium (≥15 cmol/kg of soil) and high CEC (>20 cmol/kg of soil). While the performance of sulla entries was good in the current ungrazed study, *H. coronarium* cv. Aokau performed poorly under grazing in adjacent plots (Lodge *et al*. 2012), highlighting the risks associated with using relatively short-term assessments from ungrazed plots as a basis for selecting species that are ultimately required to be productive and persistent as long-term grazed pastures.

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Table 1. Mean herbage mass (kg DM/ha) in spring 2003–2005 of entries sown in 2003 at the Tamworth Agricultural Institute (TAI).

Entries (species and cultivar)	Common name	Herbage mass (kg DM/ha) 2003	2004	2005
Trifolium brachycalycinum cv. Antas	Subterranean clover	5977	3323	3818
T. brachycalycinum cv. Clare	Subterranean clover	5414	2103	4425
T. brachycalycinum cv. Rosedale	Subterranean clover	5062	2133	4163
T. subterraneum cv. Junee	Subterranean clover	3465	1301	3353
T. subterraneum cv. Nungarin	Subterranean clover	1961	1192	2726
T. subterraneum cv. Izmir	Subterranean clover	2255	1388	3504
T. subterraneum cv. Urana	Subterranean clover	3277	2653	3798
T. subterraneum cv. Coolamon	Subterranean clover	3053	1542	3614
T. glanduliferum cv. Prima	Gland clover	5565	4352	3168
T. incarnatum cv. Caprera	Crimson clover	4646	3853	1665
T. hirtum (95 GCN)	Rose clover	3491	4415	3958
T. hirtum cv. Hykon	Rose clover	1634	2865	3602
T. isthmocarpum	Moroccan clover	4760	4034	2711
T. vesiculosum cv. Cefalu	Arrowleaf clover	5067	6975	3122
T. vesiculosum cv. Zulu	Arrowleaf clover	5532	8421	2102
T. resupinatum cv. Nitro	Persian clover	6098	5499	2722
T. resupinatum cv. Prolific	Persian clover	5496	3858	2904
T. michelianum cv. Frontier	Balansa clover	3538	4215	2952
T. michelianum cv. Paradana	Balansa clover	4042	2068	1868
T. purpureum (pur-A)	Purple clover	7549	9439	4220
T. purpureum (139465)	Purple clover	6875	8604	4233
Lotus ornithopodioides (1)		3598	2865	2933
L. ornithopodioides (6)		5280	4789	1647
L. ornithopodioides (17)		5383	3310	1171
Medicago truncatula cv. Caliph	Barrel medic	3582	1815	4121
M. truncatula cv. Jester	Barrel medic	4049	2851	5047
M. tornata x *M. littoralis* cv. Toreador	Disc/strand medic	3611	1870	3509
M. orbicularis cv. Bindaroo	Button medic	2097	1559	3350
M. ploymorpha cv. Cavalier	Spineless burr medic	4080	2190	3912
M. polymorpha cv. Scimitar	Spineless burr medic	3548	1426	3954
Trigonella balansae (SA 5045)		5691	3797	1966
Ornithopus compressus cv. Charano	Yellow serradella	3716	3823	3052
O. compressus cv. King	Yellow serradella	3684	4859	3328
O. compressus cv. Santorini	Yellow serradella	4186	4621	2012
O. compressus cv. Yelbini	Yellow serradella	3837	3995	3549
O. sativus cv. Cadiz	Pink serradella	5043	5522	779
O. sativus cv. Erica	Pink serradella	5280	4445	2095
O. sativus cv. Margurita	Pink serradella	5437	5307	2977
Biserrula pelecinus cv. Casbah	Biserrula	2759	5382	2895
B. pelecinus cv. Mauro	Biserrula	4409	5549	2669
Hedysarum coronarium cv. Aokau	Sulla	6162	11118	5496
H. coronarium cv. Necton	Sulla	6246	11234	5949
H. coronarium cv. Sparacia	Sulla	6408	10810	3790
H. coronarium cross	Sulla	6764	9785	4471
H. carnosum (SA 34401)	Sulla	3837	7746	2588
H. carnosum (SA 35358)		6316	10838	4954
H. flexuosum (SA 35361)		7295	8019	4944
H. flexuosum (SA 35362)		7356	9184	5623
Mean		4675	4853	3363
Average SED		434	768	671

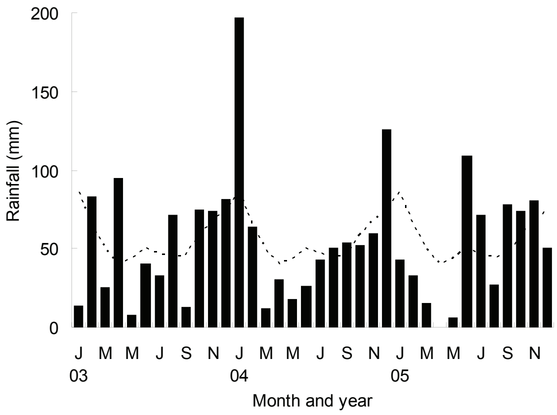

Figure 1. Actual monthly rainfall (mm, bars) for the Tamworth Agricultural Institute (2003–2005) together with the long-term average monthly rainfall (mm, dotted line) for Tamworth.

Changing frequencies of occurrence of two annual medics on soils of the grazing lands of central New South Wales - 50 years of observation

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Abstract
A 50-year survey was conducted of the frequencies of occurrence of two annual medics, *Medicago minima* (L.) Bart. and *M. laciniata* (L.) Mill., on six common soil types of the Macquarie region of central New South Wales. Observations were made on eight occasions at 25-60 positions in ~350 paddocks. None of the paddocks had ever been cultivated. The presence or absence of the two medics at each position (in quadrats 400 sq. cm in area) was recorded. The proportion of quadrats occupied by each medic was an index of its frequency of occurrence.

Over the past 50 years, *M. laciniata* has replaced M. minima as the dominant medic in the western part of central-western New South Wales. *M. minima* was widely distributed on all soil types but, over the survey period, its frequency of occurrence declined in almost all situations. *M. laciniata* was more frequent in the more westerly, drier parts of the region and almost entirely confined to brown acid soils, grey and brown soils of heavy texture and red-brown earths. On these soils, the frequency of *M. laciniata* approximately doubled over the 50-year survey period. Based on frequencies of occurrence, *M. laciniata* was more tolerant than *M. minima* of a decline in the winter component of annual rainfall.

Although the frequency of occurrence of *M. laciniata* in central New South Wales has increased in the past 50 years while the frequency of *M. minima* has declined, we are convinced that the two events are unrelated. This is because of our observations that the frequency of *M. minima* declined throughout the region during the 50-year survey period, even in those parts where *M. laciniata* did not occur. We speculate that the decline of *M. minima* may be a natural phenomenon that occurs as a relatively new species comes to terms with its new environment.

Key words: Annual medics, frequency of occurrence, *Medicago laciniata*, *M. minima*.

Introduction

Species of *Medicago* are exotic to Australia. Most species of the genus that have become naturalised arrived in this country by accident. The mode of their introduction is not known with certainty, but there is plausible speculation (Brockwell *et al*. 2008). Many of the ships that brought early settlers to Australia also carried sheep, cattle and horses; sometimes burrs were in the fleece or on the coat. Stocks of hay to feed the animals were often replenished at Atlantic ports, the Canary Islands, and the Cape of Good Hope. Invariably, this hay contained pods and seeds of legumes. Cocks *et al*. (1980) present an informative review of the entry of wild plants into Australia.

By the middle 1860s, annual species of *Medicago* (medics) were well established in semi-arid parts of western New South Wales (Pastoral Times 1866). They became of considerable importance to rural industry (Beadle 1948) despite the adherence of their burrs to fleece constituting vegetable fault in wool. In 1958, Andrew and Hely (1960) made a comprehensive survey of the frequencies of occurrence of medic species on soils of the Macquarie Region (Premier's Department 1948) of central New South Wales. Related surveys have been conducted on a number of occasions since. This paper and the accompanying poster record the changing frequencies of two medics, *M. minima* (L.) Bart. – small woolly burr medic, *M. laciniata* (L.) Mill. –cutleaf medic, over the 50-year period 1958-2008. The work was done mostly in the Macquarie Region but some observations were made further west. The methods of Andrew and Hely (1960) were used throughout.

Methods

Observations were made on eight occasions (1958, 1959-60, 1962, 1986, 1987, 2000, 2001 and 2008) at 25-60 positions in approximately 350 paddocks on the six major soil types of the region. The presence or absence of the two medics at each position (in quadrats ~400 sq. cm in area) was recorded. The proportion of quadrats occupied by each medic was an index of its frequency of occurrence.

The soil classification of Downes and Sleeman (1955) was used by Andrew and Hely (1960) in the initial survey and we have used it ever since. Of course, soil classification has been progressively modernised since 1955 and the nomenclature of Downes and Sleeman (1955) is now outdated. Various, more recent nomenclatures are shown in Table 1.

Results

M. minima occurred with varying degrees of frequency on all six soil types (Table 2a). *M. laciniata* was essentially confined to brown acid soils, grey and brown soils of heavy texture and red-brown earths. Over the 50 years of our survey, the frequency of *M. laciniata* approximately doubled on these soil types whereas the frequency of *M. minima* declined on all six soils and in almost all other situations (Table 2a, 2b, 2c, Table 3). *M. laciniata* was most frequent in the more westerly, drier part of the region (Table 2b, Table 3), and was more tolerant than *M. minima* of declining winter rainfall (Table 3).

Table 1. Classification of the 6 main soil types in the survey area (Downes and Sleeman 1955), and subsequent re-classifications.

Downes & Sleeman (1955)	Stephens (1962)	Northcote (1979)	Isbell (1996)
Red-brown earth	Red brown earth	Red brown earth	Chromosol
Grey & brown soils of heavy texture	Grey & brown soils of heavy texture	Grey, brown & red clays	Vertosol
Black earth	Black earth	Grey, brown & red clays	Vertosol
Brown acid soils	Brown soils of light texture	Red earth	Chromosol
Red loam	Krasnozem	Euchrozem	Chromosol
Solodic soils	Solodized soil	Yellow earth	Sodosol

Table 2. Changing frequencies of occurrence of Medicago laciniata and M. minima (a) on the same soil types (over 28 years), (b) on the same soil combinations (over 42 years), and (c) at exact same sites (over 22 years).

Percentage of quadrats occupied by each medic was the index of its frequency of occurrence. For each soil type (soil combination) and each year of observation, values for medic frequencies are arranged in pairs - M. laciniata upper, M. minima lower. A value in bold font indicates that that value is significantly greater than its companion value (P <0.05).

(a) Medic frequencies on SAME SOIL TYPES												
Soil type	Red brown earth		Grey & brown soils		Black earth		Brown acid soils		Red loam		Solodic soils	
Year	1958	1986-7	1958	1986-7	1958	1986-7	1958	1986-7	1958	1986-7	1958	1986-7
M. laciniata	19.2	47.8	13.4	**36.7**	0.1	0.9	**87.7**	**70.7**	0.0	0.0	0.0	0.0
M. minima	**58.7**	34.9	**48.3**	20.2	**43.0**	**27.9**	57.0	30.9	**78.4**	**52.0**	3.0	10.2
(b) Medic frequencies on SAME SOIL COMBINATIONS (of brown acid soils)												
Soil combina-tion	Nangery		Giridale B		Giridale A		Nyngara Whitbarrow		Nyngara Pangee		Nyngara Bogan	
Year	1959	2001	1959	2001	1959	2001	1959	2001	1959	2001	1959	2001
M. laciniata	1.5	12.4	30.0	**73.6**	10.2	**56.0**	35.3	**60.4**	2.7	**34.4**	57.0	**70.4**
M. minima	**15.4**	15.2	46.2	1.2	**24.4**	2.4	**65.2**	20.0	15.3	15.6	65.8	11.6
(c) Medic frequencies at EXACT SAME SITES												
Soil type	Red brown earth		Grey & Brown soils		Black earth		Brown acid soils		Red loam			
Year	1986-7	2008	1986-7	2008	1986-7	2008	1986-7	2008	1986-7	2008		
M. laciniata	61.6	44.8	32.0	6.0	0.0	4.0	0.0	52.0	0.0	0.0		
M. minima	36.8	24.0	0.0	0.0	56.0	24.0	33.3	14.3	73.3	38.7		

Discussion

Over the past 50 years, *M. laciniata* has replaced *M. minima* as the dominant medic in the western part of central-western New South Wales. Although the frequency of occurrence of *M. laciniata* increased while the frequency of *M. minima* declined, we are convinced that the two events are unrelated. This conviction is based on our observations that *M. minima* frequency declined throughout the region during the 50-year survey period, even in those parts where *M. laciniata* did not occur. We speculate that the decline of *M. minima* may be a natural phenomenon that occurs as a relatively new species (~150 years for *M. minima*) comes to terms with its new environment. (*M. laciniata* is believed to have arrived in central New South Wales more recently than *M. minima* - cf. Vincent 1954.) An analogous condition, known as medic decline, affects *M. littoralis* and *M. truncatula* in the Eyre Peninsula of South Australia (e.g. Howieson *et al*. 2000)

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Table 3. Changing frequencies of occurrence of Medicago laciniata and M. minima in 4 geographical zones located along a south/north transect between 146 °42' E and 146 °54' E.

Percentage of quadrats occupied by each medic was the index of its frequency of occurrence. Winter rainfall and winter component of total annual rainfall both diminished from south to north. In each zone medic frequencies are arranged in pairs - M. laciniata upper, M. minima lower. A value in bold font indicates that that value is significantly greater than its companion value (P <0.05).

Zone - south (1) to north (4)		1	2	3	4
Most appropriate rainfall recording station		Bobadah	Miandetta	Girilambone	Gongolgon
Latitude		32°18'S	31°39'S	31°18'S	30°21'S
Mean Rainfall (mm)	Annual	393	402	408	394
	Winter	183	167	176	166
Medic frequency	*M. laciniata* 1960	21.6	13.9	9.8	5.1
	M. minima 1960	41.3	34.6	19.2	12.8
	M. laciniata 2000-01	**54.0**	**60.0**	**75.6**	**60.0**
	M. minima 2000-01	20.4	12.0	2.4	1.6

Development and evaluation of new *Trifolium subterraneum* ssp. *brachycalycinum* crossbreds

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Abstract

Trifolium subterraneum subspecies *brachycalycinum* has potential wide adaptation in acidic to alkaline soils. In particular, the late season ssp. *brachycalycinum* cultivar Antas has proven to be very productive across a range of environments in South Australia including Petersville, Anlaby, Neales Flat and Kybybolite. However, the later maturity of Antas and relative soft seed is known to reduce its persistence in low rainfall environments and in rotation with crops. A cohort of Antas and Mintaro crossbreds were produced with the aim of increased levels of hard seed and earlier flowering. Field evaluation across four sites has highlighted the increased hard seededness and early maturity of several lines whilst matching the high productivity of Antas.

Key words: subterranean clover, annual pasture legume.

Introduction

Trifolium subterraneum ssp. *brachycalycinum* have historically been considered suitable for neutral to alkaline soils (Katznelson and Morley 1965). More recent results have shown they can also outperform traditional subclover cultivars on acidic soils (Dear *et al*. 2003, Dear *et al*. 2007, Lodge and Harden 2007, Conboy 2011). Among the ssp. *brachycalycinum* cultivars the late season cultivar Antas has proven to be very productive particularly on the grey calcareous soils (high free lime content) of the Yorke Peninsula (Trevor Dillon, pers. comm.) and the more acidic soils of western and south western Victoria (Conboy 2011). However, Antas due to its late maturity and relative soft-seededness, has not persisted at some lower rainfall localities or when grown in rotation with cereals. High productivity and the ability to grow on highly calcareous to acidic soils are valuable attributes of Antas that are worthy of inclusion for new earlier flowering hard seeded cultivars of ssp. brachycalycinum. As part of the activities of the Australian Pasture Alliance (APA - a partnership between Seedmark and SARDI), a series of Antas and Mintaro crosses were made with earlier flowering parents and higher levels of hard seed. This paper reports the preliminary results of the cohort in field evaluation trials at four sites.

Methods

Antas was crossed with Mintaro, Rosedale, Clare and two breeders lines in 2006. In addition, crosses were made between Mintaro and two breeders lines. These crossbred lines have undergone selection as spaced plants at the Waite campus and Turretfield Research Centre, South Australia until 2009. Seventeen lines (B series) were short-listed based on early flowering than Antas (range from early to mid-late), strong vigour, large leaf size and higher levels of hard seed than the cultivars Antas and Clare. A further two lines (EB series) are derived from early flowering individual plant selections from the cohort that delivered the cultivar Mintaro.

The cohort of 19 lines was evaluated in the field under sward conditions for the first time in 2010 and 2011. Trials were sown at four sites in South Australia (Anlaby, Petersville, Neales Flat and Kybybolite). Anlaby, Petersville and Neales Flat were sown late May to early June 2010 and Kybybolite was sown early June 2011. Treatments were arranged using the design package SpaDes (Spatial Design Generator). Plots measured 1.5 m x 3 m with three replicates at Anlaby, Petersville and Neales Flat. The plots at Kybybolite measured 2 m x 3 m with five replicates. Rainfall, soil pH and soil texture at each trial site is outlined in Table 1.

The lines will undergo at least three years of field evaluation. Measurements included establishment (not shown), dry matter (DM) production, days to flower, seed yield, hard seed levels and disease score (eg. powdery mildew). Neales Flat, Anlaby and Petersville were crash grazed after the winter dry matter assessment in the second year and then the stock were removed and regrowth scored for spring production. Dry matter was measured using either the visual ruler method or rising plate meter and the data was transformed to kg DM/ha. Means of fixed variety effects were calculated using spatial linear mixed models performed by Genstat 11(Lawes Agricultural Trust, Rothamsted).

Table 1: Trial site locations, average annual rainfall, soil pH and soil texture

Site	Ave. annual rainfall (mm)	Soil pH (surface 10 cm)	Soil texture
Anlaby	475	7.50 (in $CaCl_2$)	Clay loam
Petersville	425	8.00 (field kit)	Clay loam
Neales Flat	375	6.00 (field kit)	Red brown earth
Kybybolite	545	6.04 (field kit)	Sandy loam

Results and discussion

Data was averaged across sites for days to flower and hard seed (Figure 1 and 2 respectively) and is derived from un-replicated selection plots grown at the Waite Campus and Turretfield Research Centre and a replicated sward trial at Kybybolite. The cohort revealed a wide range of days to flowering from 86 days to 131 days. All lines flowered earlier than Antas, 12 lines flowered earlier than Clare and seven lines flowered earlier than Mintaro. Hard seed levels ranged from 28% to 90% and all lines except B45 and B66 had harder seed than Antas. Seventeen lines had harder seed than Clare and only three lines were harder than Mintaro. EB1 was extremely hard seeded. Contrary to information on Antas (Wurst 2004), our results have shown Clare to have harder seed than Antas. Higher hard seed levels will increase resilience to false seasonal breaks (Nichols and Dear 2007) and increase the ability to regenerate after cropping (Reeves 1987).

EB1 had the highest seed yield at the end of the first year (Figure 3) and many lines had similar seed production to Antas. Dry matter production in the establishment year is not presented in this paper. Antas had the highest winter production in the second year, but line B42 had very similar winter production (Figure 4). The poor winter production of EB1 may be partly attributed to very hard seed. Lines B42, B32, B34 and Clare had higher second year spring production compared to Antas (Figure 5). It is worth noting that Antas was no longer in the top performing group by the end of the second year at the driest site Neales Flat (data not shown).

Powdery mildew (Erysiphe polygonii) was observed at all four sites during the 2011 growing season (data not shown). Powdery mildew made an early appearance during April due to the early seasonal break; this was particularly evident at the Neales Flat site. Mintaro and many lines with Mintaro parentage were susceptible along with the lines EB1 and EB25. Rosedale was low to moderately affected. No powdery mildew was observed on Antas, Clare and Antas crosses. Clare has been noted for its resistance to powdery mildew (Barbetti and Nichols 1991), but they did not test Antas.

Conclusions

Preliminary results from our trials have shown that many of the lines have inherited the high productivity of Antas with flowering times ranging from early to mid- late. In addition, most lines have harder seed than Antas. The cohort could potentially result in three new commercial cultivars being released representing early, mid and late maturities. Further evaluation is required before a decision can be made.

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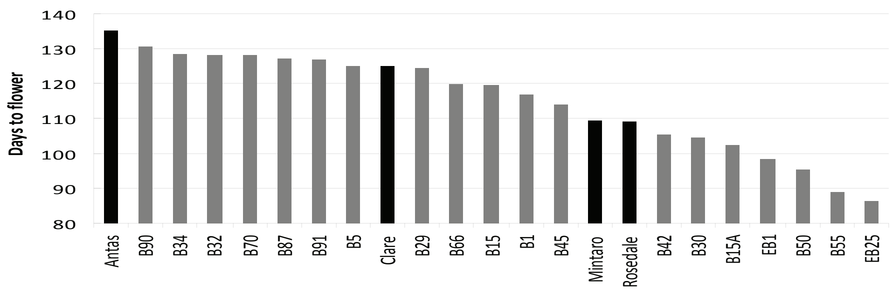

Figure 1: Mean (across three sites) days to flower from sowing for the Antas crossbreds (early May sowing at Waite and Turretfield and early June sowing at Kybybolite, when 50% of spaced plants had at least one open flower at Waite/Turretfield and when sward plots had started flowering at Kybybolite).

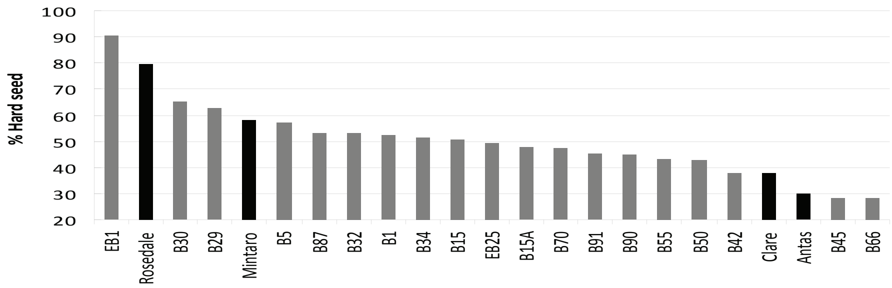

Figure 2: Mean (across three sites) percentage hard seed after four months storage in a alternating temperature cabinet set at 60°C/15°C.

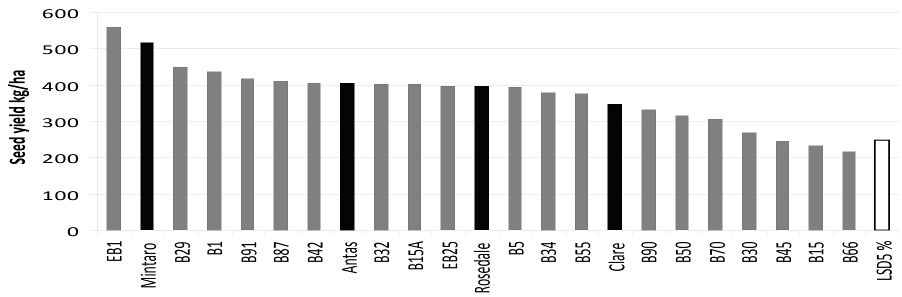

Figure 3: Mean (across two sites) seed yield (kg/ha) at the end of the first year

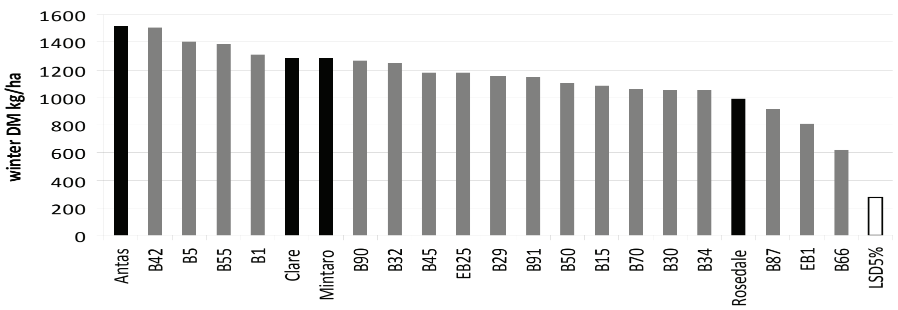

Figure 4: Mean winter dry matter production (kg/ha) (across three sites) in the second year.

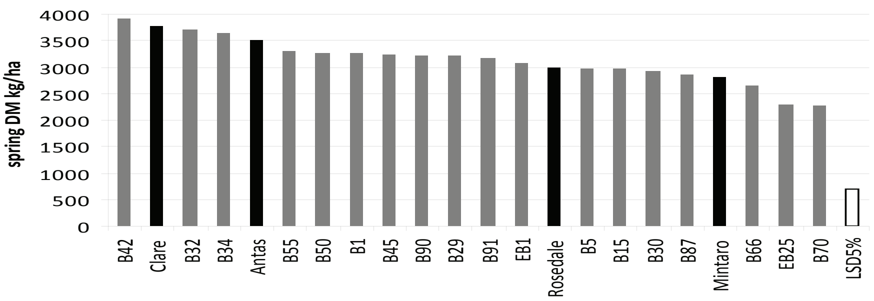

Figure 5: Mean spring dry matter production (kg/ha) (across three sites) in the second year.

The evaluation of temperate perennial pasture legume species for a low to medium rainfall environment in Tasmania.

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Abstract

In response to the need to find better adapted and more persistent perennial legumes for dryland pastures in cool temperate low to medium rainfall (500-750 mm) regions, 24 species of perennial legume were evaluated for production and persistence under sheep grazing at two replicated sites in Tasmania. The sites: Cressy, annual average rainfall 628 mm and Jericho, annual average rainfall 570 mm are representative of the target region. The work identified three alternative species in Talish clover (*Trifolium tumens* Steve. ex M.Bieb., *Trifolium ambiguum* M.Bieb. and lucerne x yellow lucerne hybrid (*Medicago sativa* L.subsp. *sativa* x *Medicago sativa* L. subsp. *falcata* (L.) Arcang.) as well adapted to the environmental conditions. White clover (*Trifolium repens* L.), a species commonly sown in the low to medium rainfall region failed to survive at both sites. Other alternative species identified as worthy of further consideration include *Trifolium physodes* Steve. ex M.Bieb. sulphur clover (*Trifolium ochroleucum* Huds.), alsike clover (*Trifolium hybridum*), narrowleaf trefoil (*Lotus tenuis* Waldst et Kit) and birdsfoot trefoil (*Lotus corniculatus* L.).

Keywords: Drought tolerance, adaptation, persistence, temperate pastures, perennial legumes

Introduction

White clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), strawberry clover (*Trifolium fragiferum* L.) and lucerne (*Medicago sativa* L.subsp. *sativa*) are the four most widely used perennial legume species in grazing systems across Tasmania's low to medium rainfall (500-750 mm) region. In this environment all of these species have adaptational deficiencies limiting their persistence. *T. repens* is at its natural limit of climatic adaptation, performing best in areas receiving more than 700 mm mean annual rainfall (Dear and Ewing, 2008). *T. pratense* has a low tolerance to moisture stress and will not survive long, dry summers. *T. fragiferum*, is more drought tolerant than *T. repens* (Dear *et al*. 2003), but is best adapted to wet or saline soils and will not survive long, dry summers. The persistence and use of *M. sativa* subsp. *sativa* is restricted by factors including water logging, acid soils and unfavourable grazing management.

Attempts to find alternative perennial legumes for temperate regions in Australia have met with little success. Lolicato (1997) evaluated a large number of perennial legume species but found nothing to match the persistence of *M. sativa* subsp. *sativa*. Li *et al*. (2008) evaluating 47 species of perennial legumes and herbs in a range of mixed farming zones across southern Australia also found no perennial legume to match the overall persistence of *M. sativa* subsp. *sativa*.

The objective of this study was to evaluate the persistence and production of a range of perennial forage legume species collected from environments similar to the target area, with the long term goal of providing producers in the target environment with well adapted alternative perennial pasture legume cultivars.

Methods

The early stages of this project saw over 1000 accessions and breeding lines, representing 80 species representing the Genera's *Anthylis*, *Astragalus*, *Coronilla*, *Dorycnium*, *Hedysarum*, *Lathyrus*, *Lotus*, *Medicago*, *Melilotus*, *Trifolium* and *Vicia*, screened and characterised for their potential to fit into the target environment. From this initial screening work, 7 commercial cultivars and 63 lines, representing the most promising material selected from 24 species representing 8 genera were sown into a randomised complete block design with 4 replications at two sites described in Table 1.

All lines were sown as seed, with sowing rates ranging from 5 kg/ha to 50 kg/ha dependant on the seed size of the line. Seed was scarified and inoculated with the appropriate strains of rhizobium prior to sowing. The seed beds were prepared by rotary hoeing in August 2005. In September 2005 the lines were sown by mixing the seed with moistened sand and surface broadcasting by hand into 1m x 2m plots, covered by hand raking and then rolled. Both sites received 300 kg/ha of 0-6-17 NPK prior to sowing, with a maintenance dressing of 200 kg/ha of 0-6-17 NPK applied in autumn 2007.

Seedling density was measured in two quadrats (0.25m x 0.25m), 4 weeks after sowing. Plant frequency (%) of each line was used as a measure of persistence. Frequency assessments were taken after the autumn breaks of 2007 and 2010. A square quadrat of steel mesh with 100 cells (each 0.1m x 0.1m) was placed in a fixed position on the ground at each assessment time. For each plot, cells containing a portion of a live plant crown of the sown species were recorded and the total number of cells containing a live crown was used to estimate frequency of occurrence. Seasonal dry matter assessments were made in years two and three. Dry matter production at Cressy was assessed either visually or by cutting one 0.25m^2 quadrat per replicate and oven drying the samples at 100^{o}C. The percentage of sown species was assessed visually in each plot. Visual ratings were used to assess herbage production at Jericho where severe drought conditions resulted in

Table 1. Site details

Attribute	Jericho	Cressy
Latitude	42°22' 16.36" S	41°43' 57.76" S
Longitude	147°18' 57.19" E	147°03' 58.80" E
Elevation (m)	399	147
Mean annual rainfall (mm)	570	628
Mean maximum temperature (oC)	15.5	18.2
Mean minimum temperature (oC)	5.0	5.6
Soil texture	clay loam	sand
pH (water)	5.6	5.3
Colwell P mg/kg	9	46
Colwell K mg/kg	212	188

low herbage production. Both sites were grazed on a rotational system to fit in with the collection of seasonal herbage production data.

Results

Drought conditions prevailed at both sites for three of five years of the study. In 2006, 2007 and 2008 annual rainfall at the Jericho site was 56, 36 and 25 percent respectively, below the long term average. Over the same period Cressy was 40, 30 and 20 percent respectively, below the long term average (Table 2).

For seedling density, all lines of *Astragalus chinensis*, *Trifolium africanum*, *Trifolium burchellianum* and *Trifolium medium* recorded low seedling numbers, resulting in poor swards with low plant densities at both sites. Germination and early establishment of the remaining lines resulted in good swards in the initial year at both sites. Frequency data from the Jericho and Cressy evaluation sites has been combined for presentation in this paper. Results from frequency assessments confirmed the poor persistence of the three major *Trifolium* species *T. repens*, *T. pratense* and *T. fragiferum* in this environment with frequency percentages of 0, 7 and 3 respectively after 5 years (Table 3). The most notable feature in the data is the performance of the M. sativa subsp. *sativa* x *M. sativa* subsp. *falcata*, *T. ambiguum* and *T. tumens* lines, with the best lines of the species recording frequency percentages of 92, 55 and 65 respectively after five years. This is equal to or better than the *M. sativa* subsp. *sativa* control cv. Prime with a mean frequency percentage of 60. *Lotus corniculatus* persisted well at both sites up to 2007, however, three years of drought at Jericho resulted in the species suffering a large decrease in plant numbers. *L. corniculatus* continued to persist at Cressy. Based on frequency two other species worthy of further consideration are *T. physodes* and *T. ochroleucum*, recording frequency percentages of 33 and 28 respectively across the two sites after five years.

Dry matter production data is presented as a mean species and best line ranking, based on data from four dry matter ratings and four dry matter cuts for Cressy and eight dry matter ratings for Jericho (Table 4). The *M. sativa* subsp. *sativa* control cv. Prime ranked the highest at the Cressy site, while one of the *M. sativa* subsp. *sativa* x *M. sativa* subsp. *falcata* lines was the highest ranked at Jericho. A line of *T. pratense* was very consistent across both sites ranking 2nd and 3rd respectively at Cressy and Jericho.

Other species to rank in the top ten at one of the sites included *L. tenuis*, *T. hybridum* and *T. tumens*. These ranked higher than the two major species T. repens and T. fragiferum at both Cressy and Jericho.

The results of the dry matter cuts taken between 19th January 2006 and 15th June 2007 are not included in Table 4, however, the *M. sativa* subsp. *sativa* control cv. Prime produced the most dry matter for the period with a total of 10,087 kg DM/ha harvested. The four next productive lines were representatives from *T. pratense*, *M. sativa* subsp. *sativa* x *M. sativa* subsp. *falcata*, *T. hybridum* and *L. tenuis* with 8669, 8209, 6901 and 6784 kg DM/ha respectively.

Table 2. Rainfall data for the years 2005 to 2009

	Jericho		Cressy	
Year	Rainfall (mm)	Variation from the long term mean (%)	Rainfall (mm)	Variation from the long term mean (%)
2005	569	0	792	+26
2006	249	-56	379	-40
2007	366	-36	441	-30
2008	426	-25	500	-20
2009	687	+20	634	+1

Table 3. Species average and maximum establishment counts and frequency data taken from a combined site analysis

Species	Number of lines	2005		2007		2010	
		Seedling density (plants/m^2)		Frequency (%)		Frequency (%)	
		Mean	Highest	Mean	Highest	Mean	Highest
Astragalus chinensis	1	17	17	1	1	0	0
A. falcatus	1	135	135	15	15	3	3
*Coronilla varia**	8	46	104	13	26	10	16
Dorycnium hirsutum	1	180	180	8	8	1	1
Hedysarum coronarium	1	360	360	14	14	0	0
*Lotus corniculatus**	4	284	373	32	66	14	31
L. tenuis	1	388	388	58	58	1	1
*Medicago sativa**	1	138	138	64	64	60	60
M. sativa x falcata	2	298	316	86	87	91	92
Trifolium africanum	1	30	30	1	1	0	0
*T. ambiguum**	6	238	381	34	50	31	55
T. burchellianum	2	39	47	2	3	0	0
*T. fragiferum**	3	165	184	34	40	2	3
T. hybridum	2	318	344	12	20	0	0
T. medium	1	39	39	1	1	0	0
T. montanum	1	89	89	10	10	1	1
T. ochroleucum	3	290	342	34	39	21	28
T. pannonicum	2	260	271	4	5	1	1
T. physodes	10	194	339	21	47	14	33
*T. pratense**	7	303	377	36	41	4	7
*T. repens**	3	134	228	6	13	0	0
T. rubens	1	302	302	20	20	9	9
T. tumens	7	382	507	57	68	42	65
Vicia cracca	1	61	61	13	13	12	12
LSD (P=0.05)		61		12		10	

*including a commercial cultivar

Conclusion

This study not only provides an assessment of the relative potential adaptation and production of the species tested to dryland pastures in cool temperate low to medium rainfall (500-750 mm) regions of Tasmania, but also in similar environments around the world. The adaptation shown by the lucerne hybrid *M. sativa* subsp. *sativa* x *M. sativa* subsp. *falcata*, *T. ambiguum* and *T. tumens* in this study, highlight the considerable potential these species have for long term dryland pastures across the target region. *L. tenuis*, *T. hybridum* and *T. pratense*, although lacking the persistence, were identified as species with potential for higher rainfall zones due to their excellent dry matter production. As a result of this and previous work commercial cultivars are now being developed in Tasmania for these six species.

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Table 4. Rankings (1-highest to 70- lowest ranking) for 2006 and 2007 dry matter production

Species	Number of lines	Jericho		Cressy	
		Species mean	Best line	Species mean	Best line
Astragalus chinensis	1	69	69	69	69
A. falcatus	1	66	66	64	64
*Coronilla varia**	8	57	47	58	46
Dorycnium hirsutum	1	45	45	51	51
Hedysarum coronarium	1	6	6	27	27
*Lotus corniculatus**	4	34	12	29	18
L. tenuis	1	7	7	14	14
*Medicago sativa**	1	8	8	1	1
M. sativa x falcata	2	2	1	6	5
Trifolium africanum	1	67	67	68	68
*T. ambiguum**	6	31	13	47	37
T. burchellianum	2	68	65	66	66
*T. fragiferum**	3	35	24	22	18
T. hybridum	2	22	14	9	8
T. medium	1	68	68	61	61
T. montanum	1	58	58	69	69
T. ochroleucum	3	27	22	35	28
T. pannonicum	2	51	48	34	30
T. physodes	10	39	30	45	32
*T. pratense**	7	10	3	10	2
*T. repens**	3	43	25	24	16
T. rubens	1	56	56	35	35
T. tumens	7	21	10	20	15
Vicia cracca	1	63	63	36	36

* including a commercial cultivar

'CloverCheque' identifying on-farm limitations to clover performance in Eastern Victoria.

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Abstract

Nutrient deficiencies of clover pastures are widespread across south-east Australia. In many cases, these nutrients are not applied due to issues associated with cost, detection and clover responsiveness. Recent pasture trials in East Gippsland looking at improving clover performance indicated the need for a number of nutrients including boron. However, monitoring boron's impact on clover performance is complex. Thus a pilot on-farm project with a beef group was established in 2010 to evaluate clover performance on four properties with different soil types. Monitored were seed reserves (sub clover), clover content, soil nutrient levels, rhizobia levels and rhizobia effectiveness. The initial results indicated that all paddocks had low sub clover seed reserves and clover content. Two paddocks were found to be boron deficient. With the others, one had low calcium levels, the other herbicide contamination. The two paddocks impacted by boron deficiency were found to contain sub clover rhizobia that were only 20-30% effective. Zinc deficiency was also found to impact clover growth at three sites. In all paddocks, by spring 2011, there was a significant improvement in clover content, vigour and overall production. This program, if used more widely would enable: Producers to better monitor clover performance; Advisors to improve soil data interpretation and Researchers to better define the relationship between boron deficiency and rhizobia effectiveness.

Key words: on-farm, clover, boron, zinc, rhizobia, nitrogen fixation.

Introduction

Grassland farmers rely on two 'free kicks' to operate a profitable business. One, natural rainfall, the other, nitrogen derived from growing healthy clover pastures. Rainfall is readily monitored, however, nitrogen input via clover is assumed to occur.

In East Gippsland, Victoria prior to 2002, clover production data was measured for two years across 86 properties as part of the Triple P (Pasture Productivity Program) program. Results indicated that at over half the sites, a poor clover growth response to applied fertilizer (nitrogen excluded) occurred even though nutrient application was based on a standard soil test. Research investigations into the reasons for this outcome have been conducted since this time.

Results indicated that boron deficiency is the likely reason for both clover decline and lower levels of nitrogen fixation. They also indicated that best outcomes were achieved when this nutrient was applied to old established pastures after freshly inoculated clover seed was sown.

These findings were used to trial an extension project to validate these research outcomes and enable the participating farmers to learn how to monitor clover performance. Four East Gippsland beef farms on different soil types were used. The paddocks selected were ones where farmer estimates indicated that animal production levels had declined by half over the last ten years, even though fertiliser was applied regularly based on a soil test.

Methods

With funding from Woolworths Sustainable Farming Program, this extension program was established in 2010 to enable these farmers to improve clover performance. It was conducted on three farms (B- s*hale origin*, V- *deep coastal sands* & M- *coastal sand over gravel* associated with the Orbost Landcare Group and on an additional farm (Bairnsdale – B/dale – *coastal sand over clay*) identified with similar clover issues.

This ongoing two year project sought initially to define the current clover performance on farm, seek out the problems associated with its growth, and then generate actions needed to improve clover performance. Two similar adjacent paddocks on each property were used, one being a control, that was managed as for the rest of the property, the other, the treatment paddock.

At its start in autumn 2010, the subterranean (sub) clover seed reserves of each site were determined from fifty 2.5cm3 surface soil cores taken at random. Using a similar coring method, soil was collected from each site and sent to the Australian Inoculants Research Laboratory in Gosford, NSW, to check the levels of sub clover rhizobia present.

For soil nutrients, a standard 10cm soil sample was taken from each site and sent to the Incitec Pivot soil testing laboratory at Werribee, Victoria. Botanical composition measures were taken in autumn (May) by using a point analysis of the species present at 50 random spots in each paddock. Dry matter production (DM) was estimated by monitoring pasture growth between the five annual inspections along with that consumed by stock during these intervals.

For each treatment paddock, the top soil was taken from the top 5cms (20cm x 20cm area) across 10 random spots and used to check the nitrogen fixing efficiency of sub clover rhizobia. Each soil sample was sieved, blended with 60 gm of superphosphate and 30 gm of Yates trace element mix + 2gms of borax, then placed in 12-140 size plastic pots. Ten Leura sub clover seeds were sown into each pot, six being the control, the others having 5gm /pot of the sub clover rhizobia culture containing WSM1325 added. The pots were watered daily and clover growth (gms wet weight) determined using three separate harvests at around two monthly intervals.

Based on seed and soil test data, nutrients were autumn applied over the whole treatment paddock. Apart from paddock B, 200kg/ha of 3:1 super potash + copper 0.5% + molybdenum 0.25% + boron 1% was applied. With paddock B, Buchan lime at 2.5 tonne/ha was autumn applied due to this soils low calcium level. The area was topdressed three months later with the above fertiliser. Before topdressing commenced, half of each treatment paddock was drill sown to freshly inoculated Leura sub (5kg/ha) and Demand White clover (2kg/ha). This was done 5-7 days after the paddocks had been sprayed with Roundup 360 (1.5litres/ha).

Results

Investigations to date indicate that poor sub clover vigor issues were due to:

• Herbicide contamination at the B/dale site. This was due to the producer feedlotting cattle on both paddocks for the two previous years with cereal hay and straw that had been sprayed (chlorsulfuron) to remove broadleaf weeds when the crop was actively growing. After eliminating pest and disease as issues, normal sub clover seedling growth occurred after the soil was treated with chlorine to detoxify this chemical. This trial site was discontinued.

• Low sub clover seed reserves. (Table 1) were present at all sites at the start of the program. These low levels were used as a guide to add boron to the fertiliser mix. It is anticipated that all levels will be above normal levels (>200kg/ha) in the treatment area by autumn 2012.

• Initially there was a low content of clover in all pastures; however, it has improved (Table 1) particularly with sowing treatment. White clover has now reappeared, and it is now a significant species in the sown pasture areas.

• Plant nutrition deficiencies were present in all soils (Table 2), thus the need for lime at the B site due to low calcium levels and the need for phosphorus, sulphur and potassium at all sites. Past research indicates that copper and molybdenum are needed regularly on these soils. Recent trials have suggested that if the B:Ca ratio > 10, then boron deficiency is likely (Hamilton 2008). The clover in the V and B/dale sites showed leaf symptoms resembling Zn deficiency in the rhizobia pot tests and this was confirmed with paddock test strips using this nutrient. These sites have had several applications of lime in the past. Zinc deficiency was also found to occur at the other two sites, thus it was applied to all the treatment paddocks in 2011.

• Rhizobia levels were low at site B (Table 3). This may be due to the low calcium and pH levels which are known to impact on rhizobia survival (Peoples and Baldock 2001). However, this small rhizobia population was still effective in fixing nitrogen (Table 3).

• Rhizobia effectiveness was found to be an issue at two sites (Table 3). Thus it could be expected that at sites V and M, new seed and rhizobia would significantly improve clover performance. This was not determined as the grazing of the sown and unsown areas at the same time did not enable these differences to be determined.

Table 1. Sub clover seed levels (kg/ha) and clover content (%) in autumn 2010/2011.

Treatment	Time	Farmer			
		B	V	M	B/dale
Seed (unsown)	2010	34	24	52	4
Seed (sown)	2011	54	42	152	
Conten (unsown)	2010	16	12	14	2
Content (sown)	20011	30	54	40	

Discussion

Whilst this project was not designed to generate research knowledge, it does use past research information (Hamilton 2008) to identify issues that need to be addressed to restore clover performance across a range of soils in Eastern Victoria.

Low sub clover seed levels have been associated with boron (Dear and Lipsett, 1987) and calcium (Ozanne and Howes, 1974) availability in the soil as well as their interaction (Tisdale and Nelson, 1973). Low levels of clover growth associated with poor nitrogen fixation have been found in a number of studies across Australia. (Riffkin et al. 1999; Sanford et al. 1994; Peoples et al. 1998) This issue has also been identified in East Gippsland (Hamilton 2008) and in this program. The reasons for this, as indicated in these studies, are variable (disease, pest and nutrient) or unexplained, however, one known nutrient reason is boron deficiency (Bonilla et al. 2009).

To date there has been a significant improvement in sub and white clover growth (Table 3) and seed set at most sites. The B site has been slow to respond to treatment, however, this can be attributed to the slow impact from the broadcast lime treatment. It is expected that the sub clover performance at this site will take at least three years to fully recover.

The relationship between lime use and boron (Sherrell 1983; Dear and Lipsett 1987) and lime use and zinc (Bolland et al. 2002) on clover vigor has been defined. The relationship between calcium derived from lime on boron and zinc availability to legumes needs to be better defined. If this can be achieved using soil data, then these nutrient deficiencies will be more readily diagnosed.

The issue of poor rhizobia efficiency in fixing nitrogen has been identified at several other sites in East Gippsland and because of this, more research is needed to verify this interaction.

In East Gippsland, this 'CloverCheque' project has validated known information and provided additional new information on issues that impact on clover, pasture and hence animal performance. Its use in other locations could be expected to have similar benefits.

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Table 2. Soil nutrient levels.

Soil test	B	V	M	B/dale
pH (water)	5.2	5.3	5.5	6.6
pH (CaCl²)	4.3	4.4	4.7	6.0
Texture	CL	SL	SL	CL
P (Olsen)	9	13	11	15
S (KCl)	5.8	7.4	5.5	8.8
K (Avail)	45	130	78	140
Ca (Avail)	1.8	7.5	4.8	6.0
Zn (ppm)	0.42	6.1	1.1	1.5
Cu (ppm)	0.25	4.0	0.17	0.25
B (ppm)	0.41	0.65	0.45	0.75

Table 3. Sub clover soil rhizobia levels, growth increase due to added rhizobia, and estimated increase in dry matter over control, due to treatments in spring 2011.

Treatment	Measure (2010)	Farmer			
		B	V	M	B/dale
Soil rhizobia	cells/gm	13	6896	3262	9375
Added rhizobia	growth >	26%	70%	82%	27%
Treatment area	Dry matter >	1600	3800	3300	

Posters

Successful lucerne establishment in Western Australia

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Abstract

Companion planting (cover cropping) lucerne (*Medicago sativa* L.) with barley compared to being sown as a monoculture was studied at seven sites in the Western Australian grain-belt. Lucerne plant density and biomass production in the calendar year of sowing was less when sown with a companion crop (19-26 plants/m^2) than when sown as a monoculture (34-35 plants/m^2). However, lucerne density declined at an increased rate in the second and third calendar year from a higher initial plant emergence resulting in comparable densities (12-18 plants/m^2). Failures in establishing the lucerne at a satisfactory density were due to unsuitable soil types. Successful lucerne establishment from low seeding rates combined with acceptable yields from a companion barley crop supports the increasing adoption of lucerne pastures on suitable soils in Western Australian mixed farming systems.

Keywords: cover crop, alternate row.

Introduction

Establishing perennial pastures with annual field crops (cover cropping) is a compromise method of pasture establishment used primarily to allay some of the costs and initial production losses associated with pasture establishment. It may also provide some seedling protection from windblasting (Bee and Laslett, 2002).

In the low to medium rainfall region of South Australia Potter (1965) recommended sowing lucerne at 1 - 2 kg/ha at 0.35 m spacings behind the covering tines of the seeder which were between every second 0.175 m spaced sown crop row. Taylor *et al.* (1992) successfully established perennial pastures by sowing the pasture and crop in each alternate row (0.175 to 0.2 m spacing's between each plant species) and adjusting sowing depth and fertilisers commensurate with the individual crop requirements. This study aimed to determine the seeding rates and establishment strategies that would result in adequate lucerne plant densities at the break of the season following establishment.

Materials and methods

In 1999 experiments were established at seven sites throughout the grain belt of Western Australia bounded by latitudes 29^{0}S to 34^{0}S and longitudes 116^{0}E to 134^{0}E, at Buntine, Quairading, Dumbleyung, Katanning, Jerramungup, Cascade and Wittenoom Hills (Figure 1). All sites were located within the 325 to 400 mm annual rainfall isohyets. Soil types varied from shallow duplex (sand over clay/clay loams) to deep sands with and without gravel content. Surface soil pH was in the range 4.5 to 6.0 (CaCl2).

The sowing commenced on 3 June with the final experiment sown on 8 July. The sites were sown through a 6 row cone seeder with double disc openers with trailing press wheels. Fertiliser was applied at all sites through the seeder at 10 kg/ha of P with rates of K from 0 to 50 kg/ha as recommended by the commercial "CSBP Soil Analysis Service".

The lucerne cultivar Sceptre and the barley cultivar Stirling were sown at all sites. An application of glyphosate (675 g a.i./ha), 24-D ethyl ester (400 g a.i./ha) and Trifluralin (560 g a.i./ha) was applied to all sites immediately preceding the sowing. Experimental design was a randomised complete block and treatments were replicated 3 times. The sowing rates and row configurations evaluated in the cover crop experiments established at the 7 sites are presented in Table 1.

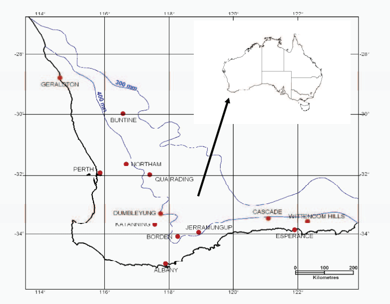

Figure 1. Location of experimental sites

The plant density and herbage production measurements were taken with 4 x 0.25 m^2 quadrats (quadrat size of 1 m by 0.25 m) which were placed accurately on plots to cover 5 x 0.2 m spaced rows. Lucerne plant counts were collected from 1 m^2 (4 x 0.25 m^2 quadrats/plot). Herbage samples were generally collected from the same quadrats at the same time as plant densities. They were sampled at a height of 1 cm and sorted into species. The biomass samples were then dried at 90^{0}C for 48 hours and weighed. Grain yield was measured in December by machine harvesting the whole plot and weighing the sample. The measurements were taken during August (plant density) and December 1999 (plant density and biomass), March 2000 (plant density and biomass) and May 2001 (plant density).

Analysis of variance (ANOVA) using Genstat Release 6.1 (PC/Windows 2000) was carried out on plant density, pasture biomass and field crop grain yields.

Results and Discussion

The seven sites had near to or below average rainfall (deciles 3 to 5) between April and November 1999. From December 1999 to March 2000 it was well above average, decile 9, and from March 2000 to May 2001 below average, decile 1 to 3.

The lucerne plant numbers were higher with the monoculture sowings than the lucerne barley @ 60 kg companion sowing until May 2001 when numbers were similar. The lucerne monoculture sown at 4 kg/ha @ 0.2 m row spacing produced more biomass at both measurement times than other treatments. Barley sown at 0.2 m row spacing produced higher yields than when sown at wider row spacings.

The outcomes from this study provide a guide as to the likely establishment success of lucerne sown with or without a cover crop in the Western Australian cropping belt. While sowing lucerne as a monoculture compared to sowing in alternate rows or in direct row competition with a barley crop increased initial lucerne numbers it resulted in an increased rate of population decline over the subsequent 20 months. All treatments had greater then 20 plants m^{-2} at the break of the season following establishment which was considered successful by Latta *et al*. (2002) and comparable lucerne numbers irrespective of the previous establishment treatment when the lucerne was fully established.

The grain yields produced by the field crops as cover crops were correlated with both seeding rate and row width, from the limited data available there was no evidence to suggest that the lucerne competed against the cover crop to the extent whereby yields were reduced.

Conclusions

This study supports informed decision making in regards to establishing lucerne in the medium rainfall grain-belt of Western Australia. Primarily it shows that lucerne sown at 2 kg/ha with suitable seeding technology into soils with no obvious production constraints will establish successfully over a range of environmental conditions.

Secondly it shows that lucerne can establish successfully when sown with a cover crop when seasonal conditions produce late spring or summer rains to allow on-going growth and development following the senescence of the cover crop. Lucerne can be successfully established through blocking every second or more of the seeding tynes, thus reducing the seeding rate by 50% or more. This technology with lucerne sown at 2 kg ha in the barley free rows provides the catalyst for the widespread sowing of lucerne in the grain belt.

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GenStat Release 6.1 (PC/Windows 2000) Copyright 2002, Lawes Agricultural Trust (Rothamsted Experimental Station)

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Table 1. Sowing rates (kg/ha) and row configurations of lucerne (L) and barley (B) of the seven cover crop experiments

Lucerne	Barley	Lucerne (L) Barley (B)
4	0	L L L L L L
2	0	L L L
2	60	LB B LB B LB B
2	60	L B L B L B
2	30	LB B LB B LB B
2	30	L B L B L B
2	15	L B L B L B
2	30	L L B L L B

Table 2. Mean lucerne plant density (plants/m2), biomass production (t DM/ha) and barley grain yields (t/ha) from seven sites in response to sowing rate@row width of lucerne and barley

Treatment		(plants/m²)				(t DM/ha)		(t/ha)
Lucerne	Barley	Aug. 99	Dec. 99	Mar. 00	May. 01	Dec. 99	Mar. 00	Dec. 99
4@20	0	52	34	32	18	0.36	0.44	
2@40	0	51	35	26	15	0.22	0.27	
2@20	60@20	25	19	18	12	0.11	0.18	2.1
2@40	60@40	27	20	18	12	0.14	0.18	1.6
2@20	30@20	37	24	22	13	0.14	0.17	1.8
2@40	30@40	36	26	21	14	0.17	0.17	1.5
2@40	15@40	37	27	23	15	0.19	0.19	1.6
2@20	30@60	40	26	25	13	0.24	0.29	1.6
LSD (P=0.05)		16	12	11	nsd	0.18	0.13	0.2

The response of lucerne to limiting and non-limiting irrigation strategies.

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Abstract

Dairy farmers in northern Victoria need to develop more flexible forage options because of reduced and variable annual irrigation water allocations related to climate variability. An experiment is currently being conducted at Tatura, Victoria, to determine the dry matter yield, persistence and water-use of lucerne under a range of limiting and non-limiting irrigation strategies. Nine irrigation treatments have been imposed ranging from full irrigation to no irrigation in either a single, or over consecutive, irrigation seasons. Over the first irrigation season (2009/2010) there were significant differences in dry matter yield (5.0 to 15.5 t DM/ha) and irrigation water-use (0.9 to 9.6 M/ha) between treatments. However, over the second irrigation season (2010/2011) the differences between treatments were less discernable because of the abnormally high spring and summer rainfall that delayed the onset of the irrigation season and reduced the number of irrigations that were applied. This field experiment will be continuing until 2014 so that clear recommendations can be made to farmers who wish to optimise their forage production under potentially limiting water supplies.

Key words: variable irrigation, flexible forage production, water use efficiency

Introduction

The dairy industry in northern Victoria relies on irrigation water to grow a large proportion of their feed inputs. However, over the last 15 years, irrigation allocations have been substantially lower and more variable than for the preceding 20-30 years. Potential climate change scenarios indicate an overall decline in the water available for irrigation in northern Victoria, with reductions in river flows of 5–35% by 2070 predicted (Anon 2004). One option for dairy farming businesses to remain viable in these circumstances is to grow areas of alternative forages such as lucerne, which yield well under irrigation, persist in dry conditions and respond when water is again available. Lucerne, when fully irrigated and cut for hay or silage, can have yields of 17–26 t DM/ha (Greenwood *et al.* 2006) with most production occurring in the hotter seasons. Under dryland conditions, the density of lucerne is usually much lower, but yields of 3–11 t DM/ha are still possible (Hirth *et al.* 2001). However, there is little information on how quickly lucerne density declines without irrigation, as well as its subsequent productivity after a prolonged period of water stress. The aim of this research is to determine the DM yield and persistence of lucerne, and its water use, under a range of irrigation treatments that might be imposed by dairy farmers when faced with variable water allocations.

Methods

This experiment is being conducted at the DPI Tatura Centre (36°26' S, 145°15' E, elevation 110 m) on a red-brown earth (Stace *et al.* 1968). A moderately winter-active lucerne (SARDI 7) was sown at 20 kg seed/ha into a prepared seedbed on 1 May 2009. Irrigation treatments commenced in spring 2009 and the experiment will continue until autumn 2014.

Experimental treatments and design

The experimental design is a randomized complete block of 9 irrigation treatments each represented in 4 blocks, giving a total of 36 plots each with dimensions of 12 m by 70 m. The treatments are:

(1) Full irrigation
(2) Full irrigation until a harvest in January in years 1,2, 3,4
(3) Full irrigation until a harvest in November and then no irrigation until the following irrigation season in years 2, 3, 4
(4) Full irrigation until a harvest in January and then no irrigation until the following irrigation season years 2, 3, 4
(5) Dryland for 1 year in year 4
(6) Dryland for 2 consecutive years in year 2, 3
(7) Dryland for 2 years in years 1, 4
(8) Dryland for 3 years in years 2 , 3, 4
(9) Full irrigation, using a sub-surface drip system (SSD), aiming to keep the soil water deficit between 30 and 50 mm.

With the exception of the Subsurface drip (SSD) treatment, all treatment plots are being irrigated by border-check at an interval of 75–90 mm evaporation less rainfall (E–R). Irrigation of the SSD plots is scheduled to maintain the soil water deficit between 30 and 50 mm. The site is routinely monitored for weeds and pests with intervention only in accordance with best management practice.

Plant measurements

The plots are harvested to a height of 60 mm when there are new shoots at the base of the crown which are less than the cutting height of 60 mm (Bourchier 1998). At harvest, 3 swathes (1.1 m wide by approximately 6 m long) are cut from each plot, using an autoscythe. Samples taken after cutting are used for determining nutritive characteristics and botanical composition. Following the final harvest of each irrigation season, the frequency of lucerne plants is being assessed by recording the presence or absence of lucerne plants within 0.15 x 0.15 m square cells within 0.9 x 0.9 m quadrats. The fraction of soil surface covered by vegetation (fc), as observed from overhead, and the average height of the forage, is visually estimated for each plot by one or two observers every 1–2 weeks.

Soil measurements

Samples for soil chemical analyses (0–100 mm depth) are collected from each of the plots in early October of each spring, commencing 2009. Fertiliser rates are determined with the aim of maintaining Olsen P levels at greater than 25 ppm, Skene K at greater than 200 ppm and CDP S at greater than 15 ppm. Soil water content is measured weekly to 1.2 m for Treatment 9 and every 3 months to 2.5 m in the other 8 treatments. Additional measurements are taken every 4–6 weeks during periods in which there are no irrigations and following heavy rain (>50 mm).

Water measurements
The depth of the local groundwater table is measured using a testwell installed in each plot. Volumes of irrigation water applied to and runoff from, each plot are measured at each irrigation. Water productivity is calculated as the ratio of forage output to water inputs.

Climatic data
An automated weather station is located within the experimental area to collect weather data and to determine potential evapotranspiration (Allen *et al*. 1998).

Statistical analysis
The forage yield, water use, nutritive characteristics and water productivity data are compared using analysis of variance. All statistical analyses are performed using Genstat 10 (Payne *et al*. 2007).

Results and Discussion

Dry matter yields in Year 1 (2009/10) ranged from 5.1 to 14.6 t DM/ha, with the yield from the subsurface drip irrigation treatment (9) not differing from the full irrigated border check irrigated treatment (1) (Table 1). Yields in Year 2 (2010/11) ranged from 12.1 to 14.1 t DM/ha with only the treatments that were not irrigated in 2010/11 yielding less than the other treatments (Table 1). The yields in 2010/11 were lower than may have been expected as high rainfall resulting in one less harvest in spring / early summer than was anticipated as the experimental site was too wet to harvest. To date there is no difference between treatments in lucerne plant frequency.

The total water use (irrigation plus rainfall less runoff plus depletion of soil water) in 2009/10 was 6.8 ML/ha greater in the fully irrigated treatments (1 and 9) than in the treatment receiving a single spring irrigation (7) (14.9 vs. 8.1 ML/ha). The total water use in 2010/11 ranged between 11.0 ML/ha for the non irrigated treatment (5) to 12.9 ML/ha for the full irrigated treatment (1). Water use in the treatments that were dried off in either spring (2) or summer (3 and 4) was intermediate. Despite the high rainfall during the 2010/11 irrigation season, the soil water content declined in all of the treatments that were not irrigated for the full season, with the greatest decline of 1.8 ML/ha in (6).

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Table 1. The effect of irrigation treatment on lucerne dry matter yield in 2009/2010 and 2010/2011

	Dry matter yield (DM) (t/ha)									
Treatment*	1	2	3	4	5	6	7	8	9	LSD (P=0.05)
2009/2010	14.8	12.0	15.1	14.8	15.2	15.3	5.0	15.5	14.4	1.33
2010/2011	13.1	13.0	13.4	13.3	12.1	13.2	14.1	12.4	13.1	0.77

* For treatment descriptions see methods section

Performance of *Hedysarum coronarium* cv. Aokau (sulla) under grazing in northern New South Wales

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Abstract

Herbage mass, plant frequency and seedling regeneration of *Hedysarum coronarium* cv. Aokau (sulla) was assessed under sheep grazing treatments at Tamworth, NSW from 2003 to 2005. Treatments were either continuous grazing or grazing when plants reached a height of 50 or 150 mm, together with three spring exclosures that allowed flowering and seed set in the first year (2003) only, the second year (2004) only, or in both years. The main significant effects were the decline in Aokau herbage mass and plant frequency over time, with there being no significant effects of grazing treatments on herbage mass, plant frequency or seedling regeneration. By December 2005 (<32 months after sowing) the average Aokau plant density was <2 plants/m2. Grazed Aokau sulla was shown to be a biennial with poor regeneration and persistence and marked summer dormancy in the summer rainfall zone of northern NSW.

Key words: *H. coronarium*

Introduction

Short-term (2–3 year) national (Li *et al*. 2008), regional (Boschma *et al*. 2010; Boschma *et al*. 2011) and local evaluation studies (Boschma *et al*. 2012) in northern New South Wales (NSW), highlighted the relatively good performance of *Hedysarum coronarium* (sulla) compared with a range of other annual and perennial legumes, particularly on soils with high calcium and cation exchange capacity (CEC). However, sown pasture persistence and productivity is a major issue for many producers on the North-West Slopes, where rainfall is summer dominant and the climate is variable (e.g. Lodge *et al*. 2003; Lodge and McCormick 2010). Most of the initial studies with sulla have been in ungrazed or intermittently grazed/cut plots and its performance under grazing is largely unknown in northern NSW. Such information is essential before wider adoption and sowings can be recommended. This paper describes the results of a grazing experiment on *H. coronarium* cv. Aokau at the Tamworth Agricultural Institute (TAI).

Methods

The experiment was conducted at TAI, located 5 km south-east of Tamworth, NSW (31°09' S; 150°59' E; elevation 434 m; AAR at Tamworth, 672 mm). The site was on a Red/Brown Chromosol soil in a paddock with a long history (>50 years) of crop and pasture rotations (mainly winter cereals and lucerne). Weeds were controlled in the six months prior to sowing and four weeks before sowing 1.7 L/ha of trifluralin (400 g a.i./L) was applied and incorporated with harrows. The experimental area was sown on 2 May 2003 to Aokau sulla (5.5 kg/ha of seed inoculated with a commercially available rhizobium), the only commercially available cultivar at the time. Single-superphosphate (200 kg/ha) was applied at sowing, but not in subsequent years. The area remained ungrazed until August 2003, when a site (~1 ha) was selected, fenced and plots were pegged so that spring exclosure treatments could be applied before flowering. At early flowering (early September 2003) the mean herbage mass of Aokau in the experimental area was ~2500 kg DM/ha, plant frequency was 100% and plant density was ~40 plants/m^2. Despite average or above average monthly rainfall from October 2003 to February 2004 (Table 1), Aokau did not grow and the initial grazing treatments were not implemented until June 2004.

The study was a randomised complete block design in three replicates with three grazing treatments and three different exclosures from grazing in spring, spatially adjusted, giving a total of 27 plots. Individual plots (each 15 x 6 m) were electrically fenced with removable sections at either end, so that plots (2430 m^2) and the surrounding area of 6570 m^2 (total area 0.9 ha) could be communally grazed by Merino wethers at an expected stocking rate of 10 sheep/ha. Grazing treatments were to: 1) continuously graze; 2) graze when Aokau plants reached ~50 mm in height, and, 3) graze when plants reached ~150 mm in height. Superimposed on these treatments were three spring exclosures that allowed flowering and seed set in: 1) the first year (2003) only; 2) the second year (2004) only, and 3) in both years. In these treatments, sheep were excluded from plots from early September to the end of December each year to allow seed set.

Monthly rainfall was often below average (Table 1) and continuous grazing could not be maintained throughout the experiment. Dates that grazing commenced and the numbers of days grazed and sheep grazing were: 18 June 2004 (30 days, 10 sheep); 12 August 2004 (3 days, 9 sheep); 3 September 2004 (1 day, 350 sheep); 6 October 2004 (2 days, 7 sheep); 8 October (8 days, 20 sheep); 18 October (21 days, 20 sheep); 15 December 2004 (1 day, 300 sheep); 24 January 2005 (1 day, 300 sheep), and 7 September 2005 (1 day, 350 sheep).

Herbage mass (kg DM/ha) was assessed on the eight occasions that sheep were removed from or placed onto the experimental plots (which were mainly associated with the spring management exclosure treatments) and was always <300 kg DM/ha after September 2004. Herbage mass was assessed in five quadrats (0.4 x 0.4 m) sampled in two strata (each 3 x 15 m) in each plot. Quadrats were assessed ~2 m apart on the centre line of each stratum by two experienced observers (one observer per stratum). In each quadrat, the observer scored total herbage mass on a continuous 0–5 scale and estimated the percentage of Aokau. At each sampling time each assessor also scored 15 calibration quadrats (0.4 x 0.4 m) for total herbage mass and the percentage of sulla. These quadrats were selected to cover the range in herbage mass across the experiment. Calibration quadrats were harvested to ~10 mm above ground level, sorted into Aokau and other species and each portion dried at 80oC for 48 h before weighing. For each assessor, scores and percentage estimates were regressed (linear or quadratic, R^2>0.80) against actual herbage mass and the percentage of Aokau to estimate its herbage mass.

Plant frequency was assessed 7–10 days after sheep had grazed the experiment in autumn and spring each year in two permanent quadrats (each 1 x 1 m), located in an area with good initial plant density in each stratum. Quadrat locations were permanently marked by positioning two white pegs in opposite corners of the quadrat frame with their tops ~10 mm above ground level. Using the marker pegs, a square quadrat of steel mesh with 100 cells (each 0.1 x 0.1 m) was placed in a fixed position on the ground surface at each sampling time. For each quadrat, cells containing a portion of a live plant of Aokau (presence) were recorded and the proportion of occupied cells was used to estimate plant frequency. Counts of Aokau seedlings in the permanent quadrats in each plot, taken at the same time as plant frequency, were used to estimate seedling regeneration (seedlings/m^2).

Table 1. Mean monthly rainfall (mm) at the Tamworth Agricultural Institute for 2003 to 2005 and the long-term average (LTA) monthly rainfall for Tamworth, NSW.

Month	2003	2004	2005	LTA
January	13.4	196.4	42.8	86.1
February	82.8	64	32.8	64.5
March	25.0	11.4	15.2	49.6
April	94.6	30.0	0.0	40.5
May	7.6	17.4	6.0	43.4
June	40.4	25.6	109.2	50.1
July	32.4	42.4	71.2	46.7
August	70.8	50.6	26.8	44.2
September	12.8	53.5	77.8	46.3
October	74.2	52.3	73.3	58.7
November	73.4	59.4	80.7	68.0
December	81.2	125.8	49.8	74.7

Test of significant differences among treatments were assessed at the 5% probability level and data were analysed using the AREPMEASURE directive in Genstat (version 9), which provided analyses of variance for repeated measurements while allowing the block structure of the design to be specified. The model was fitted to square-root transformed Aokau herbage mass data (kg DM/ha) for three sampling times, June and November 2004 and September 2005. At all other times the data contained a high proportion of zero values. Examination of residuals indicated that plant frequency and seedling regeneration data did not require transformation. Treatment terms were spring management, grazing and their interactions.

Results and discussion

Compared with the performance of *H. coronarium* in 1–2 year-old ungrazed, infrequently defoliated monocultures (e.g. Boschma *et al*. 2011, 2012) the performance of Aokau sulla in the reported grazing study was disappointing and by December 2005 (<32 months after sowing) the average Aokau plant density was <2 plants/m^2, despite high soil calcium and CEC. Irrespective of grazing treatment or spring management, the largest effect was the decline in Aokau herbage mass and plant frequency with time (Table 2). From mid June 2004 to early October 2005, continuously grazed plots were only grazed for a total of 2000 sheep grazing days compared with an expected total of 4620 grazing days (i.e. 462 days x 10 sheep/ha). In our study, Aokau appeared to behave as a biennial, with the original sown plants failing to persist for three years and recruitment of new plants failing, despite grazing being excluded for >4 months of the year. Also disappointing was the low herbage mass production of Aokau, despite above average monthly rainfall in spring 2003–early summer 2004 and December 2004, supporting suggestions that it is either summer dormant (de Koning *et al*. 2003), or semi-dormant in summer (Dear *et al*. 2003). Seedling regeneration was also poor (<10 seedlings/m^2, Table 2) and did not result in the successful recruitment of new plants. Similar results occurred in a cutting frequency/cutting height study, but have not been reported here.

The lack of persistence reported in our study may have been related to rainfall being below average for 56% of the period and the relatively low water holding capacity of the soil at the site. However, such extended periods of dry conditions are not unusual on the North-West Slopes of NSW (e.g. Lodge *et al*. 2003) and highlighted the disadvantage under these conditions of short-lived perennials that need to recruit from seedlings compared with longer-lived perennials. Further, producers would expect to be able to frequently graze sulla pastures, particularly in winter-early spring and may not be prepared to exclude livestock or reduce stocking rates for extended periods to promote seed-set and seedling recruitment.

In an evaluation study of perennial legumes and herbs sown in 2003 on a soil with high calcium and CEC near Manilla in northern NSW (Boschma *et al*. 2010), the total cumulative herbage mass of *Medicago sativa* cv. Sceptre (lucerne) was 33750 kg DM/ha (for 10 harvests over two years) compared with 4210 kg DM/ha for Aokau sulla, indicating the superior performance of lucerne in this environment.

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Table 2. Mean herbage mass (square-root kg DM/ha), plant frequency (%) and seedling regeneration (per m²) for Aokau sulla and the significant effects of time and the interaction of time and spring management. All other effects were not significant. Values in parentheses are the untransformed means, kg DM/ha.

Sampling date	Time	Spring management			
		Seed set in 2004 and 2005	Seed set in 2004	Seed set in 2005	l.s.d (*P=0.05*)
		Herbage mass - square-root kg DM/ha			
June 2004	25.2 (635)	25.3 (640)	25.8 (666)	24.6 (605)	4.70[A]
November 2004	15.6 (243)	19.6 (384)	9.4 (88)	17.9 (320)	
September 2004	10.2 (104)	7.2 (52)	8.8 (77)	14.8 (219)	
l.s.d (*P=0.05*)	2.39		4.15[B]		
		Frequency - %			
May 2004	66.9	-	-	-	
September 2004	55.5	-	-	-	
June 2005	7.1	-	-	-	
September 2005	8.5	-	-	-	
l.s.d (*P=0.05*)	5.33				
		Seedling regeneration - seedings/m²			
May 2004	0.5	0.3	0.8	0.4	4.52[A]
September 2004	4.6	6.7	6.7	0.4	
June 2005	7.0	9.0	3.3	8.6	
September 2005	3.2	3.8	1.1	4.5	
l.s.d (*P=0.05*)	2.34		4.05[B]		

[A]For comparing means across all spring management treatments and sampling dates

[B]For comparing means within a spring management treatment

Screening potential new perennial pasture legumes for tolerance to aluminium and manganese toxicities

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Abstract

There are currently few perennial legumes widely adapted to the high rainfall regions (600-800 mm) of southern Australia. There are several perennial legume species that have undergone development in recent years, which may be commercialised in Australia for use in these regions, including *Trifolium tumens*, *Lotus corniculatus*, *T. ambiguum*, *Bituminaria bituminosa* and *Cullen australasicum*. This study compared seedling tolerance of 23 perennial legume genotypes, as well as *Cichorium intybus*, to aluminium (Al) and manganese (Mn) stress, both of which commonly occur in acidic soils. Tolerance was assessed by observing seedling root and shoot growth after 23 days in hydroponic solution at 5 concentrations of Al and Mn, respectively. Results revealed that *Lotus pedunculatus* was the most tolerant to Al and *B. bituminosa* was most tolerant to Mn toxicities respectively. C. australasicum was the least tolerant species to toxicities of Al, and *Medicago sativa* was the least tolerant species to toxicities of Mn. Al rankings for legumes from most tolerant to least based on the reduction of root length relative to control within each genotype is as follows; *L. pedunculatus*, *T. repens*, *T. tumens*, *L. corniculatus*, *B. bituminosa*, *T. ambiguum*, *T. pratense*, *M. sativa*, *T. fragiferium* and *C. australasicum*. Mn rankings using reduction in shoot weight relative to the control are in decreasing order; *B. bituminosa* ssp. a*bomarginata*, *L. corniculatus*, *T. ambiguum*, *C. australasicum*, *T. pratense*, *T. tumens*, *T. fragiferium*, *L. pedunculatus, T. repens* and *M. sativa*.

Keywords: Alfalfa, acid tolerance, lime

Introduction

Soil acidification within Australia is a significant problem with ~50% of agricultural soils having pHCa values ≤ 5.5 (de Caritat *et al*. 2011). The extent of the problem is such that it is estimated to affect an area 8-9 times that of dryland salinity (Australian Natural Resources Atlas, 2001). The largest affected areas occur in New South Wales (~37 M ha), Western Australia (~21 M ha), Victoria (~14 M ha) and Queensland (~11 m ha). Soils with pHCa values ≤ 4.8 are of the greatest concern within these areas (~ 12 to 24 M ha total) due to the adverse affects of aluminium (Al) and manganese (Mn) toxicities and deficiencies of calcium, magnesium, phosphorus and some trace elements associated with low pH (Australian Natural Resources Atlas, 2001). The use of species tolerant of soil acidity in conjunction with lime application has been shown to be the most effective method of dealing with soil acidity (Scott & Fisher 1989).

There are currently few perennial legumes broadly adapted to the 600-800 mm rainfall zone of south-eastern Australia - most of which is on acid soils. Lucerne (*Medicago sativa*), is limited in this zone by soil acidity and winter waterlogging (Dear & Ewing, 2008). Periodic drought in this region limits the use of white clover (*Trifolium repens*) and subsequently often acts as an annual. In recent years there has been a significant push to develop new pasture species that fill this gap in the Australian landscape. New cultivars of *Lotus corniculatus*, *T. tumens*, and *T. ambiguum* are likely to reach the Australian market within the next 1-3 years. *Bituminaria bituminosa* and *Cullen australasicum* are species previously identified as being of interest but are less developed and less certain to be commercialised in the immediate future. The objective of this study was to assess the relative tolerance to Al or Mn toxicities for a range of newly developed perennial legume species, cultivars and accessions.

Methods and Materials

Description of germplasm

Seedlings of 23 perennial legume genotypes and one *Cichorium intybus* cultivar, Puna, were assessed in this study (Table 1). Cultivars were sourced from commercial suppliers and cultivars currently being commercialised of *T. tumens* and *T. ambiguum* were supplied by Tas Global Seeds (Launceston, TAS). Accessions of *B. bituminosa* and *L. corniculatus* were provided by Dr D. Real (Dept. Agriculture and Food, Western Australia) and one of the authors (G. A. Sandral); accessions of *C. australasicum* were sourced from the South Australian Genetic Resource Conservatorium (S. J. Hughes).

Experiment 1 – aluminium screening

The Al screening experiment was a split plot design with three replicates. Main plots were each a 45 L container of nutrient solution (see below) containing one of 5 concentrations of Al: 0, 25, 50, 75 and 100 µM. Within each container (main plot), a grouping of 10 seedlings of each genotype was grown in individual modified eppendorf tubes, representing the 'subplot' in this experimental design. Replicates were separated in time due to space constraints with approx 5 days between the harvest of one replicate and the start of the next. Relative rankings of Al tolerance (Table 1) were determined by the percentage decline in root length of the highest concentration of Al relative to the control for each of the genotypes.

Experiment 2 – manganese screening

The Mn screening was conducted as above for the Al screening using a re-randomised split plot design. There were 5 concentrations of Mn, 0, 0.25, 0.5, 0.75 and 1 mM each in a 45 L container. Relative rankings of Mn tolerance (Table 1) were determined by the percentage decline in shoot growth of the highest Mn concentration relative to the control for each of the genotypes.

Nutrient Solution

All experiments were conducted in a temperature controlled laboratory set at 20°C for two weeks to help control algal growth, then at 23°C for the final week to increase growth of the seedlings for harvest. Light was artificially provided above the plants at an average photosynthetic photon flux density of 340 ± 70 μmol s^{-1} m^{-2} on a 16/8 hr day/night cycle. Plants were held in individual tubes placed in screens suspended above 45 litre containers containing 44 litres of aerated basal nutrient solution. The concentration of nutrients in the basal nutrient solution was (μM): 500 Ca; 2000 N (300 NH4, 1700 NO3); 500 K; 201.1 SO4; 200 Mg; 50 PO4; 23 B; 10 Fe; 9 Mn; 0.8 Zn; 0.3 Cu; and 0.1 Mo. Deionised water was added to the containers when necessary to account for evaporation and transpiration losses. The pH of the solutions were maintained at pH 4.5 in $CaCl_2$ over the course of the experiment and adjusted as necessary using 1 M HCl. The respective Al and Mn treatments were added to the nutrient solutions at the beginning of the experiment as $Al_2(SO4)_3$ or $MnCl_2$ solutions. The nutrient solution and treatments were completely replaced after 16 days.

Experimentation

Seeds were scarified and had a staggered planting within their tubes in the nutrient solution culture according to their speed of germination. Slower germinating species (*T. tumens*, *T. fragiferum*, *T. pratense*, *T. repens*, *T. ambiguum*, *L. coniculatus*, *L. pedunculatus*) were planted on day 1 of the experiment, medium germinating species (*M. sativa*, *C. intybus*) were planted on day 3 and fast germinators (*B. bituminosa*, *C. australasicum*) were planted on day 5. At day 23 all seedlings were harvested with seedlings being removed from the eppendorf tubes, individual root lengths measured, then all plants separated into roots and shoots. Individual roots and shoots from each subplot were bulked, dried at 70°C for 24 hrs and weighed.

Results

Experiment 1

There was a significant (P <0.001) difference in the percentage reduction in root growth at the highest Al concentration level (100 μM), relative to the control (0 μM Al) between species (Table 1). The two genotypes of *C. australasicum* were the most sensitive to Al toxicity. The 10 genotypes of *B. bituminosa* had differential tolerances to Al with the percentage reduction in root length ranging from 72.1 to 86.5 % ranking from the middle to the bottom cultivars. Three genotypes of *L. corniculatus* (LC07AUF, LC07AUYF and LC07AT) were more tolerant of Al than *B. bituminosa*. The genotype LC07AS was not as tolerant as the other genotypes of *L. corniculatus* and 7 genotypes of *B. bituminosa*. Using the most tolerant line of each genotype, the rankings of for Al stress in decreasing order were; *L. pedunculatus, T. repens, T. tumens, C. intybus, L. corniculatus, B. bituminosa, T. ambiguum, T. pratense, M. sativa, T. fragiferium* and *C. australasicum.*

Experiment 2

There was significant interaction (P <0.001) in the percentage reduction in shoot weight at the highest Mn concentration (1 mM), relative to the control (9 μM Mn) between species (Table 1). *B. bituminosa* was the most tolerant of Mn toxicity. Differential tolerances to high Mn was more evident within the genotypes of *C. australasicum*, and *L. corniculatus* than for Al tolerances. *C. australasicum* line SA4966 was more tolerant to high Mn than SA42965 (73.0 and 86.4 % respectively). *L. corniculatus* line LC07AS (50.5 %) was also more tolerant than the other 3 genotypes of that species (64.9, 77.9 and 78.5 %). Using the most tolerant genotypes the rankings of most to least tolerant for Mn stress were; *B. bituminosa, L. corniculatus, T. ambiguum, T. pratense, C. australasicum, T. tumens, T. fragiferium, L. pedunculatus, T. repens, C. intybus* and *M. sativa*.

Discussion

Aluminium tolerance in *L. pedunculatus* and *T. repens* is well documented, and these two ranked as the most tolerant in the Al screening. *M. sativa* was sensitive to Al and along with *T. fragiferium* was the most sensitive of the released cultivars. Relative to *L. pedunculatus* and *M.sativa*, *C. intybus* was tolerant, but *T. pratense* was sensitive to Al. Of the new cultivars, *T. ambiguum* and three genotypes of *L. corniculatus* were the most tolerant of Al while *B. bituminosa* and *L. corniculatus* (LC07AS) had an intermediate tolerance of Al. *C. australasicum* was sensitive to Al and ranked last of all genotypes. There would seem to be some potential for increasing Al tolerance of *B. bituminosa* through selective breeding due to large differences in root growth between individuals within, though some of this affect may be attributable to differences in vigour.

The sensitivity of *M. sativa* to acidity was shown in the Mn experiment where it ranked last in terms of tolerance to high Mn. The other released cultivars including *L. pedunculatus, T. fragiferium, T. pratense, T. repens,* and *C. intybus* had an intermediate or sensitive response to high Mn. *B. bituminosa* was very tolerant to high Mn relative to *M. sativa*. There was less variation within the accessions tested for *B. bituminosa* suggesting reduced opportunity to select for tolerance to Mn toxicity than Al toxicity. The soon to be released *T. ambiguum*, *T. tumens* and three *L. corniculatus* genotypes had an intermediate tolerance to high Mn. *L. corniculatus* (LC07AS) had a higher Mn tolerance ranking and was as tolerant as the *B. bituminosa* genotypes.

In many environments of the high rainfall zone the dual stresses of Al and Mn exist. These stresses can reduce seedling numbers of sown species (Hayes *et al.* 2012), reduce plant growth (mainly Mn) and restrict root exploration (mainly Al) effectively reducing available soil water. The combined impacts of these effects with defoliation from grazing livestock can result in the complete disappearance of sown sensitive genotypes. It is therefore important for broad scale adaptation to acid soils that genotypes have good tolerance to both Al and Mn stress. Of the new genotypes screened, *B.*

bituminosa and *L. corniculatus* were the most tolerant to toxicities of both Al and Mn. For tolerance to Al and Mn the most consistent accessions for *B. bituminosa* were 6, 10, and 27 while for *L. corniculatus* , LC07AT was the most consistent compared to *M. sativa* which was sensitive to both Al and Mn. *T. tumens, C. intybus* and *T. repens* had a high tolerance to Al but were sensitive to high Mn. *M. sativa, C. australasicum* and *T. fragiferium* were consistently sensitive to toxicities of Al and Mn and will require a wider search of the germplasm to determine if there are tolerant genotypes available.

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Table 1: Rankings and percentage of reduction of root length due to Al at 100µM or reduction of shoot weight due to Mn toxicities at 1 mM, respectively, in nutrient solution culture relative to the its corresponding control for each cultivar/accession

Species	Cultivar/ accession	Al tolerance ranking	Root length % reduction due to Al	Mn tolerance ranking	Shoot weight % reduction due to Mn
B. bituminosa	2	21	86.5	11	64.7
	4	14	80.5	2	44.3
	5	16	83.8	5	52.1
	6	6	72.1	6	52.4
	9	11	75.7	4	51.8
	10	10	75.6	8	54.3
	17	20	85.6	7	53.2
	27	9	74.7	10	57.2
	42	12	79.2	9	55.7
	53	18	84.8	1	38.1
C. australasicum	SA4966	23	87.5	15	73.0
	SA42965	24	88.8	22	86.4
L. corniculatus	LC07AUF	8	73.4	17	77.9
	LCO7AUYF	5	68.6	18	78.5
	LC07AS	15	83.7	3	50.5
	LC07AT	7	73.1	12	64.9
T. ambiguum	Kuratas	13	79.4	13	66.5
T. tumens	Permatas	3	62.4	16	76.2
M. sativa	SARDI10	19	84.8	24	89.9
L. pedunculatus	Maku	1	28.1	20	82.0
T. fragiferium	Palestine	22	87.2	19	81.0
T. pratense	Astrid	17	84.1	14	72.3
T.repens	Haifa	2	60.2	21	85.8
Chichorium intybus	Puna	4	41.7	23	89.4
l.s.d (P=0.05)		-	14.48	-	16.93

Yield, final population and emergence of seed treated lucerne (*Medicago sativa* L.) sown on five dates.

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Abstract

Dryland seedling lucerne establishment was examined at Lincoln University, Canterbury, New Zealand. Stands were sown on 4 October, 4 November, 2 December 2010 and 10 January and 7 February 2011 with four seed treatments; bare seed, ALOSCA ® granules, coated seed and peat inoculant. Accumulated crop dry matter (DM) yield decreased with each successive sowing from 15 t/ha to 2.5 t/ha for the latest sowing date. Lower DM yields were explained by less intercepted light and a change in DM partitioning from shoots to roots. The latest sowing grew 1.84±0.26 kg DM/ °Cd compared with 3.84±0.22 kg DM/ °Cd for the earliest sowing. Coated seed produced a higher (P<0.001) initial plant population of 331 plants/m^2 which was 68% of the 490 seeds/m^2 sown. It also had the highest population 12 months later although seedling numbers had declined by 27%. Thus, adequate plant populations were established from all sowing dates and seed treatments including the bare seed control.

Key words: alfalfa, inoculation, sowing date

Introduction

Development of successful lucerne establishment practices is important for seedling survival and stand persistence. In New Zealand, lucerne sowing is generally in early spring or summer to coincide with reliable rains and moderate soil temperatures (Wynn-Williams 1982).

At sowing, lucerne seed is usually treated with *Sino-rhizobia meliloti* containing inoculum to ensure effective nodulation and nitrogen fixation. The objective of this study was to determine the effect of sowing date, and therefore soil moisture content, and inoculation method on initial emergence, seedling survival and dry matter production.

Methods

Experimental design

A dryland field experiment of 'Stamina 5' lucerne was established as a split plot randomised complete block design with five sowing dates (4 October, 4 November, 2 December 2010, 10 January and 7 February 2011) as the main plots. The four inoculation treatments; a bare seed control, ALOSCA ® granules, lime coating and peat inoculum were sown in four replications as the subplots giving a total of 80 plots.

Experimental site

The experiment was located at the Lincoln University Field Service Centre, Canterbury, New Zealand (43°38'S and 172°28'E, 11 m a.s.l) within 0.98 hectares (140 x 70 m) of flat land. The soil is a Wakanui silt loam (*Udic Ustochrept,* USDA Soil Taxonomy) with 1.8- 3.5 m of fine textured material overlying gravels (Cox 1978). The paddock had previously been sown to lucerne/ryegrass from 2004-07, brassicas in 2008 and a short rotation ryegrass from 2009-10. The field was deep ploughed on the 1st of September 2010 after which 20% Sulphur Super was applied at a rate of 20 kg/ha P and 50 kg/ha S following soil test results. It was then harrowed, rolled and boom sprayed with Treflan® E.C trifluralin (5%) at 3 *l*/ha for weed control before plots were established. A consistent bare seeding rate of 10.5 kg/ha was used which meant 17 kg/ha was established for the coated seed treatment because the coat represented 60% by weight. An Øyjord cone seeder was used to sow 14 row plots of 4.2 × 7 m with 0.5 m gaps between plots.

Seed, inoculation and sowing

The peat slurry used produced a uniform adhesive coating around the seed. ALOSCA ® granules impregnated with *Sino-rhizobia meliloti* were mixed with bare seed at the recommended rate of 10.5 kg/ha in the drill at sowing. The coated seed was a commercially available seed treatment that contained *Sino-rhizobia meliloti*, a contact fungicide against *Pythium,* molybdenum and lime. At sowing seeds were drilled in the order of; a bare seed control followed by the coated seed, ALOSCA ® and peat. The hoppers were air pressure cleaned after coated seed sowings to maintain sterility. The unsown plots were left fallow between sowing dates.

Measurements

Gravimetric soil moisture

Gravimetric soil moisture content was measured from the top 10 mm of soil on the day of each sowing. A collective sample weighing up to 250 g of soil from each treatment subplot was oven dried at 95 °C for at least 48 hours to constant weight. Values ranged from 7 % to 14% gravimetric soil moisture across sowing dates.

Seedling emergence, initial population and established population

The number of emerged seedlings (spade leaf visible) per m^2 was determined from 2 random 1 m long drill row sections fixed per plot and observed every three days. The initial plant population was derived from these emergence counts. For each establishment crop, defoliation was delayed until 50% of plants had visible flowers. The established population was examined in the spring of the second season by counting plant taproots along a meter of drill row in each plot (November 2011).

Dry matter (DM)

Lucerne dry matter yields were measured from 0.2 m^2 quadrats cut every 7-10 days from each plot and oven dried at 65 °C to constant weight.

Results and discussion

Initial and established populations

Despite the different soil moisture regimes, all crops emerged in 10 to 35 days or after 113 °Cd. This was because a rainfall event of at least 4.6 mm occurred within 5 days of each of the 5 sowing dates. Initial emergence and established population counts 12 months later were highest (P<0.001) for coated seed (Fig 3.1) although it also had the largest decline (27%) in seedling numbers over the establishment phase.

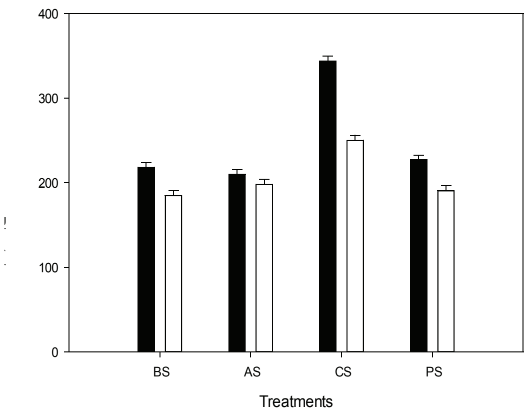

Figure 1. Mean emerged (black bars) and established (white bars) plant populations for 'Stamina 5' lucerne treated with a bare seed control (BS), ALOSCA® (AS), coated seed (CS) or peat inoculant (PS) at Lincoln University, Canterbury in 2010

This suggests the occurrence of self thinning through plant death during autumn and winter growth periods. Teixeira *et al*. (2007a) observed a similar reduction in the plant population of 'Kaituna' lucerne from 130 plants/m^2 in June 2002 to 60 plants/m^2 in September 2004. Sims (1975) reported that stands with higher emergence populations suffered the highest plant mortalities during growth and ultimately established a final plant population of only 45.7% of seed sown due to competition and self thinning.

Total seedling dry matter yield

Despite the differences in plant populations, accumulated crop dry matter (DM) yields only differed (P<0.001) amongst sowing dates and not seed treatments (Fig 3.2). The 4th of October sowing gave the highest accumulated DM yield of 15 t/ha which was 83% more than 2.5 t/ha from the 7th of February sowing. The differences in yield can be attributed to the differences in the duration of growth and therefore light interception between crops. Similar first year yields have been reported by Teixeira *et al*. (2011) for this site. Seasonal declines in lucerne DM yields from spring to autumn are also consistent with reports by Frame et al. (1998). Lucerne sown on the 10th of January and 7th of February experienced favourable soil moisture conditions above field capacity (18%) but had the lowest (P<0.001) cumulative DM yields of 4 and 2.5 t/ha, respectively. The rate of growth from these two crops was 1.7 kg DM/ °Cd and lower than the 3.9 kg DM/ °Cd found for spring sown crops. The difference was probably a direct response to short photoperiods (Teixeira et al. 2008) and/or low temperatures and consequent changes in source/sink relationships (Teixeira et al. 2007b) leading to preferential allocation of assimilates below ground for these seedling crops (Teixeira et al. 2011). The consistency of emergence and production from the bare seed control relative to the other treatments was unexpected. Previous cropping history suggests there was sufficient soil nitrogen mineralised to maintain crop growth for this initial season or sufficient indigenous rhizobia for successful nodulation and effective nitrogen fixation to maintain crop growth.

Conclusions

- Early spring and late summer sowing resulted in lower percentage stand establishment
- Emerged populations after a complete season of growth showed no differences between sowing dates.
- Coated seed gave a higher initial and established plant population but there were no differences in total DM.
- Crops without inoculation with *Sino-rhizobia meliloti* were successfully established in this location.

Acknowledgments

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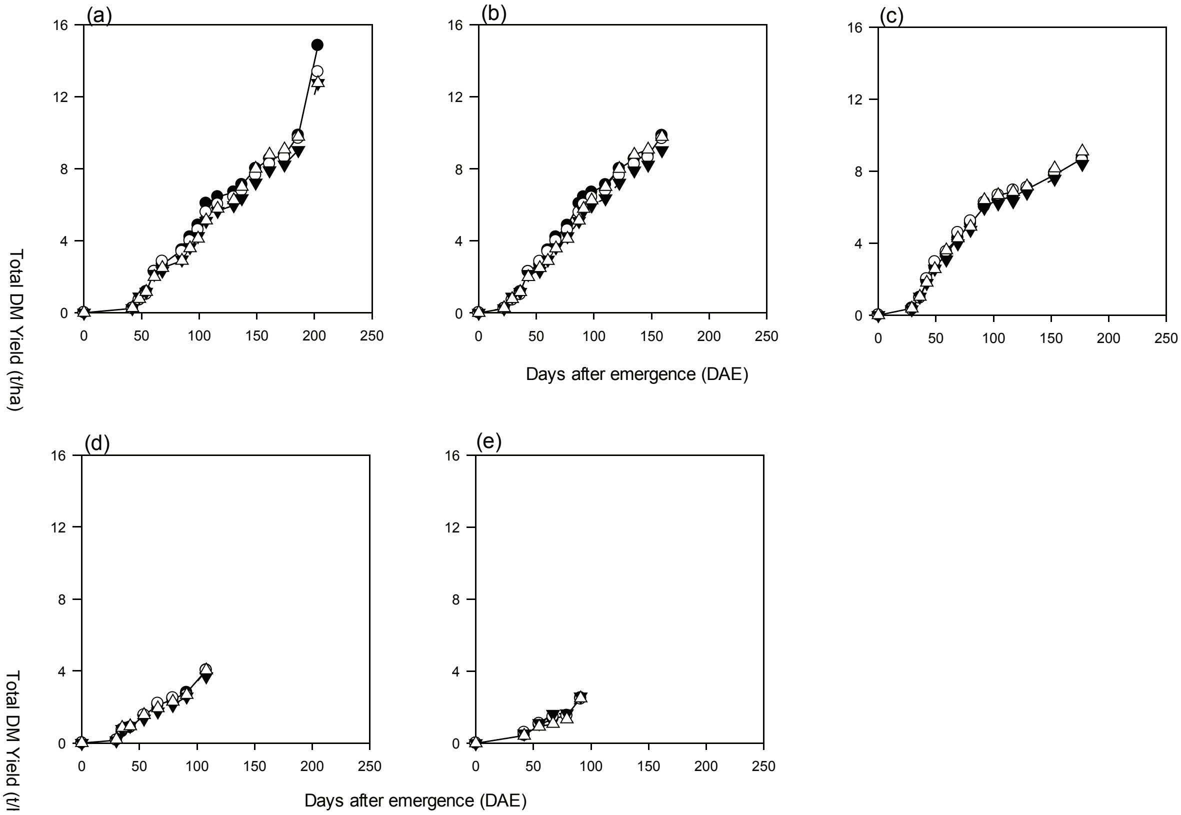

Figure 2. 'Stamina 5' lucerne dry matter (DM) accumulation of seedling crops over the growing season when sown on five different dates (a-e; (4/10/2010), (4/11/2010), (2/12/2010), (7/02/2011), (10/01/2011)) treated with a bare seed control (o), ALOSCA® (•), coated seed(⊠)or peat inoculant (⊠) at Lincoln University, Canterbury, New Zealand in 2010.

Boron deficiency of Subterranean clover (*Trifolium subterraneum*) - improving its detection and amelioration in old established pastures

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Abstract
Boron deficiency of subterranean clover is known to impact on herbage production, root growth, seed set, seed viability, nitrogen fixation and DNA synthesis. Past reports indicate that the extent of this deficiency in Australia is confined to its high rainfall tableland and coastal soils. However, its use on clover pastures growing on such soils is not common. This is due to issues associated with its detection and impact after its use. Past pasture research indicates that immediate clover responses to boron are not common. Where boron deficiency has been detected in old established pastures, the clover response has been delayed and occurred some time (years) after the initial application Two pasture experiments (2005 & 2007) were established near Bairnsdale (Victoria) on acidic high rainfall soils to better define boron's impact on old established subterranean clover pastures. Based on these experimental outcomes, the use of both freshly inoculated subterranean clover seed and boron significantly improved clover performance when introduced into old established pastures known to be boron deficient. The reasons for this result are discussed.

Key words:boron, sub clover, seed, inoculation, deficiency, gene regulation.

Introduction.

Reports of boron deficiency in plants across Australia are confined, in the main, to the lighter textured soils occurring over the slopes of the Great Dividing Range stretching from north Queensland to Tasmania (Jackson and Chapman 1975). Very few of these reports refer to its deficiency in white and subterranean clover (sub clover).

A key reason for the poor diagnoses of boron in clover based pastures is the lack of recognisable plant symptoms (Dear and Lipsett 1987). Also, a clover response to boron in the year of application is not a common occurrence. This outcome was reported across a range of sites known to boron deficient (Anderson 1952, Spencer and Barrow 1963, Hamilton 2008).

Following identification of boron deficiency in Eastern Victoria (Hamilton 2008), boron was applied across the three known deficient properties (800ha) at 2.5kg/ha in 2005. An immediate response was observed on all pastures that had been sown to sub clover within the last ten years. No response occurred in the older sown 'improved' pastures apart from areas close to old sheep stock camps.

With this, and other evidence (Lambert *et al*. 1997) suggesting that boron deficiency was common across large areas of high rainfall clover pastures, two experiments were established to validate these findings and determine more effective ways to predict its deficiency.

Methods

The two experimental sites were located on a property at Sarsfield (East Gippsland) known to be boron deficient. The sites consisted of old established pastures sown in 1982 to inoculated Seaton Park sub clover on a grey sandy loam soil of alluvial origin. Site 1 (no previous boron application) was established in 2005. Site 2 was established in 2007 on an area that had boron (2.5 kg/ha) applied in 2005. Both treatment areas had a sub clover content of around 50%, had lime applied twice over the previous 20 years (total of 5t/ha) and were fertilised annually with superphosphate (12 kg/ha P) and potassium (24 kg/ha). The trace elements copper and molybdenum were applied at five yearly intervals and zinc once every 15 years.

Experiment 1 had four replications of a control (nil) and four boron treatments applied in autumn 2005 and 2010. The treatments were 1.5, 3, 6, 12 kg/ha of water soluble boron applied as Granubor. Each treatment area was 4 x 5m. Annual basal fertiliser was the same as for the paddock.

Experiment 2 had four replications of five nutrient treatments. Each treatment area was 4 x 15m with annual basal fertiliser as for the paddock without potassium. Treatments were a control and two annual potassium treatments (autumn 50kg/ha K and an autumn/spring application each of 50kg/ha K) with and without boron. Boron at 2.5 kg/ha (water soluble) was autumn applied in 2007 only.

Across each nutrient strip, there was a control (unsown) and two sown treatments. The two sown treatments were 10kg/ha Leura subterranean clover seed plus or minus inoculant (rhizobia WSM1325). The seed was direct drilled into a seedbed sprayed five days before with 1.5 litres/ha 360 Roundup. These strips were 5x48 m in size. Each sowing treatment was replicated twice.

All nutrient treatment areas at both sites were 4 x 5m. This allowed strips of cobalt sulphate (150gms/ha) to be applied to half. No sub clover response occurred to this treatment.

During the growing period, stock were excluded from both experimental areas, but over the summer months the area was opened to rotational grazing by sheep. Total rainfall for this period was: 2005-680mm, 2006- 480mm, 2007 - 850mm, 2008- 610mm, 2000- 460mm, 2010-700mm. Dry matter (DM) determinations were obtained using 5 quadrat cuts (0.3^3) across each treatment. Other data was collected from each site as indicated (Hamilton

Posters

2012). Soil analysis data for the sites is presented in Table 1.

Results

In experiment 1, no response to any treatment occurred in 2005 and 2006. Boron toxicity symptoms in sub clover leaves were noted on plots treated with the highest boron rate. Because of this lack of response, two replications of the experimental site were sprayed in autumn 2007 with 1.5 litres/ha Roundup 360, then direct drilled with inoculated Leura sub clover seed (10 kg/ha).

Responses to boron treatment were measured in 2007 (Table 2), but only where the seeding treatment had been applied. No nutrient responses occurred in the unsown treatments. Dry winter/spring conditions in 2008/09 prevented measurements, however, responses to boron treatment were measured in 2010 (Table 2). Pasture was harvested in spring 2010 across all replications, as they were now all responsive to the boron treatments. A significant herbage and seed response to boron application, up to 6 kg/ha, was recorded.

Where 12 kg/ha of elemental boron was reapplied in 2010, a significant clover response to this treatment occurred, however, unlike the first application of this amount of boron, no toxic impacts to sub clover were observed.

In experiment 2, a response to boron application only occurred on the areas seeded with sub clover. It was best when the seed was inoculated. No response was recorded to boron in the unsown treatment despite a satisfactory content of sub clover. Potassium application added to the boron response in the seeded areas (Table 3).

In the unsown area, pasture yields were much lower than that in the sown treatments. There was a small response to potassium were autumn/spring applications was used regardless of boron treatment. However, where the spring potassium application was applied to the boron treated areas, a severe burn to the leaf edges of sub clover appeared soon after application, indicating a toxic impact from this extra potassium addition.

Discussion

These results indicate that when boron deficiency has been undetected and prolonged in established sub clover pastures, new seed is needed to generate an immediate response to boron use. Plants derived from sub clover seed generated under boron deficient conditions took some years to respond to boron application. This result suggests changes to the genetics of the old sown seed and subsequent plants derived from them. Conversely, when new sub clover was subjected to high levels of boron, the initial impact was toxicity. However, over time (5 years), the plants subjected to the same application rate for the second time were not impacted. This again suggests changes in genetic regulation of plant growth.

Boron deficiency in legumes impacts on plant DNA & RNA synthesis (Salisbury and Ross 1992), seed production and seed weight (Dear and Lipsett 1987), root growth (Bouma 1969), nitrogen fixation (Bonilla *et al*. 2009) and herbage production. Thus ,when deficient, its impact on clover growth, soil nitrogen levels and overall pasture production is significant.

Boron's ability in impact on plant DNA synthesis suggests that when sub clover is subject to either a deficiency or excess, it adapts to available levels by using an absorption regulation mechanism (Chapman *et at*. 1997). This enables the plant to cope with these extreme events by altering its genetic code over time. In doing this, it may also impact on sub clover's ability to recognise and host efficient N fixing rhizobia (Bonilla *et al*. 2009).

Thus in a deficient situation, sub clover plants regulate their growth to the low levels of available boron and thus need less other nutrient to grow: phosphorus (Robertson and Loughman, 1974), potassium (toxic impact in experiment 2), and nitrogen (Bonilla *et al.* 2009). Because of this reduced growth, boron levels in the herbage appear at normal levels as indicated in Table 2 (clover tissue tests 2005). However, at the same time when boron was applied at 6 kg/ha in experiment 1, there was plant uptake of this nutrient, but no dry matter response as the genetic codes are regulated to dealing with a deficiency situation.

This changes in time after boron is applied. In these experiments, the response occurred five years after boron application in experiment 1 and three years in experiment 2. Note that the boron levels in the tissue derived from newly sown sub clover seed growing without added boron (control treatment) indicated boron deficiency. Thus on the same site, the tissue levels of boron can vary due to plant seed source – tissue derived from old seed sufficiency, new seed deficiency.

Added to this, with boron deficiency, the rhizobia-legume dialogue fails, and the bacterium is recognised as a pathogen by the plant (Reguera *et al.* 2010). This dialogue failure may be a cause for the decline in the nitrogen fixing ability of sub clover rhizobia. This decline has been found to occur on several boron deficient soils in Eastern Victoria (Hamilton 2012), however, the use of inoculated sub clover seed has overcome this problem.

These results suggest that boron deficiency in sub clover will have serious long term impacts to the productivity of pastures, and it is difficult to detect. Thus more research is needed to better understand the issues raised and develop better cost effective outcomes.

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Table 1. Soil details for the Sarsfield experimental sites.

Texture	Location	pH (water)	pH ($CaCl_2$)	P (Olsen) m/kg	K (Avail) mg/kg	S (KCl) mg/kg	Ca meq/100g	B mg/kg	Ca:B
Sandy loam	Expt 1	5.2	4.5	12	59	3.7	5.0	0.45	11.1
Sandy loam	Expt 2	5.9	4.9	11	87	3.6	4.0	0.21	19

Table 2. Trial 1 results. Boron rate impact on herbage yield (kg DM/ha), sub clover tissue (mg/kg), soil levels(mg/ha) and seed yield (kg/ha).

Boron kg/ha	Herbage Yield kg DM/ha		Tissue B			Soil B			Seed yield g seed/g soil	
	2007	2011	2005 unsown	2007 sown	2011 all reps	2005	2007	2010`	2007	2010
Nil	631	1359	22	13	26	0.45	0.33	0.30	30	56
1.5	714	1376		17	20		0.33	0.39	22	43
3.0	829	1756		19	44		0.40	0.39	96	68
6.0	1048	2853	43	27	29		0.45	0.42	104	102
12.0	723	2350		95	190		0.96	0.58	94	71
l.s.d 5%	151	281								

Table 3. Trial 2 results. Impact of nutrient and sowing treatment on sub clover growth.

Treatment	Unsown	Sub seed	Sub +inoc
P+S	467	884	930
P+S+Mo	504	958	901
P+S+Mo+K1	534	963	1030*
P+S+Mo+K2	634*	967	1323**
P+S+Mo+K1+B	564	1194*	1182*
P+S+Mo+K2+B	666*	1020**	1740***
Mean	562	998*	1184**
l.s.d 5%=66 nutrient treatment, 99 sowing treatment			
Sub clover %	52	72	73

Improving the seed retention of *Trifolium michelianum*

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Abstract
Bolta balansa clover (*Trifolium michelianum*) is a high performing, late-season cultivar which is widely sown in southern and eastern Australia. Bolta is prone to seed losses through excessive shattering which has resulted in reluctance by pasture seed growers to produce this cultivar. Consequently, there is likely to be a dramatic reduction in the production of this otherwise well adapted cultivar, leading to a substantial increase in the cost of seed and reduced utilisation. To overcome this problem, the current research aims to develop a new cultivar of balansa clover of similar maturity as cv. Bolta with significantly increased seed retention and other improved agronomic traits such as better early season dry matter yield and seed yield. Thirteen experimental lines and cultivars Bolta and Paradana were sown (2010) in three field experiments located in South-East South Australia (1 site) and Western Victoria (2 sites). Plant characteristics deemed important have been measured and a number of promising experimental lines with significantly improved: seed retention; early season dry matter yield; and seed yield, compared with cv. Bolta, have been identified. A final year of field evaluation in 2012 will be used to validate these findings, after which it is anticipated that a new cultivar will be ready for commercialisation. This outcome will remedy a substantial industry problem and provide seed producers and graziers with a productive and superior alternative to cv. Bolta.

Key words: annual pasture legume.

Introduction
Balansa clover is a pasture legume of considerable agricultural importance across southern and eastern Australia. It is particularly well adapted to waterlogged environments and it is widely used for both short term fodder production and as a component of permanent pastures. There have been three balansa clover cultivars released through Australian public breeding efforts, namely cv. Bolta (Craig 1998), cv. Paradana (Anon. 1985) and cv. Frontier (Craig *et al.* 2000). Bolta is the latest maturing of the three cultivars, flowering approximately 10 days later than Paradana and approximately one month later than Frontier.

High levels of seed production are critical to the success of balansa clover, as with other annual pasture legumes where long term persistence is required. However the commercial success of annual legume cultivars is also closely allied to high levels of harvestable seed, being a product of seed yield and the ability to capture this seed through the harvesting process. Excessive seed shattering is one of the most important factors contributing to low harvestable seed yields.

Anecdotal evidence from seed producers of Bolta balansa clover suggest that commercial seed production of this cultivar is being adversely affected by seed losses through excessive shattering. Seed producers are becoming increasingly unwilling to grow this cultivar, citing low harvestable seed yields and profitability as the reason behind their decision. Consequently, cv. Bolta is at risk of a substantial increase in the cost of seed which would reduce the use of this otherwise very productive cultivar by livestock producers. Preliminary data collected by Craig (unpublished data) confirm the claims made by seed producers. The cultivar Bolta was shown to retain a significantly lower percentage of its total seed production in the head prior to harvest than either cv. Paradana or cv. Frontier. Consequently, a number of single plant selections from within three parent lines were made to attempt to overcome this problem. Preliminary screening has reduced these selections to a short-list of 13 experimental lines. This paper reports on the agronomic performance of these experimental lines and their prospects for commercialisation.

Methods
Site and experiment details
Sixteen entries were sown at field experiments in the Kybybolite district of South Australia (36°55'S; 140°55'E), and at Apsley (36°58'S; 141°50'E) and Nareen (37°24'S; 141°28'E) in Western Victoria in May 2010. The entries included 13 experimental lines of similar maturity to cv. Bolta that were derived from three parents namely cv. Bolta, cv. Paradana and a wild accession introduced from Turkey, two lines of certified cv. Bolta and one line of certified cv. Paradana.

Recent average annual rainfall (2007-2010) across the Kybybolite and Apsley sites was approximately 545 mm, and 674 mm for the Nareen site (Bureau of Meteorology 2011; R Hawker 2011, pers. comm.). The pH of the surface soil at all three sites was 6.3 (in H_20). The surface soil texture at the Kybybolite and Apsley sites was a light sandy clay loam and at the Nareen site was a clay loam (McDonald *et al.* 1990).

Entries were sown in plots measuring 2 m x 3 m, with five replicates laid out in a randomised block design. Plots were hand-sown with 20 kg/ha of inoculated, lime-pelleted seed. Red-legged earth mites and annual grasses were controlled as required. Fertiliser was applied as 2:1 superphosphate:potash and trace elements mix at the rate of 150 kg/ha to each site in winter every year. All dry herbage was removed (raked or grazed) from the plots in February 2010 to ensure good regeneration.

Measurements
Dry matter (DM) was assessed using a calibrated rising plate meter. Plots were mown or grazed to a height of

approximately 5 cm after those assessments whereby the average height of the plots exceeded approximately 30 cm.

Flowering date was determined visually at the Apsley site in 2010 by recording the date which best represented when each plot was in full flower by one assessor to ensure consistency of scoring across plots.

Seed yield, retained seed and shed seed were determined at the Nareen site in February 2011 by harvesting a ¼ m^2 quadrat from each plot. A mechanical shearing handpiece was used to cut the plants close to the ground in order to harvest the retained seed. The shed seed was subsequently collected from the ground by a vacuum harvester. The retained seed and shed seed were thoroughly dried in ovens at 60^0 C. Retained seed was extracted from the other plant material by processing through a thresher followed by an aspirator. The shed seed were separated from the dirt and debris (drawn up during the vacuum process) by floating the seed in a concentrated solution of calcium chloride and were then subsequently dried. Seed yield was determined by summing the retained and shed seed portions of the balansa clover plants. Harvestable seed is a function of retained seed and seed yield.

Statistical analyses
All field data was analysed spatially as linear mixed models ($P<0.05$) using GenStat (14^{th} Edition) (Payne *et al.* 2009). Data for the two cv. Bolta entries was averaged and the mean values have been presented.

Results and discussion

DM yields
Most experimental lines and cv. Paradana produced significantly more DM than cv. Bolta during the autumn assessments (Table 1). The highest performing lines produced 68 % more than cv. Bolta at Kybybolite (B35/99/08) and Nareen (B35/99) in April and May 2011 respectively. A number of experimental lines also produced significantly more DM than cv. Bolta during the winter assessments (Table 1). This was particularly evident at Nareen in June 2011 where 11 experimental lines produced an average of 40 % more DM than cv. Bolta. At the spring assessment, seven of the experimental lines produced significantly more DM than cv. Bolta; this increase was an average of 29 %. B20/99 was the highest producing entry on that occasion, with 39 % more DM produced. Ten of the experimental lines also produced significantly more DM than cv. Paradana at the spring assessment. Across the season, the 13 experimental lines had a higher DM yield ranking than cv. Bolta, with the ranking results showing, on average, 20 % more production. The across season rankings also showed cv. Paradana produced 16 % more DM than cv. Bolta (Table 1).

Cv. Paradana being earlier maturing than cv. Bolta is expected to have better early season DM yields and poorer late season DM yields. The current results show cv. Paradana and a number of experimental lines demonstrate a similar performance in the autumn and winter data sets. This finding shows these experimental lines have closed the gap on the early season superior production of cv. Paradana. The spring assessment showed a number of experimental lines to out-perform cv. Paradana which exemplifies the reduced growth of cv. Paradana later in the season. It is anticipated that had the current research been conducted in a later-season environment without a focus on early season growth, the superior DM production of the experimental lines and cv. Bolta compared with cv. Paradana towards the end of the season would have been further illustrated.

Table 1. Dry matter yield (kg/ha) of 13 late maturing balansa clover experimental lines

Entry	Apsley Aug. 2010	Kybybolite Sept. 2010	Kybybolite April 2011	Nareen May 2011	Nareen June 2011	Kybybolite June 2011	Apsley Aug. 2011	Ave.
Bolta	980	2417	727	545	813	1899	1801	1312
Paradana	1151	2223	1127	896	1310	2267	1674	1521
B1/08	1090	2676	752	703	1016	2038	2187	1495
B10/99	1012	2775	1130	855	1099	1905	1570	1478
B10/99/08	844	2772	1044	819	1155	1932	1815	1483
B20/99	1054	3351	910	821	1204	2059	2186	1655
B33/99	1150	3050	1074	853	1138	1985	1995	1606
B34/99	1109	3298	993	792	1148	2089	2107	1648
B34/99/08	1117	2944	1101	835	1047	2246	2043	1619
B35/99	1109	2710	1128	914	1079	2116	1874	1561
B35/99/08	1077	2971	1221	858	1302	2022	1870	1617
BT9 3/00	928	3189	997	740	1058	2034	2577	1646
BT9 4/00	1063	2939	827	679	973	1909	2599	1570
P11/99/08	1146	3001	892	779	1216	2074	2151	1608
P13/99/08	1179	2999	999	749	1054	1874	1158	1430
l.s.d (P=0.05)	188	551	240	92	208	235	337	N/A

N/A: not applicable

Posters

Days to flowering

With the exception of B34/99, all the experimental lines flowered within a day of cv. Bolta which flowered at 163 days after sowing (Table 2). Consequently 12 experimental lines have suitable maturity to replace cv. Bolta. Cv. Paradana flowered 158 days after sowing. The difference in maturity between cv. Bolta and cv. Paradana is more contracted (five days) than the ten days previously documented (Craig 1998). This contraction in flowering dates is in line with other subclover research that the authors have undertaken (Pescott and Craig 2011). An explanation for this finding is not clear, however, a contraction in days to flowering appears to be consistent across species and data sets and may reflect changes in the climate and seasons that have recently been experienced.

Seed yield and seed retention

No significant differences in seed yield were recorded. While a number of experimental lines produced up to 46 % more seed than cv. Bolta (B10/99/08), these differences proved to be insignificant (Table 2). The unusually wet summer that we experienced in 2010/2011 may have led to more seed shattering in some lines and subsequently created a high level of variability (residuals) in the data. It should be noted that previous data (Pescott 2011) on the same germplasm has identified higher seed yields in some experimental lines compared with cv. Bolta. However, importantly, all lines in the current data set produced seed at levels that would be considered adequate for commercial production. Despite the unusually wet summer, nine experimental lines had a significantly higher percentage of retained seed than cv. Bolta, which in most cases was at least twice that of cv. Bolta (Table 2). The percentage of cv. Paradana seed retained was also significantly higher than for cv. Bolta which confirms industry concerns about the seed production of cv. Bolta. Of particular note is the data set of harvestable seed which represents the quantity of seed retained on the plant at the time of harvest. Cv. Paradana and three experimental lines were found to have significantly more harvestable seed than cv. Bolta. On average, these three experimental lines had over 200 % more harvestable seed (Table 2). A number of the other experimental lines had more than twice the harvestable seed compared with cv. Bolta but the high residuals in the data again meant these differences were not significant.

Conclusions

This paper has confirmed that the high levels of seed shattering in cv. Bolta are the primary cause of seed harvesting issues in this cultivar. Importantly, the existence of a number of experimental lines of similar maturity as cv. Bolta that possess heightened levels of retained seed give confidence to the seed industry that the current issues can be addressed. While the best of the experimental lines do not appear to possess the quantities of harvestable seed of cv. Paradana, they still represent a significant improvement on the levels produced by cv. Bolta. The improvements in early season pasture growth demonstrated by a number of the experimental lines compared with cv. Bolta represent another valuable improvement, with increases in productivity at this time of year being highly sought after by the grazing industries. It is anticipated that the performance of the experimental lines in 2012 will mirror the results presented in this paper, in which case it is most likely that one line will be put forward for commercial release in 2014.

Acknowledgments

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Table 2. Days to flower, seed yield, retained seed and harvestable seed of 13 late maturing balansa clover experimental lines.

Entry	Days to Flower Apsley 2010	Seed Yield Nareen Feb. 2011	Retained Seed (% total yield) Nareen Feb. 2011	Harvestable Seed (kg/ha) Nareen Feb. 2011
Bolta	163	616	11	71
Paradana	158	977	33	355
B1/08	164	892	16	145
B10/99	163	698	23	174
B10/99/08	163	897	26	225
B20/99	162	810	26	215
B33/99	162	814	22	202
B34/99	161	556	24	129
B34/99/08	162	699	21	155
B35/99	162	595	15	88
B35/99/08	163	743	22	185
BT9 3/00	164	760	11	76
BT9 4/00	163	696	15	110
P11/99/08	162	651	25	180
P13/99/08	164	846	24	200
l.s.d (P=0.05)	1.4	n.s.	9	130

n.s.: not significant

A morphological and cytological analysis of interspecific hybrids: *Trifolium repens* L. × *T. uniflorum*

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Abstract
A significant constraint to white clover vegetative persistence is summer moisture stress. An important characteristic in *Trifolium uniflorum* is its robust root system. Successful hybridisation of these two species has resulted in the generation of fertile hybrids. Our paper reports on a study conducted to evaluate plant morphology and cytology of F_1 and backcross 1 (BC_1) to white clover. The hybrid progeny evaluated consisted of 7 random F1s, 32 random BC_1s and 17 individual genotypes that represented 5 elite white clover cultivars. Clones from each of the 56 individual genotypes were planted in a sand pit using a RCB experimental design with 3 replicates. The experiment was planted in March 2006 and was completed in April 2007 immediately after measuring a number of shoot and root traits. Marker chromosome counts of F_1 and BC_1 individuals confirmed hybridity. Meiotic configurations of the F_1s and BC_1s indicated close homology between the two species. There was significant (P<0.05) phenotypic variation for all the traits measured among the progeny. Pattern analysis of the genotype-by-trait data helped identify 4 distinct progeny groups. The group that had superior vigour and morphology, for both root and shoot traits, consisted of two BC_1 progeny. The F_1 progeny were confined to the group with the lowest mean expression for root and shoot traits. The BC_1 individuals which had similar shoot morphology to white clover but also a robust root system similar to T. uniflorum are currently being integrated into a breeding programme.

Key words: *Trifolium* spp., interspecific hybrids, cytogenetics, embryo culture.

Introduction
White clover (*Trifolium repens* L.) is one of the most widely distributed forage legumes in moist, temperate regions of the world. The most valuable characteristics of white clover in comparison to other forage legumes are its rapid growth, high feed quality, efficient nitrogen fixation and high seed production (Williams 1987). Despite being a perennial, stands of white clover often decline in the second or third year due to the death of the primary tap root (Westbrooks and Tesar 1955; Brock and Tilbrook 2000). The death of the primary tap root and reliance on weak stolon roots make white clover plants more susceptible to a number of stress factors, including drought (Bryant 1974; Spencer *et al.* 1975), viruses (Barnett and Gibson 1975; McLaughlin and Pederson 1985; Alconero *et al*. 1986; Ragland *et al.* 1986), nematodes and other root chewing insects (Yeates *et al*. 1973; Skipp and Gaynor 1987; Mercer 1988; Pederson and Windham 1989; Gaynor and Skipp 1987).

T. uniflorum is a long lived single crown species from the Mediterranean region. It lacks the productivity and robust stoloniferous growth habit of white clover but it grows in dry, rocky places and is characterised by deep thick taproots and thickened, sturdy stems. Hybridisation between white clover and *T. uniflorum* was first achieved by Pandey (1957) and subsequently by Evans (1962), Gibson *et al.* (1971). Pandey *et al.* (1987) evaluated the F_1, F_2 and backcrosses to *T. repens* both cytologically and morphologically. The hybrids were found to be fertile and to have strong root systems, suggesting potential value for breeding stronger roots into white clover. However, these hybrids were not developed further. The objective of the current research was to generate new hybrids between *T. repens* and *T. uniflorum* and to characterise them both morphologically and cytologically, and to assess their agronomic potential.

Methods

Hybridisation and embryo rescue

Seeds of *T. uniflorum* and *T. repens* parent species were obtained from the Margot Forde Forage Germplasm Centre, Palmerston North, New Zealand. Hand pollinations were conducted using *T. repens* as female parent and *T. uniflorum* as male parent. All F_1 hybrids were obtained through embryo culture using the techniques of Williams *et al.* (2011). Most backcross one (BC_1) progenies were obtained by hand crossing F_1 plants (as either male or female) to *T. repens*. A few BC_1 families were obtained using *T. uniflorum* as recurrent parent.

Cytological Observations

Somatic chromosome counts, meiotic configurations in pollen mother cells (PMC) and pollen stainability of F_1 and BC_1 plants were carried out using techniques described by Hussain and Williams (1997).

Sandpit experiment

A large excess of stolon cuttings were taken from seven F_1, 32 BC_1 and 17 *T. repens* genotypes for propagation in the glasshouse on 13 February 2006. On 23 March 2007, the rooted cuttings with visually uniform root and shoot sizes were planted into a large area of coarse sand of 45 cm depth. The experiment was a randomised complete block design with three replicates. Plants were arranged on 60 cm grid with two rows of plants per replicate. During the experiment, data were recorded on leaflet length and width, length of longest stolon, stolon thickness, stolon number, node number on longest stolon, nodulation (0 = no nodules, 5 = many nodules) and plant size (1= very small, 9= very large, more than 40cm from the centre of the plant to the edge of the dense spread) and nodal rooting (0 = no nodal rooting, 5=very frequent nodal rooting). In April 2007, plants were destructively harvested and data were recorded on root length, number of roots 2mm thick, root and shoot dry weight. Standard ANOVA analysis methods were used to statistically determine significant effects and standard errors of the means (SEM).

Results and Discussion

T. repens x T. uniflorum F_1 crosses

Sixteen F_1 hybrids were produced through embryo culture. The morphological features of F_1 hybrids were somewhat intermediate between the two parents but more like *T. uniflorum* with shorter internodes and few florets in the inflorescences, infrequent nodal rooting and rooting near the crown. To generate good numbers of embryos, F_1 hybrids were produced using *T. repens* as female parent.

First backcross (BC_1)

Five F_1 hybrids were reciprocally backcrossed with 4 genotypes of white clover from 4 different cultivars as recurrent parents. BC_1 seeds were obtained without the use of embryo culture. In total 10 different BC_1 progenies were developed. Seven of the 10 had white clover as female parent and three had an F_1 as female parent. Seed set was considerably less in backcross where F_1 were used as female parent (9-11 seeds/100 florets) than those where white clover was used as female parent (32-48 seed/100 florets). Another BC_1 progeny was obtained by pollinating an F_1 with *T. uniflorum*. Seed set in this cross was also low (7 seeds/100 florets). Most of the F_1s were self compatible, and so it was desirable to use them as male parents in backcrosses to white clover. Alternatively, a distinct leaf mark on white clover was used to confirm the hybridity of those backcrosses where *T. repens* was used as male parent.

Cytological evaluation of F_1 and BC_1

Five F_1 and five BC_1 were evaluated for somatic chromosome counts and were all tetraploid ($2n=4x=32$). Hybridity of the F_1 plants was confirmed by the presence of three satellited chromosomes, 2 derived from the *T. uniflorum* male parent and one from the *T. repens* female parent. Meiotic chromosome configurations of the five F_1 and five BC_1 consistently revealed both trivalent and quadrivalent formation, indicating close homology between the genomes of the two species and that genetic exchange between the two species is possible.

Phenotypic analysis

Analysis of variance indicated that there was significant ($P<0.05$) genotypic variation among the hybrids for all of the morphological traits measured. Shoot DW for the white clover control plants ranged from 3.7 to 23.3 g/plant while the F_1 plants were markedly smaller (0.9 to 3.4 g/plant). The BC_1 plants ranged widely from 1.5 to 21.4 g/plant, the best hybrids being similar in size to the best elite white clovers. Below ground, the F_1 hybrids were poor in root DW (0.6 to 1.4 g/plant) but had the highest ratio of thick roots to total roots (1.7 to 4.8 thick roots per g/root). Elite white clover root DW ranged from 2.9 to 11.1 g/plant, but the ratios of thick roots were lower at 0.1 to 1.0 thick root per g DW root. The BC_1 hybrids covered a wide range of root system sizes from 1.1 to 13.3 g/plant and ratios of thick roots of 0.7 to 3.3 thick roots per g DW root. Thus the root systems of the best BC_1 hybrids were similar in DW to those of the best elite white clovers but generally showed more thick roots.

These results indicate that backcrossing of the F_1 hybrids to *T. repens* resulted in BC_1 progeny superior in the expression of key traits to the F_1 parents. This provides evidence that key morphological traits can be improved through successful interspecific introgression followed by backcrossing. In the future, backcrosses derived from F_1s generated from crossing diverse germplasm of parental species are expected to provide novel genotypic recombinants and variation. This novel BC_1 genetic variation will provide a breeding pool for further targeted genotypic selection.

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Application of marker assisted selection in white clover (*Trifolium repens* L.)

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Abstract
Our paper is focused on a preliminary proof of concept study to evaluate marker assisted selection for a key stolon trait, node number (NN), within a random mating white clover breeding population. Microsatellite (SSR) markers identified from previous quantitative trait locus (QTL) studies were selected to screen a random sample of 450 phenotyped individuals from a breeding population to establish marker: trait associations for NN. Using phenotype and marker indices, a series of five contrasting polycross combinations, each comprising 22 individuals, were developed based on: 1) high phenotype value (P_H); 2) markers associated with high phenotype (M+); 3) markers associated with low phenotype (M-); 4) high phenotype individuals with markers associated with high phenotype (P_HM+); and 5) low phenotype individuals with markers associated with low phenotype (P_LM-). Progeny from the within selection polycrosses were evaluated for NN in a pot experiment along with members of the breeding population. There were significant ($P<0.05$) differences among the selection groups for NN. Mean NN for the selection groups P_H, M+ and P_HM+ were significantly ($P<0.05$) higher than the M- and P_LM- selection groups. These preliminary results demonstrate the applicability of QTL-linked SSR markers to select for increase in stolon NN in white clover breeding pools, and these selections are currently being validated by assessing the polycross progeny in a perennial ryegrass (*Lolium perenne* L.) sward under sheep grazing.

Key words: Genetic gain, marker aided breeding, divergent selection

Introduction

White clover is a significant perennial forage legume in temperate pasture systems (Frame and Newbould 1986) providing key attributes such as high nutritive value, palatability and fixation of atmospheric nitrogen. The perennial behaviour of white clover and its ability to spread horizontally and colonise pasture ecosystems (Harris and Thomas 1973) is determined by the stolon, often referred to as its structural/vegetative unit (Thomas 1987). The stolon, a surface creeping stem, is made up of a series of nodes separated by internodes. Each node has the ability to form the basis of a new plant unit as it can develop nodal roots, carries a trifoliate leaf and has an axillary bud which, during vegetative growth, may produce a lateral stolon branch (Thomas 1987). Rooted nodes, therefore, provide a mechanism to enhance the viability and persistence of stolon sections.

Unreliable vegetative persistence of white clover in the sward is a common problem in New Zealand and Australia, especially under summer moisture stress conditions (Barbour *et al.* 1996; Archer and Robinson 1989). Improving the ability of white clover populations to persist through stolon survival is a significant breeding objective, particularly as white clover plant regeneration based on stolon survival results in more productive swards in comparison to those based on annual seedling regeneration (Archer and Robinson 1989). Genotypic selection based on key morphological traits associated with stolon growth and development is fundamental to the success of improving vegetative persistence. However, measurement of stolon traits under sward conditions is laborious and is also associated with large experimental error and genotype-by-environment interaction effects (Jahufer *et al*. 1999).

Molecular marker technology provides plant breeders with a new set of diagnostic tools to track genetic variation. Application of marker-assisted selection (MAS) enables indirect estimation of trait expression independent of environmental effects and also increases precision of selection (Collard *et al*. 2005). Identification of molecular markers associated with quantitative trait loci (QTL) with significant influence on the expression of key stolon traits will enhance the selection process in white clover breeding programs. QTL discovery and development within the Pastoral Genomics program at AgResearch has successfully identified QTL for a range of above and below ground morphological traits in white clover (Jones *et al*. 2006). Our paper is focused on part of a preliminary proof of concept study to evaluate MAS for a key stolon trait, node number (NN), within a random mating breeding population using SSR markers subtending QTL for NN identified in a biparental QTL discovery population. The approach taken in our study is based on previous work on white clover seed yield (Barrett *et al.* 2009) which showed that markers associated with QTL may be used to screen unrelated populations and identify new marker alleles that have a significant effect on population performance for that trait.

Methods

Our study consisted of the following phases:

a) Phenotyping for NN in a breeding population: A random sample of 450 seedlings, from an early generation breeding population of cultivar Kopu II, was established under glasshouse conditions in July 2008, then transplanted into 15 cm diameter pots containing potting mix and placed outdoors in mid October 2008. In early November 2008, a phenotyping experiment was established using a row-column spatial experimental design with repeated clonal checks. In March 2009, two stolons, with the apical ends intact, of minimum 15 cm length, were sampled from each of the 450 plants and repeated clonal checks. The number of nodes were counted along from the apex of each 15 cm stolon sample. Analysis of the phenotype data was conducted using the variance component analysis procedure, Residual Maximum Likelihood (REML) option, in GenStat 7.1 (2003). A completely random linear model was used in the analysis using the REML algorithm. The final adjusted phenotypic means, Best Linear Unbiased Predictors (BLUPs), were based on adjustment for random error across columns, rows and repeated clonal checks.

b) Determining marker:trait association: To identify SSR marker allele:trait associations, DNA was extracted from leaf material of the 450 plants (Red Extract-N-Amp Plant Kit, Sigma) and screened with a set of six SSR markers selected using *a priori* knowledge, such as subtending NN QTLs or markers that had exhibited trait association in previous pot experiments. SSR amplification was performed as described (Barrett *et al* 2008). Marker:trait associations were identified using both a Kruskal-Wallis non-parametric one way analysis of variance, implemented in MapQTL® 4.0 software (Van Ooijen *et al*. 2002) and a linear regression analysis (Genstat 7.1, 2003) on a data set comprising the genotype and BLUP phenotype data. Five SSR alleles with significant (*P<0.0001*) trait associations were used to develop divergent selection indices for increased and reduced NN.

c) Phenotype and genotype-directed crosses: Using phenotype and marker indices, a series of five contrasting polycross combinations, each comprising 22 individuals, was developed based on: 1) high phenotype value (P_H); 2) markers associated with high phenotype (M+); 3) markers associated with low phenotype (M-); 4) high phenotype individuals with markers associated with high phenotype (P_HM+); and 5) low phenotype individuals with markers associated with low phenotype (P_LM-). High and low phenotype refer to the high 5% and low 5% of individuals in the random sample for NN expression, respectively. The selected 22 genotypes within each phenotype/marker combination were polycrossed using pollen-free bumble bees during summer 2009/2010, and seed from the half-sib (HS) families was harvested within each of the crossing categories.

d) Evaluation of half-sib (HS) phenotypes: In November 2010, 10 random progeny from each of the HS families within each selection category were established in a pot experiment. A random sample of 50 individuals from the Kopu II breeding population was also included. All seedling and experiment establishment procedures including NN counts were similar to those described above. NN was counted in March 2011. The experimental design was a row-column design with repeated checks and data analysis performed as described above.

Results and discussion

Phenotypic and genotypic characterization of the Kopu II breeding pool:
There was significant (*P<0.05*) genotypic variation (σ^2_g = 189±31) for the trait NN, among the 450 white clover plants in the random sample. This was also evident from the range (46 to 123 nodes m^{-1}: *l.s.d.*$_{0.05}$ = 23) of expression for NN among the 450 plants. The BLUP adjusted phenotypic means were used to select the high and low 5% groups of 22 individuals each.

Marker selection and crossings:
The number of alleles present in the population of 448 early generation Kopu II genotypes for each of the six SSRs ranged from 7 to 63 with an average of 26.3±8.84 alleles/SSR and a total of 158 alleles. As to be expected, single-locus SSRs had fewer alleles in the population than those which amplify from with multiple loci. The allele information was used to generate a matrix combined with BLUP phenotype data for marker:trait association analysis. Using both Kruskal-Wallis and regression analysis, highly significant (*P<0.0005 – 0.0001*) allele:trait associations were identified. Both methods generated very similar results with only slight changes in the order of significance for lower ranked allele:trait associations. Five SSR alleles with significant (*P<0.0001*) trait associations were used to develop divergent selection indices for increased and reduced NN. Plants carrying beneficial marker alleles exhibited a 19% increase in trait mean for NN compared with those without the beneficial marker alleles. These indices, along with the phenotype indices, were used to develop five contrasting polycrosses as described above. The HS progeny of these were assessed for NN in a pot experiment.

Half sib family progeny response to phenotypic and marker aided selection:
There were significant (*P<0.05*) differences observed among the selection groups for NN (Table 1). Mean NN for HS family progeny from polycrosses based on phenotype or markers associated with increased NN (P_H, M+ and P_HM+) were significantly (*P<0.05*) higher than those from polycrosses based on reduced NN (M- and P_LM- selection groups). Furthermore, progeny from P_H and P_HM+ had a mean NN significantly (*P<0.05*) higher than the M+ based selection offspring, highlighting that selections made in combination with phenotype indices had greater genetic gain. The M+ and M- selections, however, performed as predicted and provide an opportunity for making additional crossing cycles before needing to re-phenotype to select individuals for crossing.

Table 1. Genotypic variance (σ^2_g) among the half-sib (HS) family parental selection classes and their means for the trait NN (number/meter of stolon).

Selection category	NN
σ^2_g	209±97
Top Phenotypic (P_H)	85.4^{a,b}
Top Phenotypic and Marker + (P_HM+)	76.5^{a,b}
Marker + (M+)	72.7^{b}
Marker - (M-)	61.2^{c}
Low phenotypic and marker - (P_LM-)	48.9^{d}
Early generation Kopu II	46.4^{d}
l.s.d.*(P*=0.05*)*	10.0

Means with identical superscript letters are not significantly (P<0.05) different.

Posters

The selection groups P_H, M+ and P_HM+ also had a mean NN significantly (*P<0.05*) higher than the genotypes representing the original breeding population, early generation Kopu II. This indicates potential genetic improvement for increased expression of NN relative to the original base population. The mean NN of the P_LM- selection combination HS progeny was lowest and similar to the original Kopu II population.

A key step towards application of MAS in breeding programs is identification of effective marker:trait associations in complex multi parent breeding pools. Results from progeny assessment in this preliminary proof of concept MAS experiment indicate the marker effects are heritable in complex elite populations, evidence that QTL-informed MAS in forage populations is a viable option. To further assess the gains shown by MAS, this experiment has been transferred to a sward-based field environment with a companion grass under grazing to represent more typical agronomic conditions.

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Messina (*Melilotus siculus*) – a new salt and waterlogging tolerant annual pasture legume for southern Australia

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Abstract
The commonly sown pasture legumes in southern Australia are sensitive to the combination of salinity and waterlogging. Messina (*Melilotus siculus*) is an annual pasture legume with much higher salt tolerance as both seedlings and mature plants than other legumes and with similar waterlogging tolerance to balansa clover (*Trifolium michelianum*). Glasshouse and laboratory studies have identified several mechanisms for salt and waterlogging tolerances that explain its adaptation to saline, waterlogged soils. Trials through the Future Farm Industries CRC are underway in South Australia and Western Australia to select the best adapted of 21 messina accessions for release as a new cultivar. Selection is also being conducted for a salt tolerant rhizobium strain able to persist over summer and nodulate regenerating messina plants. This paper discusses the ecology, physiology and agronomy of messina, progress towards its commercialisation as a new pasture species for saline, waterlogged soils and further work required.

Key words: salinity, waterlogging, fodder, varieties, plant breeding, rhizobia, nitrogen fixation,

Introduction
Large areas of southern Australia have become seriously affected by dryland salinity due to the clearing of native vegetation and rising watertables. Figures derived from the National Land and Water Resources Audit (2001) suggest that 1.3-1.5 million ha of agricultural land in Australia are currently affected by dryland salinity, with this area predicted to increase to 1.7-2.3 million ha by 2020. Areas affected by dryland salinity are often affected by winter waterlogging. Plants growing in such environments are subject to the additional challenge of hypoxia (low oxygen concentration), increasing their susceptibility to high shoot Na^+ and Cl^-concentrations. These adverse conditions have severe effects on plant growth and survival (Barrett-Lennard, 2003). Such environments typically contain unproductive sea barley grass (*Hordeum marinum* L.) and are only suited to livestock production.

Greater animal production and subsequent economic benefits have been shown from incorporating saltland pastures into saline land (O'Connell *et al*. 2006). Saltland pastures are generally based on the salt-tolerant grasses, puccinellia (*Puccinellia ciliata* Bor) and tall wheat grass (*Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.-C. Wang) in high rainfall areas, or saltbush (*Atriplex* species) in low rainfall areas. However, saline landscapes are generally nitrogen deficient, resulting from denitrification and the lack of adapted legumes (Rogers *et al*., 2005). Companion legumes with salt and waterlogging tolerance are, therefore, required to sustain productivity of saltland pasture systems. Inclusion of an adapted legume into these environments can substantially increase farm profitability through increased pasture productivity and quality and subsequent improved animal performance (Masters *et al.* 2001).

Self-regenerating annual pasture legumes are widely used in the farming systems of southern Australia (Nichols *et al.* 2007). However, currently used legumes, particularly subterranean clover (*Trifolium subterraneum* L.), are very sensitive to salinity (Rogers *et al.*, 2005). Annual legumes need to germinate and establish in the years following sowing, but this occurs following the opening autumn rains, when soil surface salinity levels are generally at their highest levels (Nichols *et al.*, 2008). There is clearly a need to identify annual legumes adapted to the combined stresses of salinity and waterlogging on saline soils.

Messina, *Melilotus siculus* (Turra) Vitman ex B.D. Jacks (syn. *M. messanensis* (L.) Mill.), has been identified as a very promising annual pasture legume for saline, waterlogged soils. Work is progressing through the Future Farm Industries Cooperative Research Centre (FFI CRC) towards commercialisation of the species as a new plant for agriculture, along with an adapted rhizobium strain. This paper summarises the results of studies on messina, its prospects for commercialisation and further work required.

Adaptation of messina to saline, waterlogged soils
Messina is native to saline, marshy areas of the Mediterranean basin, Iberian peninsular and east Asia (Marañon *et al*., 1989) and is naturalised in similar environments in southern Australia (Jeanes, 1996; Paczkowska and Chapman, 2000). Initial interest in messina came from a series of trials conducted across southern Australia, in which herbage production and persistence of 42 annual pasture legumes from 33 species were measured over three years at five sites that varied in extent of both salinity and waterlogging (Nichols *et al.*, 2008). Burr medic (*Medicago polymorpha* L.) was productive on well drained soils with surface (0-10 cm) electrical conductivity (EC_e) in summer >8 dS/m, while balansa clover (*Trifolium michelianum* Savi)

was productive on soils subject to waterlogging, with summer surface EC_e <8 dS/m. Messina was the only species that regenerated on waterlogged sites with summer surface EC_e >8 dS/m and has persisted on sites with EC_e >30 dS/m (Nichols *et al.*, 2008; Nichols and Craig, unpublished data).

Salinity tolerance and avoidance mechanisms at germination

Messina has a range of salinity tolerance and avoidance mechanisms at germination. It has higher salinity tolerance *per se* as a germinating seedling than other pasture legumes. Nichols *et al.* (2009) showed messina germination was not reduced by 300 mM NaCl (equivalent to 30 dS/m), while significant reductions occurred for Scimitar burr medic at 240 mM and Frontier balansa clover at 120 mM NaCl. Studies by Rogers *et al.* (2011) and Jeffery (2011) confirmed the high salinity tolerance of messina at germination, and found variation within the species. For example Jeffery (2011) found no reduction in germination percentage at 300 mM NaCl, relative to 0 mM, in 19 of the 21 messina accessions tested, while Frontier balansa clover and Jota *Melilotus albus* had <18% germination.

Messina has an ability to recover germinability after exposure to high levels of salinity. Nichols *et al.* (2009) showed messina was able to recover 31% of its potential germinability upon transfer to non-saline solution after 21 days in 600 mM NaCl. Jeffery (2011) found variation within 21 messina accessions for germination recovery following 14 days at 600 mM NaCl, with four recovering full germinability and seven with >70% germination. This compared with no germination of Scimitar burr medic or Jota *Melilotus albus* and 64% germination of Frontier balansa clover.

Seed coat impermeability (hard seeds) was shown by Nichols *et al.* (2009) to protect the seed against the toxic effects of salinity over summer. They also showed messina had a delay in the timing of hard seed breakdown (seed softening) over the summer–autumn period, compared to *T. subterraneum* and *T. michelianum*, which were ready to germinate by mid-March. This delay acts as a salinity avoidance mechanism to defer germination until late autumn-early winter, when reliable rainfall, capable of flushing salts from the surface, is more likely to occur. Jeffery (2011) found variation for seed softening patterns among 21 messina accessions. The first significant seed softening occurred after mid-April in 18 accessions and after mid-May in four of them.

Tolerance to salinity and waterlogging in the vegetative stage

The high salt tolerance of messina has been confirmed in glasshouse experiments. In a study of 19 *Melilotus* species Rogers *et al.* (2008) found 2-month old messina plants subjected to 28 d in an aerated solution of 240 mM NaCl had 89% the shoot biomass of non-saline controls, compared to 31% for Paradana balansa clover. Rogers *et al.* (2011) found variation for salinity tolerance among 29 messina accessions, with ten having >80% the shoot biomass of non-saline controls after 21 d at 300 mM; no plants of Frontier balansa clover survived. In another experiment shoot biomass of messina was 30% that of non-saline controls after 21 d at 450 mM, compared to 15% for both Paradana balansa clover and Scimitar burr medic (Teakle *et al.* 2012).

The high waterlogging tolerance of messina has been confirmed in glasshouse studies. Rogers *et al.* (2008) found messina shoot biomass after 28 d in stagnant solution (designed to emulate the hypoxic conditions of waterlogged soils) was 102% of aerated controls, compared with 99% for Paradana balansa clover and 29% for Sceptre lucerne, *Medicago sativa* L. Root biomass of messina in the stagnant solution was 119% that of the aerated solution, compared with 144% for balansa clover and 32% for lucerne. Rogers *et al.* (2011) examined 23 messina accessions and found none had shoot growth reductions >20% in stagnant solution, compared with aerated controls, while root biomass was not reduced in any accession and increased by as much as 41%. Teakle *et al.* (2011) and Verboven *et al.* (2011) showed that waterlogging tolerance of messina is aided by a highly porous form of aerenchyma, termed phellem, which develops around the roots under stagnant conditions and enables O_2 transport from the hypocotyls.

A recent study by Teakle *et al.* (2012) showed that messina is very tolerant to the combined stresses of salinity and waterlogging. New leaves were produced in messina after 14 d in stagnant nutrient solution with 550 mM NaCl (~ sea water salinity), while both Paradana balansa clover and Scimitar burr medic died after 5 d in a 400 mM NaCl stagnant solution.

Commercialisation of messina and an adapted rhizobium strain

Initial field trials on saline, waterlogged soils showed that while messina was able to set seed and regenerate, the vast majority of regenerating seedlings failed to nodulate and grow (Nichols *et al.*, 2008; Bonython *et al.* 2011). This was shown to be due to the inability of the commercial annual medic rhizobia (*Sinorhizobium medicae* strain WSM 1115) to persist over summer in the highly saline soil surface (Bonython *et al.* 2011). A field screening has identified several *S. medicae* strains with much greater ability to nodulate regenerating messina plants on saline soils, most notably SRDI 554 (Bonython *et al.* 2011). This now paves the way for development of messina as a new species for agriculture.

Field trials are currently underway to evaluate 21 accessions of messina over a 3-year period on saline, waterlogged sites in South Australia and Western Australia. Measurements include biomass production, seed production, seedling regeneration densities and nodulation ability. It is intended that the best adapted variety will be released to the seed industry in early 2013, along with the best salt-tolerant rhizobium strain.

Further research

Before messina can be released as a new species for agriculture, duty of care trials need to be conducted to minimise any risks to animal health. Messina has negligible levels of the chemical coumarin, found in other *Melilotus* species (Nair *et al.*, unpublished data; Stevenson, 1969), which can taint the flavour of meat, milk and flour and cause a haemorrhagic condition in livestock if fed mouldy hay (Masters *et al.*, 2001). Messina also has similar nutritive value to other pasture legumes (Rogers *et al.* 2008). However, animal feeding trials need to be conducted to confirm its lack of anti-nutritional factors and its value as a stock feed.

Once the best adapted accession has been selected as a new cultivar, agronomic and grazing management packages need to be developed to optimise pasture performance and animal production. Factors include establishment methods, mixtures with salt tolerant grasses, fertiliser rates, broadleaf herbicide options and grazing strategies. Seed production strategies also need to be devised. A preliminary seed harvesting trial produced seed yields of over 1500 kg/ha (AD Craig, unpublished data), indicating the high yield potential of messina and its potential for seed harvesting with a conventional cereal harvester.

Conclusions

Messina will fill the gap of a pasture legume for waterlogged environments with summer soil surface salinity EC_e values >8 dS/m. It will markedly increase the productivity of sea barley grass flats and be a companion legume to puccinellia and tall wheat grass or as an understorey legume to saltbush. Glasshouse and laboratory studies have identified mechanisms for salt and waterlogging tolerance that explains its adaptation to saline, waterlogged soils. Conservative estimates suggest messina can improve productivity by 4 dse/ha across an area of 600,000 ha, with lesser gains being achieved across another 1 million ha. A new messina variety, along with an adapted salt-tolerant rhizobium strain, will be released to the seed industry in 2013.

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Evaluation of Grazing Tolerant Lucernes

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Abstract

Recent experiments have demonstrated that lucerne (*Medicago sativa)* varieties selected for grazing tolerance will persist far better when grazed for extended periods than varieties not selected for grazing tolerance. A field experiment, with lucerne varieties representing a range of winter activity ratings, was conducted at Leigh Creek. After 2.5 years of rotational grazing the experiment was continuously grazed for 173 days from late spring to early autumn at a stocking rate equivalent to 50 sheep/ha. This intensity is not considered much higher than some farmers would adopt during a drought. Ground cover of some varieties had significantly declined by the end of the grazing period but further decline occurred throughout the following winter months while livestock were excluded. The grazing tolerant lines persisted better than most standards, even those within the same dormancy category, with some standards almost completely dead while the grazing tolerant lines were over 60% alive.

Keywords: lucerne (*Medicago sativa*), persistence, grazing tolerance

Introduction

Lucerne *(Medicago sativa)* is the most widely grown perennial pasture legume in Australia (Lodge 1991). It will persist if strict management practices are adhered to and it is well understood that, for long-term productivity, lucerne pastures require rotational grazing (Leach 1968). Numerous studies recommend a minimum spell period of around 35 days and a short grazing period (Lodge 1991). These practices should provide a reasonable balance between persistence, quality, yield and animal safety. Nevertheless, many Australian farmers find ideal grazing management techniques impracticable and hard to sustain under tough seasonal conditions.

There are a number of factors which contribute to the low rate of adoption of ideal grazing rotations as outlined by Lodge (1991). Paddocks are often too large and high costs are associated with sub-dividing and watering in broadacre pasture systems, additional management is required and perhaps there is a poor understanding of the benefits of rotational grazing. However, the greatest obstacle to adoption is that over a very long dry summer, when the only green feed on the farm is lucerne, farmers will leave their valuable stock on lucerne for extended periods despite the, known, consequences.

The adverse effects of continuous grazing on lucerne plants have been reported by Smith *et al.* (1989) and set-stocking with high stocking rates under dry Australian conditions will most likely result in a rapid decline in stand persistence (Brownlee 1973). There is a strong correlation between winter dormancy and grazing tolerance (Humphries *et al.* 2006; Bouton & Gates 2003), but there are also a number of other traits that contribute to differences in lucerne persistence under grazing. Smith *et al.* (1989) and Humphries *et al.* (2006) cite various authors who have found that grazing tolerance in lucerne has been linked to deep set crowns, decumbency or prostrate habit, subsurface budding, broad crowns, prolific and non-synchronous budding, extended periods of budding, maintenance of leaf area under grazing and maintenance of root carbohydrates.

A 6 year grazing tolerance screening program, which was conducted in the United States by Cal/West Seeds, combined elite breeding lines and successful commercial cultivars. Experiments were continuously grazed by both sheep and beef cattle for lengthy periods at high stocking rates, following the protocol developed at the University of Georgia and the North American Alfalfa Improvement Conference (NAAIC) standard test protocol (Bouton & Smith 1998). Further screening of twenty-four lines of elite material from this program, and fourteen commercially available cultivars, was conducted at two sites in Australia by Wrightson Seeds from 1999 to 2005 (Salmon & Hill 2008). After 4 years of rotational grazing, the Leigh Creek (south western Victoria) experiment was subjected to continuous grazing for 2 years (40 sheep/ha with a 2 month spell after 10 months). A similar experiment was conducted at Gundagai (southern New South Wales) where a severe drought was experienced; final selections were made from plants subjected to this extreme stress. Almost all of the US lines developed for grazing tolerance persisted better than the 'Australian' commercial cultivars. Two test lines were identified as having an ideal balance of persistence, winter activity and yield and released as 'Stamina 5' and 'Stamina GT6'.

Here we report a subsequent experiment established in 2006 at the PGGWrightson Seeds Leigh Creek Research Farm to evaluate new experimental grazing-tolerant lines, including selections taken from the 1999-sown experiments (above) and commercial cultivars.

Methods

Experimental treatments and design

The NAAIC standard test protocol to screen lucerne for grazing tolerance (Bouton & Smith 1998) was used, however there was one major exception being that sheep were used in this experiment, rather than cattle, for closer grazing. There was no leaf area maintained under severe sheep defoliation and the resulting stress on the lucerne plant was dramatic (Smith *et al.* 2000).

The trial was drill-sown on the 1st of September 2006 at the PGGWrightson Seeds Research Farm at Leigh Creek (-37°56'S, 143°95'E), south-western Victoria. The soil is a deep red Krasnozem derived from volcanic ash. The site was previously limed, sprayed with 3 lt/ha of Roundup 1 month prior to cultivation, and the seedbed was prepared to a fine tilth.

There were 20 entries comprising of 13 commercially available cultivars representing various winter activity ratings, including 'Stamina 5' and 'Stamina GT6' as the grazing tolerant control types, and 7 experimental lines of varying winter-activity ratings labelled PGWS-1 to PGWS-7. PGWS-1,2,3 and 5 were specifically developed for grazing tolerance. The seeding rate was 20 kg/ha and all seed was lime coated and inoculated and sown with a 10-row, precision cone-seeder and roller.
Plots of 1.30 x 5.08 m were laid out as a randomly allocated factorial design with 4 replicates. The plot area was surrounded by a 10 m border sown to lucerne. Drinking water, a supplement feeding area and a shade house were situated along the fence-lines and 10 m away from the trial plots.

Grazing
After reaching an initial flowering stage, the experiment was rotationally grazed/cut for 2.5 years and yields were recorded. After the 3rd October 2009 the trial was continuously grazed with 14 crossbred wethers (equivalent to 50 sheep/ha) until the 25th March 2010 (173 days). The sheep were fed a supplement of grain towards the end of the grazing period. After an assessment of ground cover was made the lucerne was chemically 'winter cleaned' (2.5 lt/ha diuron; 2 lt/ha diquat) and plants allowed to recover to ensure no depletion of the stand occurred due to chemical application. The experimental plots were then spelled until September when, after 3 days of grazing, a further assessment was made.

Measurements and Analysis
Ground cover was measured on the 18th December 2006. Subsequent measurements were made 13 and 7 days, respectively, after de-stocking in April and September 2010. These intervals allowed the lucerne to regrow to 5 – 7.5 cm height. The proportion of a 1.0 m length of drill row supporting lucerne growth was visually assessed from six randomly selected sites within each plot to provide an estimate of ground cover. Analysis of variance was carried out using the 'Statistix 2.0' program.

Results and Discussion

Differences in persistence between the grazing tolerant and the non-grazing types had become

Table 1 2006 Leigh Creek trial - Ground cover percentages (GC%) per meter row for the pre-grazing, first assessment (7th April 2010) and final assessment (8th September 2010) and cumulative yield (DM t/ha) before continuous grazing.

Cultivar	Winter Activity	Cumulative DM t/ha until 20/02/2009		Ground Cover %				
				Initial 18/12/2006	First † 07/04/2010		Final † 08/09/2010	
Non-grazing tolerant varieties								
Aurora	6	19.52	defg	94	51	gh	23	fghij
Australis	9	21.85	abc	97	29	i	5	k
Genesis	7	18.36	g	92	66	ef	28	fghi
Hunterfield	6	18.68	fg	95	70	e	47	de
Icon	6	21.40	abcd	93	38	hi	14	ijk
Kaituna	5	22.33	a	92	76	cde	36	ef
PGWS-4	6	20.42	abcdefg	94	47	gh	18	hijk
PGWS-6	7	22.25	ab	94	78	bcde	35	efg
PGWS-7	7	20.04	bcdefg	94	55	fg	25	fghi
Sardi 7	7	19.07	efg	93	43	ghi	20	ghij
SD 54Q53	4	20.61	abcdef	92	69	ef	35	efg
SD L56	5	21.54	abcd	93	72	de	32	efgh
UQL-1	7	21.21	abcde	95	38	hi	10	jk
Mean		20.56		94	56		25	
Grazing tolerant varieties								
PGWS-1	5	20.96	abcde	93	87	abc	67	c
PGWS-2	4	20.75	abcdef	89	89	abc	71	bc
PGWS-3	6	20.25	abcdefg	96	77	cde	68	bc
PGWS-5	3	19.75	cedefg	94	93	a	82	ab
Stamina 5	5	19.95	cdefg	96	92	ab	90	a
Stamina GT6	6	20.83	abcdef	97	85	abcd	61	cd
Venus	5	20.55	abcdefg	89	91	ab	76	abc
Mean		20.43		93	88		74	
LSD (0.05)		2.25		4.5	14.4		15.5	
CV%		7.8		3.4	15.1		25.9	

†Means with the same letters within a column are not significantly different (P<0.05)

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visually apparent during the first grazing period and were reflected in the April assessment (Table 1). Stand density was significantly reduced in the non-grazing tolerant types after this initial 5 months of set-stocking. Despite minimal grazing over the winter the non-grazing tolerant types continued to decline, presumably due to their depleted energy reserves, and the September assessment revealed significantly less ground cover than the April assessment.

Defoliation in autumn can reduce the level of root carbohydrate reserves in the plant, the level of winter hardiness and survival and the number of crown buds available for spring growth (Smith 1972). Winter injury from extreme cold is unlikely to occur in Australia, although autumn and winter grazing may affect long-term persistence (Lodge 1991), particularly for non-grazing tolerant types. Brummer & Bouton (1992) suggested that the ability of cultivars to produce and store high levels of total non-structural carbohydrates may enhance grazing tolerance. This physiological attribute is particularly important through the critical autumn period under continuous grazing. There are other attributes that can affect persistence of lucerne stands such as disease. While this site was situated on a free draining soil, there are many soils where lucerne is grown which are slightly heavier in texture and less well-drained. Phytophthora root rot (*Phytophthora medicaginis*) is recognised throughout Queensland and NSW river systems and flood plains (Rogers *et al.* 1978), and selection for resistance has long been emphasised as important for persistence. .

Stamina 5 was the most persistent cultivar with a 6% decline in stand density over 4 years. Some of the experimental lines selected under continuous grazing persisted well (eg PGWS-5, 3 and 2) but none were superior to Stamina 5. Almost all of the lines developed for grazing tolerance persisted significantly better than the non-grazing tolerant types, irrespective of dormancy group. The only exception to this was Hunterfield (47% final GC). All the grazing tolerant lucernes had final ground covers greater than 60%.
These results show that selection for grazing tolerance can be effective in lucerne of diverse winter activity groups. Considerable differences in persistence occurred between varieties within the same winter activity group – Stamina GT6 (61%) versus Icon (14%) both winter active 6, and Stamina 5 (90%) versus SDL56 (32%) both winter active 5. The negative correlation between higher winter activity and grazing tolerance (Figure 1) supports previous findings (Brummer & Bouton 1991; Smith *et al.* 1989). Similar results under grazing by sheep in Australia have also been recorded by Humphries *et al.* (2006) who reported final plant frequencies ranging from 0 -13 % for highly winter-active entries and 11-40% for winter dormant entries.
The consequence of subjecting highly winter active cultivars to continuous periods of grazing is clearly illustrated in Figure 2, with Stamina 5 directly compared to Australis. Further evidence of this correlation was recorded in a dry-land trial sown in 2003 at Bendigo (north central Victoria). In this trial the highly winter active varieties were the least persistent following the 4 years of almost continuous grazing (Figure 3). Hunterfield, Stamina GT6, a grazing tolerant test cultivar and Venus all performed well as seen in the subsequent 2006 Leigh Creek trial. Figure 4 shows the results of the key lines from the 1999 sown trials that were re-tested in subsequent grazing trials revealing similar results to those in Table 1 and Figure 3.

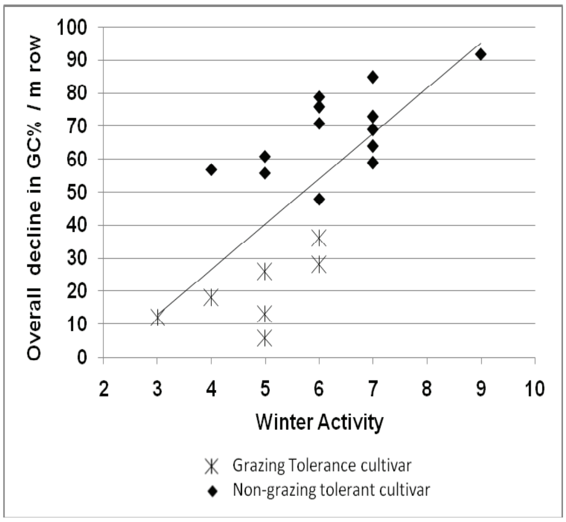

Figure 1 Relationship between winter activity rating and overall decline in stand density (GC%). R^2 = 0.499.

Improved persistence also results in reduced weed invasion (Bouton *et al.* 2001) and grazing tolerant lines have been shown to yield as well, or better, than non-grazing tolerant lines even under rotational stocking and mechanical hay harvesting (Bouton & Gates 2003). Cumulative dry matter yield for the 2.5 years before the continuous grazing treatment (Table 1) indicates that there was very little difference in overall yield for the grazing tolerant and non-grazing tolerant varieties. However, the grazing tolerant varieties are likely to produce superior yields after the grazing stress due to higher plant density (Bouton *et al.* 2001).
Most Australian farmers understand the need for appropriate spells to enable lucerne to replenish carbohydrate reserves. In times of drought, however, lucerne will be overgrazed and the benefits of grazing tolerant lucernes such as Stamina 5 and Stamina GT6 should be significant.

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Figure 2 Leigh Creek trial (2006)- Stamina 5 (left) in comparison to Australis (right). Photo 8/9/2010.

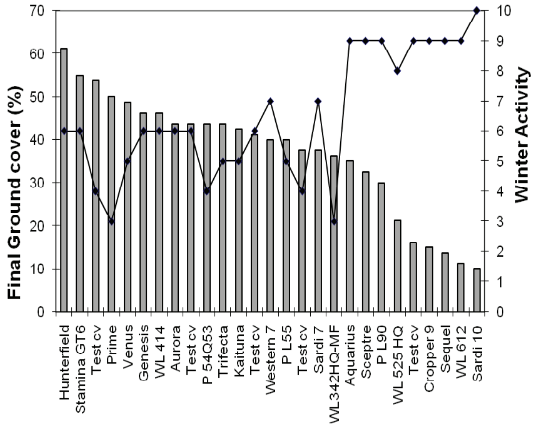

Figure 3 Bendigo lucerne trial (2003 sown) - Final ground cover percentages (left axis), measured 24-April-2004 following 4 years of continuous grazing relative to the actual winter activity rating of the variety (right axis). LSD (0.05) = 13.5, CV = 25.9%

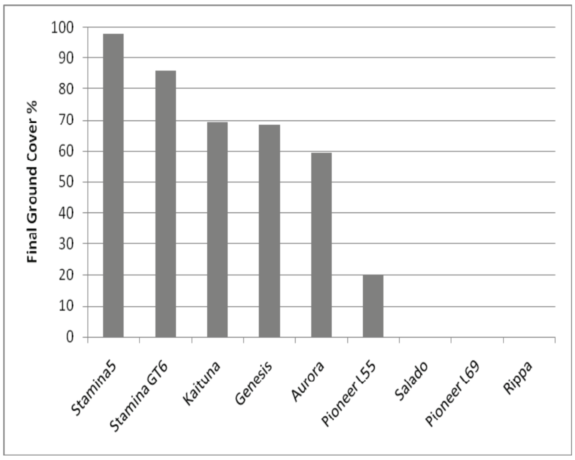

Figure 4 Leigh Creek lucerne trial (1999 sown) - Final ground cover percentages measured 17-Aug-05, following 2 years of continuous grazing. Note: there a number of other treatments in this trial that have been excluded from the dataset – but the key lines that have been repeated in the subsequent trials (2003 Bendigo and 2006 Leigh Creek) have been included, in addition to the highly winter active lines (Salado, Pioneer L69 and Rippa). LSD (0.05) = 11.8, CV = 18.1%

Spatio-temporal profile of proanthocyanidin biosynthesis in white clover (*Trifolium repens* L.) flowers

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Abstract
Proanthocyanidins (PAs) are a class of flavonoid compounds produced by plants that ameliorate pasture bloat and improve the uptake of amino acids when present at low levels in the diet of ruminants. Correlations have been found between patterns of PA accumulation and spatio-temporal expression profiles of flavonoid genes involved in PA biosynthesis in a number of plant species. We have characterized the pattern of PA accumulation in different organs, tissues, cells and cell compartments of white clover flowers at different stages of development. This pattern correlated well with the spatio-temporal expression profile of the *Anthocyanidin reductase* (*ANR*) gene, a molecular marker of the PA pathway, as well as cell-specific and cell compartment-specific accumulation of a translational fusion between the ANR protein and GFP. We present a model for developmentally-regulated and cell-specific biosynthesis of PAs and compartmentalization of PAs and corresponding monomers in white clover flowers.

Comparison of homoeolocus organisation in paired BAC clones from allotetraploid white clover (Trifolium repens L.) and microcolinearity with model legume species

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Abstract
Sequence conservation and divergence between the two sub-genomes of the allotetraploid (2n = 4x = 32) forage legume white clover (*Trifolium repens* L.) was assessed through the sequencing of homoeologous bacterial artificial chromosome (BAC) pairs. White clover is thought to have originated through the hybridisation of *Trifolium occidentale* D.E. Coombe and another, as yet unidentified, species. The two sub-genomes have therefore been designated O and P' as the latter sub-genome appears to be more distantly related to both *T. occidentale* and the other candidate progenitor, *T. pallescens* Schreber. Previous re-sequencing studies of selected protein-coding genes allowed identification of sub-genome-specific features and the subsequent selection of paired homoeologous BACs. A total of four BAC pairs were sequenced to produce 173 kb of overlapping sequence that could be compared between the two sub-genomes. Of the four regions analysed, three displayed similar patterns of conservation, in which the majority of genes are retained between sub-genomes, but nucleotide identity across intergenic space was generally low. The other sequenced region revealed high levels of conservation across both predicted coding regions and intergenic space for the first 35 kb to be analysed, following which sub-genomic structure exhibited complete divergence. The number of non-synonymous substitutions between homoeologous gene pairs permitted estimation of divergence between the O and P' sub-genomes of c. 4.2 million years. This study also included the comparison of white clover BAC sequence to the corresponding regions within the genomes of the model legume species *Medicago truncatula* and *Lotus japonicus*, as well as *Arabidopsis thaliana*. The analysis of microsynteny between the four homoeologous regions and the two model legume taxa has provided important implications for strategies of translational genomics and for developing future whole genome sequencing strategies to be applied to white clover.

Transgenic white clover plants (*Trifolium repens* L.) resistent to white clover mosaic virus

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Abstract

White clover (*Trifolium repens L.*), a perennial pasture legume, is an important component of improved pastures in temperate areas throughout the world, but its productivity and persistence is significantly reduced by white clover mosaic virus (WCMV). We have explored a transgenic approach for the development of virus-resistant white clover plants, due to the lack of naturally occurring resistance to WCMV. Transgenic white clover plants producing sense-suppression (cosuppression), antisense and hairpin RNA (hpRNA) transcripts corresponding to the WCMV replicase gene were produced and analysed at molecular and phenotypic levels. Expression of hpRNA and antisense transgenes provided high-level resistance to WCMV, while the sense transgene provided only partial resistance. The presence of small interfering RNA molecules (siRNAs) prior to virus challenge indicated that WCMV resistance was due to pre-activated RNA silencing, and was a reliable biomarker for prediction of virus resistance in these plants.

Strategies for transgenic stacking in white clover (*Trifolium repens* L.)

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Abstract

White clover (*Trifolium repens* L.), a perennial pasture legume, is an important component of improved pastures in temperate areas throughout the world. Its productivity is limited by aluminum (Al) susceptibility and low phosphorus acquisition efficiency in acidic soils, as well as susceptibility to viral diseases. Proof of concept has been obtained in transgenic white clover plants expressing individual transgenes, as follows: (i) a nodule-enhanced malate dehydrogenase (*TrneMDH*) gene under the control of a root-specific (white clover phosphate transporter *TrPT1*) promoter; (ii) the alfalfa mosaic virus (AMV) coat protein gene (*AMVCP*) under the control of the constitutive, enhanced *CaMV 35S* promoter, and (iii) a cytokinin-related isopentenyl transferase (*ipt*) gene from *Agrobacterium tumefaciens* under control of the *AtMYB32* promoter to confer Al tolerance, field resistance to AMV and enhanced seed and biomass yield, respectively. Strategies were designed for the development of elite transgenic white clover germplasm expressing these three transgenes. Transgenic white clover plants expressing combinations of these transgenes were generated and shown to exhibit transgene-encoded traits under containment glasshouse conditions for the selection of transformation events to be subjected to detailed, whole of life cycle, quantitative phenotyping utilizing a forage phenomics platform.

Genetic trait dissection for saline stress tolerance in white clover

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Abstract

White clover (*Trifolium repens* L. 2n = 4χ =32), a predominant legume component of temperate pasture, is generally regarded as a salt sensitive species. Notably, different levels of salt tolerance have been documented in white clover natural populations. Genetic trait dissection for saline stress tolerance is important to understand the underlying genetics and to improve growth performance on saline land. To identify quantitative trait loci (QTL) for salt tolerance in white clover a two-way pseudo-test cross F_1 population was derived from pair-crossing of $Haifa_2$ (salt-susceptible) and LCL_2 (a second cycle selection from cultivar Haifa for low shoot Cl^- level and salt-tolerant). Phenotypic analysis of the progeny was conducted in nutrient solution culture in greenhouse with two NaCl levels (0, 75 mM NaCl) and three replicates. Genetic linkage maps were constructed using simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers. A new linkage group (LG) nomenclature system was adopted based on alignment between the draft genome sequence of the model legume species *Medicago truncatula* and the two proposed sub-genomes (O and P') of white clover. QTL analysis revealed a total of 8 unique genomic regions on 8 LGs of the $Haifa_2$ genetic map and 6 unique regions on 5 LGs in the LCL_2 genetic map associated with plant growth under salt stress and relative growth under stress, as compared to control conditions. The proportion of phenotypic variance explained by individual QTL was from 5 to 17%. The results of this study suggest that salt tolerance in white clover is controlled by multiple QTL, each of limited magnitude. Location of these QTL provides the genetic basis and potential for pyramiding of salt tolerance genes in white clover improvement. Validation of the QTL is currently being performed in another population $F_1(S184_6 \times LCL_6)$, of which the two parental genotypes have highly divergent origins.

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