Perennial Grasses in Pasture Production Systems - Symposium

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May 15-16 2013

Australian Grasslands Association

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

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Perennial Grasses in Pasture Production Systems

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Carol Harris

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The Australian Grasslands Association is a partnership between the Grassland Society of Southern Australian and the Grassland Society of NSW.

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.

Perennial Grasses in Pasture Production Systems - Symposium Organising Committee

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Posters

Introduction

R. Salmon

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On behalf of the organising committee, I am pleased to present the proceedings of the 2013 Symposium on Perennial Grasses in Pasture Production Systems, the second iteration of the Australian Grasslands Association.

The collective knowledge of the scientific and extension community has been invited to participate in this forum to review the perennial grasses sector of the Southern pastures industry. This two day symposium aims to draw on that power to review research and development and contribute to the pursuit of future objectives for perennial grasses that should in the medium to long term.

The Australian Grasslands Association was set up as a joint initiative of the Grasslands Society of Southern Australia and the New South Wales Grasslands Society, to increase the involvement of those 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.

This year I am pleased to announce that the Western Australian group Evergreen Farming has joined this initiative as an associate, increasing the national relevance and impact of the initiative.

The primary activity of the Association is to facilitate pasture research reviews. These reviews will be on a different topic and will be performed through 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)

- To consider the long-term "Blue sky" ideas that will push the boundaries, specifically how we may make the next quantum leap?

The output of the symposium will be a summary paper, capturing and characterising the topics addressed in the presentations and reporting on ensuing discussion.

The credibility of all presented papers will be leveraged by publishing in the peer-reviewed journals. As a result this proceedings contains only abstracts of the presented papers plus the full poster papers.

Why is this important?

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

In assessing the opportunity within the pastures space for these reviews, perennial grasses were chosen as the second topic.

As background, the benefits perennial grasses confer to pasture production systems through direct contribution of biomass growth, rapid responses to the autumn 'break,' feed quality, and subsequent improvements in animal production are easily and commonly attributed to perennial grasses. Less often attributed to perennial grasses and the role they play in pastures are the environmental benefits (including ground cover, soil protection/retention, water infiltration, deep roots and soil macropores etc.) plus the business risk management benefits from having livestock operations within a cropping-focussed farming enterprise.

Despite the broad range of well-adapted species and varieties of perennial grasses used in Australia, a series of droughts have multiplied the agents of pasture deterioration (including under grazing or overgrazing at critical times, low fertility/pH, soil structure issues such as compaction, weeds, plant diseases and insect pests).

Given the recent pressures, and the gravity of the contribution of pastures to Australia's productive capacity, it is important that the industry maintains a focussed forum for discussion of specialised themes, 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. Such an opportunity will support the maintenance of technical currency and competency in such specialised themes.

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. 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.

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 - Perennial Grasses in Pasture Production Systems

Session 1

Perennial grasses in temperate, clover-based pasture – their introduction, use and development for Australia

KFM Reed, RPS Consultanting

Abstract

The use of perennial ryegrass, phalaris, cocksfoot and tall fescue in mixed pasture is summarised. Following settlement, more than a century of exploitive, extensive grazing and pasture improvement based imported seed, an active decade of Aberystwyth-influenced pasture science in the 1920s set the foundations for the modern Australian seed industry. Emphasis was on ensuring persistence. The next half century, the era of pasture improvement, delivered seed testing, ecotype assessment and phenotypic selection against a background of a dramatic rise in sheep and cattle numbers - a consequence of the widening application of superphosphate and its effect on biological nitrogen fixation by the subterranean clover-Rhizobia symbiosis. The growing recognition of phalaris and the potential value of other Mediterranean grass ecotypes was stimulated by CSIRO's new Plant Introduction Service. Such material has delivered valuable increases in seedling vigour, winter growth and drought tolerance and extended the area of adaptation of perennial grasses. The modern era commencing, from 1980, is characterised by a steep rise in the number of newly-bred proprietary cultivars, the emergence of select endophytes and tetraploid perennial ryegrass, altered fertiliser practices and major investment in the promise of Agribiosciences. Significant cooperative arrangements that have stimulated the improvement of perennial pasture are outlined. Given the race to capture expanding markets, there remain opportunities for investment in high rainfall land where well established technology has yet to be applied.

Session 2 - Developments and innovations in perennial grass agronomy and management

Quantifying the interactions between grazing interval, grazing intensity, and nitrogen on the yield and persistence of rain-fed and irrigated perennial ryegrass

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Abstract

It is generally acknowledged that sound planning and good management of the feedbase are the keys to dairy business success. However, there remains some conjecture regarding agreed grazing management practices for pasture-based dairy systems. This speculation is mostly due to lack of knowledge regarding the seasonal and management (irrigation and nitrogen (N) usage) influences on grazing management (defoliation interval and height) rules. While a number of studies have researched individual aspects of grazing management, including defoliation interval, defoliation height, and water and N use, there are few data exploring the interactions between these variables. A two year defoliation experiment was undertaken in the high rainfall zone of north west Tasmania to examine the effects of these interactions on the yield and persistence of perennial ryegrass pasture. The study was a split-split plot design with three defoliation interval (the full emergence of 1, 2, or 3 new leaves per tiller), by three defoliation height (3.0, 5.5 and 8.0 cm) by three N application rates (0.0, 1.5 and 3.0 kg N/ha.day) treatments under both rain-fed and irrigated conditions. There was a significant ($P < 0.05$) independent effect of each treatment on total yield, and a significant interaction ($P < 0.05$) between defoliation height and N application rate, and between irrigation, defoliation interval and N application. There was a significant effect of defoliation height and N application rate on tiller density. This paper presents these results and discusses grazing management recommendations that optimise both pasture yield and plant persistence.

Kikuyu-based pasture for dairy production

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Abstract

Low cost, pasture-based production systems are a key competitive advantage for the wool, meat and milk commodities of Australia.

In dairying, the profitability of pasture-based systems is closely linked to the amount of pasture (or home-grown feed) converted into animal product (Garcia and Fulkerson 2005), which in turn is a function of the amount of plant tissue harvested by the animals (i.e. 'utilisation' (Holmes et al. 2002; Garcia and Fulkerson 2005) and the efficiency of its conversion into milk (Beever and Doyle, 2007).

In the southern Australian states of Victoria and Tasmania, dairy farms are mostly reliant on perennial ryegrass (*Lolium perenne* L.) as the predominant grass species. Conversely, tropical grasses are the predominant dairy forage in summer and autumn along the coastal areas of New South Wales and Queensland (Doyle *et al*. 2000; Fulkerson and Doyle 2001). The predominant C4 grass used in coastal beef and dairy systems is kikuyu grass (*Pennisetum clandestinum*), or simply 'kikuyu' (Fulkerson and Doyle 2001). Kikuyu is a perennial tropical grass of C4 photosynthetic pathway adapted to subtropical and warm-temperate climates, believed to have originated on the highland plateau of central and east Africa at altitudes of >1,500 m and annual rainfall regimes >1,000 mm (Marais 2001). Outside Australia, kikuyu also constitutes the pasture base of dairy regions in Northland, New Zealand, Kwazulu Natal, South Africa and South (Colombia) and Central (Costa Rica) America.

Kikuyu is well known for its high yield potential, excellent response to fertility and water, resistance to trampling and persistence. Less desirable characteristics include its aggressive/dominance features resembling weed-type behaviour; relatively poorer nutritive value than other perennial grasses; mineral imbalances; animal poisoning and its susceptibility to soil-born pathogens, particularly fungi.

Most of these aspects have been extensively studied over the last 50 years and several literature reviews have been published in the 21st century. In particular, the comprehensive reviews by Marais (2001) in South Africa and Correa (Correa C *et al*. 2008a; Correa C *et al*. 2008b) in Colombia discussed positive and negative attributes of kikuyu for animal production in great detail. Crush and Rowarth (2007) on the other hand analysed results of trials in Australian and New Zealand animal production systems in an attempt to identify the difficulties of incorporating C4 grasses into high producing pasture-based systems. Overall, the current state of knowledge given in previous reviews indicates that several key challenges needs to be addressed and overcome to achieve productivity gains from kikuyu-based pasture systems in the future. These challenges are associated with dry matter production and utilisation, losses in productivity; nutritive value, dry matter intake and milk yield from kikuyu-based pastures.

In this review, we bring together the literature to highlight the opportunities to close the gap between current and potential utilisation and increase dairy production from kikuyu-based pastures.

The development of sub-tropical grass-based pastures in a Mediterranean environment

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Abstract

Perennial sub-tropical grasses are now a proven option for increasing productivity and reducing erosion on infertile sandy soils in the northern agricultural region (NAR) of Western Australia. However, developing a grazing system based on sub-tropical grasses in a Mediterranean environment has presented a number of challenges.

This paper firstly explains why sub-tropical grasses are a successful option in a region with hot, dry summers and secondly describes a body of systematic applied research and development towards developing productive, persistent grazing systems based on sub-tropical grasses.

A series of replicated pasture production and feed quality trials measured the biomass and persistence in the medium-term of the key species. Overall, the results indicate that sub-tropical grasses have a long-term role in the NAR in areas with mild winters and/or where the rainfall >400 mm. The best performed sub-tropical grasses across a range of sites were panic grass (*Megathyrsus maximus* cv. Gatton, green panic) and Rhodes grass (*Chloris gayana*).

Commercial establishment was problematic with variable results both within a paddock and between years and many early growers had one or more failures. The variable establishment was a major barrier to widespread adoption and the methods used to establish these grasses in sub-tropical environments were not applicable. Through a collaborative research approach between leading growers, grower groups and researchers a reliable, robust establishment package has been developed which has dramatically improved establishment. Many leading growers have specifically modified seeding machinery for sowing sub-tropical grasses.

Another challenge was that a number of cases of secondary photosensitisation in both sheep and cattle grazing perennial grass-based pastures have been reported in WA. Secondary photosensitisation from perennial grasses has been reported in the literature, but is uncommon. The relatively high incidence in WA appears to be related to environmental stresses and that outside the growing season the sub-tropical perennial grasses represent all or most of the palatable green feed-on-offer. Subsequent investigations have implicated high levels of steroidal saponins in signal grass (*Urochloa decumbens*) as the main cause. The recommendation is not to include signal grass in future seed mixes.

Germinable soil seed bank dynamics of Microlaena stipoides in grazing systems of south-eastern Australia

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Abstract

Microlaena (*Microlaena stipoides* var. *stipoides* (Labill.) R. Br.) is a C3 perennial grass that is native to areas of south-eastern Australia. In this region perennial grasses are important for the grazing industries because of their extended growing season and persistence over a number of years. Soil seed banks were studied because they play an important role in plant population dynamics and community structure. They serve as pools of genetic material that act as a population buffer against adverse climatic conditions.

An experiment to study seedbanks was conducted at Chiltern (S36°12', E146°35', 256 m) in north-east Victoria, on an existing native grass pasture dominated by Microlaena. An area of approximately 1 m2 around 16 plants chosen for study was sampled with 24 cores (50 mm diameter and 50 mm depth) collected and bulked in July 2010 and again in May 2011. The soil samples were spread evenly over seed raising flats containing sand/peat mix (2:1) with a layer of vermiculite and maintained in a glasshouse, under natural light and modified day/night temperatures. The samples were kept moist for periods of between 42 and 58 d. During each census, germinants were identified to the following functional groups (Microlaena, broadleaf, sedges, grass and legume) and removed. At the end of each cycle remaining seedlings were counted and water withheld. The dry soil samples were then thoroughly mixed and re-watered to initiate another cohort of germination. This cycle was repeated three times over two nine month periods.

These counts showed that Microlaena only represented low numbers in the seed bank (0.01 to 0.05% of total germinants), with the seed bank dominated by species other than Microlaena. Observations over a number of years showed that seasonal conditions had an influence on the number of Microlaena seedlings in the seed bank, with more than double the number of Microlaena seeds in the seed bank after a wet summer. Furthermore, it seems clear that any seedlings of Microlaena that germinate from the seed bank would face immense competition from other species. Consequently, management strategies for Microlaena dominant pastures need to focus on the maintenance of existing plants.

Session 3 - Developments and innovations in perennial grass breeding

Perennial Grass Breeding – continuing themes and new approaches

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Abstract

Perennial grass species forming the base of improved temperate pastures have been subject to organised breeding programmes in Australia and New Zealand for nearly 100 years. True perennity, productivity and nutritive quality have been key themes throughout. There have been major historic and ongoing changes in generation and harnessing of genetic variation and in understanding and assessment of the key trait themes. New systems of trait assessment, data capture, accessing and characterising genetic variation all offer improved perspectives. Environmental impact imperatives are a recent feature, as is critical scrutiny of historic gain and value on farm, from end user organisations. While significant effort in tall fescue, cocksfoot and some other species will be noted, the above considerations will be mostly discussed in relation to phalaris breeding in Australia and perennial ryegrass breeding in New Zealand.

Persistence traits in phalaris and recent advances through breeding

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Abstract

Persistence is consistently claimed by Australian farmers to be a high value trait for perennial grasses. Phalaris is a productive perennial grass with proven persistence in south-eastern Australia. Nevertheless factors which influence the persistence of pasture species in southern Australia related to climate (drought), soil (acidity), grazing pressure and, importantly, their interaction can reduce persistence of phalaris and other species in various situations. Both management and plant breeding provide options to improve persistence with the most durable outcomes achieved when the options are employed concurrently. Two examples of breeding to improve persistence traits in phalaris are described. A program to improve acid soil tolerance resulted first in the release of cv. Landmaster and more recently cv. Advanced AT. The higher Al tolerance of Advanced AT is of most benefit in more assured establishment on acid soils under variable moisture conditions and improved flexibility of sowing date. Cv. Holdfast GT was bred to address complaints of poor persistence under heavy grazing by cultivars of the highly-productive winter-active type, since high grazing tolerance is needed to achieve profitable returns from developed pasture land. Evidence of good persistence under grazing for cv. Holdfast GT and possible tradeoffs with productivity are discussed. Maintaining high productivity under a predicted higher incidence of drought stress (climate change) and increasing areas of acid soils presents ongoing challenges for persistence in pastures.

High resolution and throughput phenomic screening technologies offer an opportunity to accelerate pasture breeding objectives

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Abstract

Phenomics (the study of phenotypic expression) is being used to aid plant breeding in many agriculturally important crop species. The High Resolution Plant Phenomics Centre is pioneering the development and integration of new technologies to study plant architecture and function simultaneously. Capture of a range of data correlated to traits of interest such as water, nitrogen and phosphorus-use efficiency is also being automated both in controlled and field environments. Three dimensional reconstructions of plant species can then be overlaid with layers of agriculturally significant information in a way analogous to the use of geographic information systems (GIS). The resolution and speed at which plants can be analysed may allow plant breeders to identify individual plants with specific traits of interest, from amongst large numbers of individuals, such as those in recombinant inbred line populations, where the genetics are already known, but the action of genes of trait of interest may not be established. This paper outlines these technologies in the context of pasture species breeding objectives.

A molecular phylogenetic framework for cocksfoot (*Dactylis glomerata* L.) improvement

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Abstract

The recently completed molecular phylogenetic analysis of *Dactylis* germplasm has provided a clear evolutionary history of the diploid *Dactylis* from which modern tetraploid germplasm and cultivars have developed. This framework will allow us to fully use a wider range of both diploid and tetraploid germplasm for a more systematic improvement of cocksfoot.

Germplasm of many diploid and tetraploid forms is under serious threat from habitat degradation and climate warming in situ and unfortunately many forms are currently poorly represented in ex situ genebanks. It is critical that a wide range of these forms be collected for storage and that core collections are developed and maintained using molecular phylogenetic and genetic diversity information as the basic framework.

In order to apply molecular resources in an effective and balanced manner, it is important to ensure pragmatic field breeding programmes continue in all major regions. This is of major concern for cocksfoot as it is a species with limited international breeding investment.

It is crucial that viable large-scale cocksfoot breeding programmes are maintained internationally to allow adequate cultivar development but also ongoing germplasm collection, introgression from wild germplasm and application of molecular resources.

Determining the value of genetic gain in perennial grasses

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Session 3

Abstract

In contrast to forest tree and animal breeding where the potential value of genetic advances is often described in terms of the increased value of product likely to be obtained from the use of novel genetics, genetic gain in pastures is more often described as a relative advantage in overall or seasonal yield or as the relative improvement of a specific trait. The use of value based descriptors and the genetic and economic analyses that underpin them has led to increased rates of genetic gain in these species and has facilitated the marketing of novel genetics based on the likely value to producers.

The multiplicity of environments and production systems where pastures are used, the complex genetic architecture of many pasture cultivars and the indirect relationship between pasture attributes and animal production have all been cited as impediments to the adoption of these methods in pastures.

This paper will describe practical examples of the development of value based indices to describe the relative value of advances in pasture yield, quality and persistence and discuss how these models could be applied more broadly to describe the value of perennial grass cultivars and germplasm for Australian production systems.

Session 4 - Opportunities and roles for perennial grasses in a changing climate

Identifying perennial grass plant traits for future warmer and drier climates

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Abstract

There is potential to select pasture species better adapted to anticipated warmer temperatures and lower rainfall, associated with increasing atmospheric carbon dioxide (CO2) concentrations, to maximise pasture yields. This study assessed the effect of increasing three plant traits, root depth, heat tolerance and responsiveness to elevated CO2 concentrations, in perennial ryegrass (*Lolium perenne* L.) to adapt to future climates. Pasture production was simulated using the Sustainable Grazing Systems Pasture model at three sites in south eastern Australia: Hamilton (medium rainfall, temperate climate); Ellinbank (high rainfall, temperate climate); and Elliott (high rainfall, cool temperate climate). Two future climate scenarios were created at each site by scaling the historical climate (1971-2010) by +1°C with -10% rain (435 ppm CO2) and +2°C with -20% rain (535 ppm CO2). A genotype by environment interaction suggested that the plants traits most effective at increasing pasture yield differed depending on the local climate. Increased root depth was the most effective change in a single trait that increased pasture harvested at Elliott, increased heat tolerance was most effective at Ellinbank, while increasing all three traits was effective at Hamilton. When all three traits were increased at the same time the pasture production advantage was greater than the additive effects of changing single traits at Hamilton and Ellinbank. Further consideration of the feasibility of selecting multiple traits and the effects of a broader range of climate projections is required. Nonetheless, results of this study provide guidance to plant breeders for selection of traits adapted to future climates.

Selecting pasture grasses for improved survival under drought

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Abstract

Many agricultural regions of the Earth have experienced extended periods of intense drought over the last two decades and there is increasing evidence to suggest that this is associated with a changing climate. For regions with a Mediterranean-climate, including south-western Australia and the western Mediterranean Basin, many of the climate models used for prediction indicate drier and hotter environments. In south-eastern Australia, summers are predicted to receive more rain and winters become drier while the overall annual rainfall will decline. If these predictions are correct, the stresses caused by these changes may cause the collapse of the temperate pastures and rangelands of these regions unless there is intervention and/or altered management. This would seriously reduce the production of food and fibre from grazing animals and lead to a decline in the numerous ecosystem services provided by grasslands. In Australia, pastures research addressing these future climate scenarios has mainly focussed on predicting future pasture supply using various plant growth models and assessment of the ability of agricultural soils to sequester more atmospheric carbon. In Mediterranean Basin countries, forage plant research remains concentrated on annual cereals and irrigated lucerne. The focus now needs to shift to the development of more climate resilient perennial plants and agronomic systems able to use these more resilient plants efficiently. Research is also needed to identify germplasm within the major perennial pasture species with enhanced levels of drought and heat tolerance. This paper outlines methods to identify and measure two important drought tolerance traits used by perennial herbaceous plants, dehydration tolerance and summer dormancy.

Water use efficiency of winter-active and summer-active pastures and binary mixtures

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Abstract

Pasture production systems in temperate regions can focus on either winter-spring or summer-autumn production, or attempt a more even year-round production. Water used during one season may limit production in a subsequent season. Therefore there are tradeoffs between a focus on production in winter, in summer, or in all seasons. To quantify these trade-offs a field experiment was undertaken at Hamilton, Victoria, to measure the water use efficiency of a range of pasture species under rainfed conditions, sown as either monocultures or in binary mixtures. Various combinations of perennial ryegrass, tall fescue, cocksfoot, phalaris, chicory and lucerne were tested between spring 2010 and winter 2012. Treatments that did not include lucerne included subterranean clover. Soil water was measured using a neutron moisture meter and electronic sensors. Dry matter was harvested using a mower. The water use efficiency (pasture growth per unit of evapotranspiration) during winter-spring ranged from 4 kg/ha.mm for a chicory monoculture to 27 kg/ha.mm for a fescue-lucerne mixture. During the summer-autumn period, water use efficiencies ranged from nil for cocksfoot to 13 kg/ha.mm for a fescue-lucerne mixture. Binary mixtures of either cocksfoot or fescue with lucerne were able to increase the water use efficiency of summer-autumn growth without compromising winter-spring growth. This study demonstrates that some mixtures can increase the summer-autumn forage supply while maintaining production during winter and spring.

Session 5 - Quality and feed value in animal production systems

The interaction between plant physiology and pasture feeding value

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Abstract

Plant-animal interactions impact on all elements of the pasture and animal performance in grazing systems. The quality of pastures for animals can be described in terms of feeding value (FV) which is a combination of feed nutritive value (NV) and voluntary intake. There are numerous, complex interactions between plant physiology and pasture FV and NV. This review focuses on these interactions in four key areas (plant growth strategies, phenological development, pasture regrowth, and response to environmental stress), extracting key principles and illustrating how plant breeding or management may be used to manipulate such interactions to improve FV. FV is low in pastures with native species that have evolved in nutrient-poor environments, especially if there are greater proportions of C4 vs. C3 species in the sward. Reproductive development of grasses and long grazing intervals (which affect stage of regrowth) reduce the proportion of leaf and increase stem or dead matter content in the sward. This is exacerbated by environmental stresses such as warmer temperatures and water deficit. Management decisions provide a means of manipulating many of these interactions to improve the FV of pasture, especially by improving soil nutrient status, using irrigation where possible, introducing exotic perennial pasture species such as perennial ryegrass, phalaris and tall fescue, linking the timing of grazing to stage of regrowth, and carefully managing post-grazing residual sward state. Likewise, plant breeding has focused on altering the flowering date of grasses, reducing aftermath heading, and reducing lignification within the plant to improve the FV of pasture for livestock.

A forage value index for New Zealand dairy farmers

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In June 2012, DairyNZ and the New Zealand Plant Breeders Research Association (NZPBRA) launched a forage value index (FVI) system (www.dairynzfvi.co.nz). The primary purpose of the FVI is to provide an independent, economically-based, multi-trait ranking system from which New Zealand dairy farmers can identify ryegrass cultivars that are likely to deliver the best economic returns in their farm systems. Important associated objectives of the FVI are to: 1) place an explicit economic value (EV) on different plant traits to support the future development of plant breeding programmes; and 2) propose future genetic gain targets for pasture plant breeding in New Zealand.

In its initial release form, about two-thirds of the perennial ryegrass cultivars that are available commercially in New Zealand received a FVI rating. The remainder were not eligible as because insufficient trial data were available. The FVI includes only seasonal dry matter yield ratings because this is the only trait for which systematic data are available. For each of these cultivars, performance values (PV) have been calculated for yield in winter, early spring, late spring, summer and autumn using data from the National Forage Variety Trials (NFVT) in northern North Island and the rest of New Zealand, and combined with EVs ($/kg DM) for additional yield in each season. Eligible cultivars are then compared relative to the performance of all "cultivars first tested in NFVT before 1996" referred to as the genetic base. Data are expressed using a five-star system for each of the four main dairying regions in New Zealand: upper North Island, lower North Island, upper South Island and lower South Island. PV and ranking information for the five seasonal yield traits are also available for each cultivar. More NFVT data are required for un-ranked cultivars so that they meet the eligibility criteria for inclusion in the FVI. FVI and PV for a separate short-term ryegrass (annual, Italian) category are currently being estimated using similar methodology.

Over the next 5-6 years, the NFVT system will be further developed, and a programme of supporting science will be put in place, so that:

- Comparative data for other key productivity traits, particularly nutritive value and persistence, are collected for all commercially-available cultivars so that PVs for these traits can be calculated and combined with trait EV for inclusion in the FVI.

- The effects of grass-clover interactions on cultivar rankings are quantified and potentially incorporated into the ranking system. This work is being conducted in collaboration with scientists from Teagasc, Moorepark Research Centre, Ireland.

- The requirement for incorporating further traits, such as feeding value/grazing efficiency, can be assessed, and suitable phenotypic indicators for those traits identified so that cultivars can be ranked accordingly.

- The FVI system is validated in a self-contained dairy grazing systems experiment comparing low- and high-ranking material to confirm that FVI predictions translate into differences in milk production and profit. This is essential for building farmer confidence in the robustness and reliability of the FVI system.

- An on-farm cultivar proving network is established throughout NZ to compare rankings under farm management with rankings under NFVT management and to adjust FVI weightings or utilise those data as required. This is also critical for building credibility with farmers.

- The scope of the FVI system can be expanded to include forage species other than ryegrass, for example forage crops, along with environmental traits such as those with the potential for mitigating N emissions from dairy systems.

- The current rate of genetic gain being achieved in whole-pasture productivity as a result of perennial ryegrass breeding is determined, and future genetic gain targets can be proposed based on modelling the outcomes of different breeding methods. FVI-specific genomic selection indices will be developed by AgResearch using reference populations mapped for FVI phenotypes.

Agronomic advantages conferred by endophyte infection of perennial ryegrass and tall fescue in Australia

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Abstract

Perennial ryegrass and tall fescue are key grasses of sown pastures in the high rainfall zone of south-eastern Australia. Ryegrass in naturalised pastures, and in sown seed, is widely infected with Neotyphodium fungal endophytes, with toxic endophyte strains causing toxicosis in livestock. Endophyte infection is also beneficial in sown grasslands, assisting ryegrass hosts to overcome biotic stresses, and tall fescue hosts to overcome biotic and abiotic stresses. We review the literature for Australia, and present new data, to examine the agronomic effects of endophyte. Frequency of endophyte infection in old perennial ryegrass pastures and ecotype-based cultivars is high, and in all pastures increases with time, providing evidence that endophyte-infected plants have an agronomic advantage over endophyte-free plants. Within a cultivar, agronomic field experiments have compared endophyte-infected with endophyte-free swards. Endophyte significantly improved ryegrass establishment in 7 of 19 measurements taken from 12 trials. In mature ryegrass pastures, over half the experiments found advantages to endophyte infection. Tall fescues infected with a selected endophyte ('AR542') had improved agronomic performance relative to endophyte-free in a majority of experiments and on occasions was essential for tall fescue persistence. Cultivar by endophyte interactions occurred but were inconsistent. Endophyte was more important for agronomic performance in high stress environments than difference between cultivars. Relative importance of cultivar and endophyte is discussed, with elite cultivars adapted to the region, which are infected with elite endophytes, being the best avenue to capture the benefits and minimise detrimental endophyte effects on livestock. The major drivers are likely to be insect pests and drought, but evidence is limited.

New insights into the clinicopathological mechanisms and presentation of perennial ryegrass toxicosis in Australia.

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Abstract

Perennial Ryegrass Toxicosis (PRGT) is a common disease entity in Australia. Clinical signs of PRGT include abnormal behaviour, ataxia ('staggers'), ill thrift and gastrointestinal dysfunction (scours) (Cunningham 1959). PRGT is caused by toxins produced by the endophytic fungus Neotyphodium lolii, a symbiont of perennial ryegrass, that has widespread prevalence in pastures across south-east Australia and Tasmania (Gallagher *et al*. 1982, Cheeke 1995). Clinical signs range in severity from mild gait abnormalities and failure to thrive, to severe seizures, lateral recumbency and death (Finnie, Windsor, Kessell, 2011). Mild outbreaks result mainly in subclinical production losses, management and animal welfare issues for affected producers whilst severe outbreaks can involve significant stock losses. Clinical presentation is usually highly variable with season, breed, sex, age and production status all affecting severity of clinical signs in the flock or herd (Reed *et al*. 2011). A particular feature of PRGT in Australia is the occasional occurrence of large scale sheep losses suggesting other factors are influencing mortality rates here compared to other PRGT risk zones. During 2011, producers experienced a mild outbreak of PRGT across the state of Victoria that saw large numbers of affected animals associated with limited mortalities. Neurological observations identified two key gait abnormalities associated with intoxication and clinical samples taken from affected sheep showed dehydration and electrolyte abnormalities. These data suggest that changes in hydration status may be a contributory aetiological factor in years where high numbers of fatalities are associated with PRGT outbreaks in Australia.

Session 6 - What is the next quantum leap in perennial grass research?

Genomic Selection of Forage Grasses

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Abstract

Pasture plant breeding has made advances in productivity gains for farming systems, however the rate of this gain has been restricted to c. 7% per decade due to complex breeding habits and target traits. Commercial breeding has been based exclusively on phenotypic selection and has not, to date, used advanced DNA based selection techniques. Genomic selection (GS) is an advanced method for the exploitation of DNA sequence polymorphisms in selective breeding of animals and plants. The method employs advanced computational techniques to deliver a prediction of breeding values based on DNA based molecular markers that are widely distributed. Pasture plant breeding is a good target for GS as all of the key desirable traits selected for are difficult to measure and require several years of evaluation, so could be radically improved with an early prediction of performance. With recent advances in DNA based technologies it is now possible to develop and implement GS based breeding strategies. It is anticipated that the implementation of a GS based breeding scheme would radically reduce the breeding cycle and increase the rates of improvement that are possible within commercial breeding programs. The methodology behind GS along with a proposed pasture grass breeding scheme and design will be presented and discussed.

Spatial variability in soil nutrients: an opportunity to increase fertiliser use efficiency in grazing systems?

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Abstract

Poor fertiliser use efficiency has been identified as a key limitation for Australian grazing systems (Simpson et al. 2011). While the spatial variability of soil nutrients have been documented in pasture systems in other countries (McCormick *et al*. 2009; Fu et al. 2010), it has not been widely studied in Australian grazing systems (King et al. 2006). Understanding the spatial variability in soil nutrients could provide valuable insights into the potential for management strategies on a sub-paddock scale. Of particular interest is the potential for site specific management (SSM) of fertiliser through zonal management (Simpson et al. 2011). This technique is now commonly used in the cropping and horticultural industries however there remains some question regarding how this management strategy might be implemented in grazing systems (Trotter 2010; Trotter et al. 2010; Simpson et al. 2011) and its potential benefits. Two paddocks were surveyed to quantify the spatial variability in soil nutrients. The "Parkers East" paddock was a 41 ha paddock located near Kingstown on the NSW northern tablelands. The pasture is dominated by Festuca arundinacea and Trifolium repens and is grazed by steers in a rotational system; paddocks are grazed for 1 to 4 weeks followed by rest periods of 2 to 8 weeks depending on biomass thresholds. The paddock has a long history of high rates of fertiliser application. The "Kirby field" was a 47 ha paddock located near Armidale on the NSW northern tablelands. The pasture is comprised of both native and naturalised species including Bromus spp, Vulpia spp, Imperata cylindrical, Microlaena stipoides and Austrodanthonia spp. The paddock has been grazed primarily by sheep and to a lesser extent cattle for all of its known history. Recent applications of fertiliser have been 125kg ha-1 of single superphosphate every second year spread by air.

Soil cores (0 to 10 cm depth) were collected on a (1 ha) grid across each of the paddocks. Soil samples from across the "Parkers East" paddock had a range in phosphorus concentration (Colwell) from 19.3 to 110.6 mg kg-1 with a mean of 49.9mg kg-1 and coefficient of variation (COV) of 37%. The "Kirby field" ranged in phosphorus from 13.0 to 121.1mg kg-1 with a mean of 30.6mg kg-1 and COV of 59%. Other soil nutrients (nitrogen, sulfur and potassium) demonstrated similar levels of variability. The opportunity for increased fertiliser use efficiency through SSM warrants further investigation. Research is required into both the value of SSM and the techniques that might enable the development of this strategy.

Advanced Phenomics and Genomics-Assisted Breeding of Ryegrass

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Abstract

Lengthy cycles and limited selective recombination in current ryegrass breeding programs, along with inability to accurately phenotype for multiple traits, has resulted in minimal genetic gain and a predominant focus on yield improvement. Advances in next-generation automated genomics and phenomics technologies now provide opportunities for advanced genomics-assisted selection (GAS) of ryegrass, in concert with detailed phenotypic data, to accelerate genetic gain and reduce the duration of breeding cycles. A pilot GAS-based breeding program has been implemented in Italian ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*Lolium perenne* L.) in partnership with a commercial pasture breeding company. High-throughput genotyping capabilities and computational tools have been utilised to characterise breeding nurseries and provide informed breeding decisions. Genotyping systems have also been used to develop a breeder's tool for construction of cultivar catalogues, exploration of breeding histories and population differentiation. This tool can support intellectual property protection of breeder's varieties and also provide confidence of seed purity to farmers. A novel advanced phenotyping protocol suitable for application in commercial pasture breeding has been developed. This protocol quantifies plant protein and individual water soluble carbohydrates with a c.11 fold increase in sample possessing time over other commonly used methods. These tools demonstrates the potential to accelerate commercial pasture breeding programs and allow breeding for a range of traits such as superior nutrient profiles.

The Good Oil – Engineering the Accumulation of Triacylglycerol in Ryegrass

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Abstract

Forage grasses such as Lolium perenne often lack sufficient nutritive value to achieve the levels of milk and meat productivity seen in non-pasture based systems; consequently, pastorally-based farming is unable to take advantage of the full production potential present in today's livestock animals. Gram for gram, lipids have twice the energy content of either sugars or proteins; furthermore, dietary lipids are typically not degraded in the rumen instead they provide a direct source of metabolisable energy to the animal. The feed lot industry has taken full advantage of these factors by supplementing the animals' diets with lipids up to 10% of the dry matter. Our laboratory has sought to emulate this practice while maintaining a forage based agriculture, to do this we used synthetic biology to create genetic machinery that enables the synthesis and accumulation triacylglycerides (TAGs) in plant leaves, thus raising the potential energy available to the grazing ruminant. In homozygous mature leaves of the model plant Arabidopsis thaliana the technology led to increases of the total fatty acid, the CO2 assimilation rate and biomass by approximately 200%, 24% and 50% respectively. For ryegrass we further refined the construct by limiting the expression to green tissue only; the first round of transformants indicate that this has allowed us to double the fatty acid content in hemizygous leaves. The results to date and our projection for future work are presented here.

POSTER PAPERS

Twenty years of searching for an alternative temperate grass species for low to medium rainfall environments in Tasmania.

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Abstract: Between 1992 and 2012, in response to the need to find persistent and productive perennial grasses better adapted to the changing climatic conditions being experienced across dryland pastures in cool temperate low to medium rainfall (300-750 mm) regions of Tasmania, representatives from 65 species representing 14 Genera of perennial grasses were screened and evaluated for production and persistence under sheep grazing, across a range of sites in the target area. The work identified two promising alternative species. Hispanic or Spanish cocksfoot (*Dactylis glomerata* ssp. *hispanica*) proved to be a highly resilient species, able to withstand the driest conditions ever experienced in Tasmania. Also identified was the highly productive Coloured brome (*Bromus coloratus*), which showed it was well adapted to a temperate environment receiving >500mm annual rainfall. Cultivars representing both these species have recently been commercialised and are available to producers. This work also highlighted the deficiencies of a number of commonly sown species, the most notable being perennial ryegrass (*Lolium perenne*), which failed to survive years when annual rainfall fell below 500mm. A number of other species were identified as worthy of further consideration for use in this environment, particularly from within the genus *Bromus*.

Key words: Perennial grasses, temperate Australia, plant introduction

Background

Temperate perennial grasses are most widely used in pastures in New South Wales, Victoria, Tasmania, south west Western Australia and south east of South Australia. The main perennial grass species sown for grazing across these regions is perennial ryegrass (*Lolium perenne*) with the first recorded sowings dating back to the nineteenth century. Perennial ryegrass has remained popular largely due to the availability of relatively cheap seed, ease of establishment and management and its ability under high rainfall to produce a bulk of good quality feed. Under dryland conditions, however, as found across the majority of grazing enterprises in these regions, perennial ryegrass pastures have a number of limitations including poor persistence and low productivity during the summer months, creating landcare issues and a feed gap. Ryegrass is also highly susceptible to a number of pasture grub pests and has negative animal health issues due to the presence of endophytes.

In the early 1990's considerable potential existed in dryland grazing systems across southern Australia for the development and use of better adapted animal friendly perennial grass species, outside the range of traditionally sown pasture species. This need for alternative species in Tasmania was highlighted by the result of a pasture survey in the Midlands and Derwent Valley regions conducted in 1993 which showed ground cover contributed by commonly sown improved species *Dactylis glomerata* and *L. perenne* to be frequently as low as 5% (Friend 1997).

Dry summers and longer term droughts have been a regular feature throughout these regions resulting in poor productivity, lack of persistence and in some cases complete failure of current species and cultivars. A project was developed by the Tasmanian Department of Primary Industries Parks Water and Environment (DPIPWE) aimed at finding well adapted species to fit into this environment, keeping in mind and acknowledging that the environment from which a species originates will set some limits on how much further progress can be made in selecting and breeding more drought tolerant plants from within that species (Kemp 1994). The program moved to the Tasmanian Institute of Agriculture (TIA) in 1996, where it continues to this day.

Methods

The work involving the search for new perennial grasses has been in three stages.

The initial stage conducted between 1990 and 1995, saw 460 accessions representing 65 species of perennial grasses (Table 1) screened as spaced plants at the Cressy Research Farm in Northern Tasmania, for their potential use as pasture herbage plants in the target environment.

The second stage commenced in 1995. Following plant characterisation and phenotypic selection for desirable traits, 117 of the most promising lines representing 28 species of perennial grasses, including 6 commercial cultivars (Table 2) selected for preliminary field evaluation and adaptation at three sites: Jericho (Central Midlands, cold winters, hot dry summers), Hamilton (Derwent Valley, drought prone, north facing slope) and Swansea (East Coast, drought prone, summer rain). The lines were established in 5m row of spaced plants, planted 25cm apart and 1m between rows. Lines were not replicated.

At this stage, the evaluation process involved the determination of herbage production, seasonal distribution, nutritive value, palatability, sensitivity to a range of stresses including; frost, drought, grazing tolerance and susceptibility to pests and diseases and long term persistence across a range of environments. All lines were grazed heavily by sheep after each assessment for herbage production. The target environments selected for this screening work were considered "difficult" because of the combined effects that drought; cold and low to moderate soil fertility have on pasture production and persistence.

Finally, from the early performance of lines at these three sites, 56 experimental lines representing 23 species (Table 3) were selected for advanced (stage 3) testing in replicated 10m x 1.5m sward trials established in 1998 at five evaluation sites to compare the productivity and the persistence of these lines against commercial varieties. Lines were sown as a mixed sward with *Trifolium subterraneum* and grazed by sheep after seasonal assessments. The evaluation sites were situated at: Cranbrook (East Coast, drought prone, low fertility, slightly saline), Triabunna (East Coast, high fertility, summer rain), Jericho (Central Midlands, cold winters, and hot dry summers), Ross (Northern Midlands, drought prone), Cambridge (Coal River Valley, drought prone).

Outcomes

To date the perennial grass development program has resulted in the development and commercial release with Plant Breeders Rights protection (PBR) of two cultivars of Hispanic cocksfoot (*D. glomerata* ssp. *hispanica*) cvs. Uplands and Sendace, one cultivar of cocksfoot (*D. glomerata* ssp *glomerata*) cv. Megatas and one cultivar of coloured brome (*Bromus coloratus*) cv. Exceltas. The Hispanic cocksfoot cultivars Uplands and Sendace have shown the ability to survive extended dry periods in a low rainfall environment, surviving drought conditions in Tasmania's Derwent Valley where the mean annual rainfall (1995 to 2000) was 382mm. Perennial ryegrass failed to survive at this site when annual rainfall fell below 500mm. The cultivar Megatas was selected for its excellent seedling vigour and increased leafiness and vigour with a low crown. It is highly summer active producing a large bulk of highly palatable, high protein, high-energy forage all year round with a high level of digestibility and nutritive value. Megatas has little or no aftermath heading, producing leafy feed throughout summer.

The cultivar Exceltas is a summer active perennial. Exceltas out yielded all perennial ryegrass cultivars in late spring/early summer in irrigated trials conducted by the DPIPWE at Elliott Research Station, Tasmania and in dryland trials conducted by the TIA at Cressy Research Station, Tasmania.

Several other species from the genera *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Poa* and *Secale* were identified as having potential and are worthy of future consideration.

Conclusion

The superior drought tolerance and persistence of a number of alternative grass species against the commonly sown cultivars and species tested, highlights the potential to develop new cultivars from this material. It highlights the importance of collection environment in determining

Table 1. Species list for accessions screened between 1990 and 1995

Scientific name	No of accessions screened	Scientific name	No of accessions screened
Agropyron cristatum	4	Dactylis smithii	4
Agropyron desertorum	4	Dactylis woronowii	1
Agropyron hybrid	2	Digitaria argyrograpta	2
Agropyron intermedium	4	Digitaria eriantha	1
Agropyron magellanicum	2	Digitaria milanjianum	1
Agropyron trichophorum	3	Digitaria pentzii	2
Arrhenatherum elatius	3	Digitaria smutzii	3
Bromus anatolicus	2	Elymus lanceolatus	1
Bromus anomalus	1	Elymus patagonicus	1
Bromus araucanus	2	Elymus pubiflorum	1
Bromus auleuticus	1	Elymus trachycaulus	1
Bromus biebersteinii	2	Festuca arundinacea	9
Bromus breviaristatus	1	Festuca idahoensis	13
Bromus carinatus	13	Festuca longifolia	1
Bromus catharticus	17	Festuca nigrescens	1
Bromus coloratus	2	Festuca ovina	81
Bromus inermis	3	Festuca pallescens	2
Bromus macranthos	2	Festuca pratensis	5
Bromus mango	4	Festuca rubra	3
Bromus marginatus	7	Lolium perenne	27
Bromus runssoroensis	1	Phalaris aquatica	2
Bromus setifolius	3	Phalaris hybrid	5
Bromus sitchensis	3	Phleum pratense	7
Bromus spp.	18	Poa ampla	8
Bromus stamineus	5	Poa canbyi	6
Bromus syriacus	5	Poa glaucantha	1
Bromus tomentellus	14	Poa ligularis	1
Bromus tomentosus	7	Poa pratensis	4
Bromus valdivianus	6	Poa secunda	1
D. glomerata ssp glomerata	92	Psathyrostachys juncea	1
D. glomerata ssp. hispanica	22	Secale montanum	4
Dactylis marina	2	Thinopyron ponticum	2

genotype adaptation when developing new cultivars. With the failure of a number of "traditional" species and cultivars, along with the increasing problem of land degradation in low rainfall areas of Tasmania, the development of drought tolerant, well adapted alternatives must now be considered a high priority with long term persistence becoming the principal selection criterion.

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Table 2. Promising species list for field evaluated from 1995 to 1998

Scientific name	No of lines evaluated	Scientific name	No of lines evaluated
Agropyron intermedium	1	*Dactylis woronowii*	1
Agropyron trichophorum	1	*Elymus trachycaulus*	1
Arrhenatherum elatius	1	*Festuca arundinacea*	2
Bromus araucanus	1	*Festuca idahoensis*	1
Bromus auleuticus	1	*Festuca ovina*	1
Bromus biebersteinii	1	*Festuca rubra*	1
Bromus coloratus	1	*Lolium perenne*	2
Bromus inermis	1	*Phalaris aquatica*	2
Bromus macranthos	1	*Phalaris hybrid*	1
Bromus mango	2	*Poa ligularis*	1
Bromus valdivianus	1	*Poa pratensis*	1
Dactylis glomerata	74	*Psathyrostachys juncea*	1
D. glomerata ssp. hispanica	13	*Secale montanum*	1
Dactylis marina	1	*Thinopyron ponticum*	1

Table 3. Species list for advanced evaluated in sward trials

Scientific name	No of lines evaluated	Scientific name	No of lines evaluated
Agropyron trichophorum	2	*Dactylis glomerata ssp glomerata*	22
Arrhenatherum elatius	1	*D. glomerata ssp. hispanica*	11
Bromus araucanus	1	*Dactylis marina*	1
Bromus auleuticus	1	*Dactylis woronowii*	1
Bromus biebersteinii	1	*Elymus trachycaulus*	1
Bromus coloratus	1	*Festuca idahoensis*	1
Bromus inermis	1	*Festuca ovina*	1
Bromus macranthos	1	*Festuca rubra*	1
Bromus mango	2	*Phalaris aquatica*	1
Bromus stamineus	1	*Psathyrostachys juncea*	1
Bromus valdivianus	1	*Secale montanum*	1
		Thinopyron ponticum	1

The removal of *Lolium rigidum* from newly sown perennial grass seed crops with different selective grass herbicides.

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Abstract: *Lolium rigidum* is a weed in perennial grass seed production crops. Not only can its seed contaminate seed production for following generations, but it may also cross with species such as *L. perenne.*

A number of selective grass herbicides were investigated to determine their effectiveness at controlling *L. rigidum* in first year perennial grass seed crops and to estimate the amount of damage they would cause to the species of interest. The perennial grasses studied were *L. perenne*, *Dactylis glomerata*, *Phalaris aquatica* and the Mediterranean and continental cultivars of *Festuca arundinacea*. The selective grass herbicides used were Triallate (500g/L) at a rate of 1.6 L/ha; Propaquizafop (100g/L) at a rate of 300 ml/ha; Tralkoxydim (600g/L) at a rate of 255 ml/ha and Ethofumesate (500g/L) at a rate of 3 L/ha. None of the chemicals used were able to remove 100 percent of the *L. rigidum* from the perennial grasses plots, indicating that they could not be used in seed production to prevent contamination from this weed. However, some of the chemicals used, such as Tralkoxydim, removed a majority of the *L. rigidum*, indicating that further breeding work could be done to develop perennial grass species more tolerant to this herbicide at higher rates. A higher rate of this herbicide may have a greater efficiency at removing all *L. rigidum* contamination from seed production crops in early breeding generations.

Introduction

Lolium rigidum is a weed in perennial grass seed production crops (Ben Dunstan, Personal communication). Not only can its seed contaminate seed production for following generations, but it may also cross with species such as *L. perenne*. This potentially creates hybrids between the species in the following generation and may make certification of a variety difficult. *L. perenne* is a perennial grass species and has the ability to last in the field for greater than 3 years. When hybrids with *L. rigidum*, an annual species, are created the perenniality of the new plant is decreased. This is undesirable to farmers wanting a grass that can persist for a long period of time before having to resow again.

There should be no *L. rigidum* plants in perennial grass seed crops in early generations after the original parent synthetic is created. One effective way of removing *L. rigidum* from a seed production crop is via rouging, but this is a time consuming and expensive process (Ben Dunstan, Personal communication). Selective herbicides provide a cheaper management tool for the control of *L. rigidum* in perennial grass seed crops. In a previous study by Dear (*et al.* 2006) a range of selective grass herbicides were trialed to determine their efficacy on *L. rigidum* and their potential negative effect on the growth of the perennial grasses. This paper reports on an experiment investigating the chemicals identified in this previous study in a replicated field experiment on a number of commercial perennial grass species to determine if they would be effective in breeder's seed production crops for *L. rigidum* control.

Keywords: Lolium rigidum, perennial grasses seed production, selective grass herbicides.

Materials and Methods

A four replicate randomized block experiment was planted into a cultivated seed bed at Yarck, Victoria, on 9/5/12 using a small plot cone seeder. Plot dimensions were 10 x 1 metre. Half the plot was broadcast with a *L. rigidum* treatment at 25kg/ha to assess the efficiency of the different herbicides at controlling *L. rigidum* in the different perennial grasses. The other half of the plot was kept weed free to assess the damage the herbicides would have on the perennial grasses. The trial was fertilized with 100kg/ha of Di Ammonium Phosphate after sowing. The perennial grasses studied included perennial ryegrass, *L. perenne* (sown at 25kg/ha), cocksfoot, *Dactylis glomerata* (sown at 4kg/ha), phalaris, *Phalaris aquatica* (sown at 8kg/ha) and the Mediterranean and continental cultivars of tall fescue, *Festuca arundinacea* (both sown at 15kg/ha). The selective grass herbicides used were Triallate (500g/L) at a rate of 1.6 L/ha; Propaquizafop (100g/L) at a rate of 300 ml/ha; Tralkoxydim (600g/L) at a rate of 255 ml/ha and Ethofumesate (500g/L) at a rate of 3 L/ha.

The chemicals were applied on 17/7/12 when the perennial grasses were beyond the 3 leaf growth stage. A 15 litre knapsack sprayer was used to apply the chemical over a 1 metre band across the replicates and treatments. The experiment was visually assessed on 20/09/12. The area of the plots that had been kept weed free was assessed for damage from the herbicide, with a visual growth score of 1-9, with 9 having the highest growth. The area of the plots with the *L. rigidum* treatment was visually scored for the density of *L. rigidum* plants present, with 100% density meaning the plot was fully *L. rigidum* and no other species could be observed. The data was analysed using ANOVA from Genstat Version 4.1. Significance levels were at P=0.05.

Results and discussion

All the selective herbicides significantly decreased growth in perennial ryegrass (Table 1). Propaquizafop had the greatest negative effect on perennial ryegrass growth, followed by Tralkoxydim (25% and 36% growth of the control). Tralkoxydim was the only chemical to significantly decrease *L. rigidum* in the perennial ryegrass treatment; however, this was not to the 100% level of control that was desired. By selecting for increased tolerance in perennial ryegrass to Tralkoxydim, the negative effect on its growth may be decreased and the percentage of *L. rigidum* plants removed may be increased, although 100% removal may not be achievable.

Propaquizafop and Tralkoxydim (44% of the control) significantly decreased the growth of Mediterranean tall fescue. These two herbicides also significantly decreased the percentage of *L. rigidum* in the plots. Tralkoxydim was more effective at *L. rigidum* removal from Mediterranean tall fescue than Propaquizafop (12.5% and 40% plot density respectively). Therefore, future work should target the selection for resistance in Mediterranean tall fescue to Tralkoxydim at higher rates to increase the chemical effectiveness at controlling *L rigidum*.

Propaquizafop and Tralkoxydim (42% of the control respectively) significantly decreased the growth of Continental tall fescue. Ethofumesate, however, did not decrease the growth of continental tall fescue (85% of control), but significantly decreased the percentage of *L. rigidum* in plots. As Ethofumesate decreased L. rigidum levels less than Tralkoxydim (27.5% vs 10% plot density respectively), and it had less effect on the grass of interest, there may be the opportunity to increase rates to a higher level before growth is affected.

Phalaris has a number of options for *L. rigidum* control. Propaquizafop significantly decreased the growth of phalaris (19% of the control). Propaquizofop, Triallate, Ethofumesate and Tralkoxydim all significantly decreased the *L. rigidum* density in the plots. However, Tralkoxydim was significantly more effective at *L. rigidum* control than the other herbicides. Therefore, further work in the selection of the tolerance of this herbicide in phalaris should be pursued.

Propaquizafops and Tralkoxydim (0% and 36% of the control respectively) significantly decreased the growth of cocksfoot. Both also significantly decreased *L. rigidum* percentages in the plots (by 40% and 17.5% respectively). Tralkoxydim would be the only option for future work in selecting cocksfoot plants that have greater tolerance towards it, as there was no cocksfoot survival in the Propaquizafop plots. However, in another trial (Leddin, unpublished), Trifluralin, a pre-emergent herbicide, was found to give effective control over *L. rigidum* without significantly damaging cocksfoot plants.

Table 1: Effect of selective grass herbicides on *Lolium rigidum* and other perennial grasses, Yarck 2012.

Entry	Visual Growth Score (1-9)	*L. rigidum* Density
Platinum Perennial ryegrass control	7	47.5
Platinum Perennial ryegrass Propaquizafop	1.75	37.5
Platinum Perennial ryegrass Triallate	5.25	37.5
Platinum Perennial ryegrass Ethofumesate	5.25	27.5
Platinum Perennial ryegrass Tralakoxydim	2.5	12.5
Prosper Med. Tall fescue Control	2.25	62.5
Prosper Med. Tall fescue Propaquizafop	1	40
Prosper Med. Tall fescue Triallate	2	47.5
Prosper Med. Tall fescue Ethofumesate	2.5	50
Prosper Med. Tall fescue Tralakoxydim	1	12.5
Dovey Cont. tall fescue Control	3.5	77.5
Dovey Cont. tall fescue Propaquizafop	1.5	42.5
Dovey Cont. tall fescue Triallate	3	60
Dovey Cont. tall fescue Ethofumesate	3	27.5
Dovey Cont. tall fescue Tralakoxydim	1.5	10
Holdfast GT Phalaris Control	4	82.5
Holdfast GT Phalaris Propaquizafop	0.75	57.5
Holdfast GT Phalaris Triallate	4.25	60
Holdfast GT Phalaris Ethofumesate	4.5	45
Holdfast GT Phalaris Tralakoxydim	2.75	12.5
Yarck Cocksfoot Control	2.75	82.5
Yarck Cocksfoot Propaquizafop	0	40
Yarck Cocksfoot Triallate	2	70
Yarck Cocksfoot Ethofumesate	2	65
Yarck Cocksfoot Tralakoxydim	1	17.5
%CV	21.18	35
LSD (5%)	0.80	22
Mean	2.68	45

Conclusion

The growth of phalaris was the least affected of the perennial grasses studied by the application of the selective grass herbicides and cocksfoot was the greatest affected. This is similar to results from another study (Dear *et al.* 2006). This may be due to the phalaris plants being more established at herbicide application.

Tralkoxydim was the most effective chemical at controlling *L. rigidum*. Each perennial grass should therefore be further screened for tolerance to Tralkoxydim. This could potentially create a new variety that is more tolerant of Tralkoxydim at higher rates, allowing for the greater removal of *L. rigidum* from seed production crops. However, 100% control of *L. rigidum* in early generations of perennial grasses seed crops may only be achieved by a combination of management methods, such as pre-knockdown sprays and rouging of crops, combined with selective herbicide treatment, unless greater resistance in the species of interest and greater effectiveness on *L. rigidum* can be brought about by breeding. In the meantime, the use of Tralkoxydim for establishing perennial grass for pastures would only be suggested in species where growth is not adversely affected e.g. phalaris.

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Potential of common wheatgrass, *Elymus scaber*, to hybridise with wheat and produce a perennial cereal species.

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Abstract: Developing perennial cereals is seen as a method of managing landscapes at risk of soil degradation, while maintaining food security. Common Wheatgrass (*Elymus scaber*) is a native perennial which demonstrates high adaptability to varying Australian environments. As a *Triticeae* grass it may also be hybridised with wheat with the prospect of deriving a new perennial cereal crop or enabling the transfer and integration of other useful traits into wheat.

Attempts were made to hybridise five populations of *E. scaber* from differing climatic regions, with seven hexaploid (*Triticum aestivum*) and nine tetraploid (*T. durum*) wheats. These cultivars of wheat were chosen as they had demonstrated high crossability with rye (*Secale cereale*) when developing triticale (*xTriticosecale*). All crosses were conducted in the glasshouse using wheat as the female parent. Manual emasculation and pollination techniques were used. Seed set was high, but the hybrid seeds were shrivelled with degenerated endosperm and were not viable. Regression modelling indicated that there were significant ($P < 0.001$) relationships with the hybrid parents for percentage seed set. Two types of immature embryo rescue were tried allowing 8 wheat-like plantlets to be recovered. However, no *Elymus* DNA was detected in these putative hybrids, suggesting either these were rare selfs or doubled haploids following *Elymus* chromosome elimination. The potential of this species in perennial cereal development is discussed.

Key Words: Perennial wheat, hybrids, embryo rescue, tetraploid wheat, hexaploid wheat

Introduction

There is increasing interest in developing perennial cropping plants as a method to combat soil degradation caused by annual based cropping systems. Successful development of perennial cereals, through hybridisation, requires the selection of perennial parents which demonstrate significant adaptability to the target environment (Murphy *et al*. 2007). Common Wheatgrass (*Elymus scaber*) may prove useful in this role as it is an Australian native perennial grass belonging to the *Triticeae* tribe. It has a wide range of adaptability throughout southern grain producing areas of Australia (Mitchell *et al*. 2001).

Due to the remoteness in the relationships between the genomes of wheat and *E. scaber*, any attempt to hybridise the two species will encounter some difficulties. Previous attempts to produce wheat x *E. scaber* intergeneric hybrids have used hexaploid wheat as a parent and rescued embryos on media at between 4 and 20 days post pollination (Torabinejad *et al*. 1987; Ahmad and Comeau 1991; Torabinejad and Mueller 1993). It is possible to develop wheat embryos from ovules excised and cultured on specialised media 24 hrs after pollination. This method has not been applied to wide hybridisation; it potentially will permit earlier embryo rescue than the standard methods and may therefore overcome early post-zygotic incompatibility barriers. Furthermore, attempts to hybridise tetraploid wheats with *E. scaber* have not been reported. Comparisons of wide hybridisation between hexaploid and tetraploid wheats with alien species have demonstrated that some hybridisations fail to set seed with hexaploid wheat parents (Mujeeb-Kazi *et al*. 2007). This might suggest tighter control of crossability in hexaploid wheat compared to tetraploid wheat. The current study attempted to hybridise a range of high-crossability hexaploid and tetraploid wheats using *E. scaber* accessions as a pollen source. Resulting putative hybrid wheat/*E. scaber* embryos were rescued 7 to 14 days post pollination or hybrid wheat ovules were rescued 24 hours post pollination in attempts to increase hybrid plant recovery.

Methods

Cultivars of wheat used as female parents were chosen based on their high crossability with rye (*Secale cereale*) in the production of triticale (x*Triticosecale*; Sirkka *et al*. 1993). Five *E. scaber* accessions were collected from high and low rainfall zones of NSW (Table 1). These were used as male parents in the cross with the wheat cultivars. All germplasm was maintained in a glasshouse.

Female wheat parents were manually emasculated four days prior to anthesis. Only the outer florets on the spike were used for pollination, with all other florets removed. Dehiscing anthers from *E. scaber* plants were used to pollinate the maternal parent by brushing the anther over receptive stigmas. Crossed pistils were sprayed with a combination of 75 mg/l GA3 and 5 mg/l 2,4 D, at 24 and 48 hours after pollination to aid fertilisation. Seeds were dissected under sterile conditions 7 to 14 days after pollination and inspected for the presence of embryos. Recovered embryos were placed on agar-solidified Gamborg's B-5 basal medium with minimal organics (Gamborg *et al*. 1968). Embryos which germinated were transferred to McCartney bottles containing the same media and allowed to develop two leaves before transferring to soil.

A subset of crossed spikes underwent ovule culture 24 hrs post fertilisation. In this instance the ovaries were removed from the crossed wheat spike. The ovule was partially dissected leaving a portion of the pericarp still attached. These were then placed in ovule culture medium (Kumlehn and Nitzsche 1995) and the same plantlet recovery procedure as above was followed.

PCR-based molecular markers were able to amplify DNA sequences specific to *E. scaber.* These marker assays were conducted on putative hybrid plants. Data from the crossing experiments were analysed using an unbalanced analysis of variance (Genstat 11.1). Regression modelling was used to develop predictions from each response variate. Least significant differences were determined at the 5% level.

Results

Across all wheat parents 1,966 florets were pollinated using pollen from different E. scaber accessions. These developed 1,440 seeds, from which 28 embryos could be dissected onto Gamborg's media, 7-14 days post-pollination. The good early seed development indicated that early fertilisation barriers were overcome and that *E. scaber* pollen could fertilise wheat florets. Sacaba 81 had the highest percentage of seeds produced per florets pollinated (91.4%), with Langdon 91334 having the lowest (4.1%; Table 1). Pollen sourced from the Canowindra and Ganmain *E. scaber* accessions developed significantly higher percentages of seed set (approx. 80%) compared to the other three accessions. Without embryo rescue, any seed left to develop on the wheat parent was shrivelled with degenerated endosperm and was not viable. From the small number of embryos rescued, 6 plants were able to be recovered. Significantly more plants were cultured when Numinus S was used as a female parent or Canowindra *E. scaber* as a male parent in the hybridisation attempts made (Table 1).

A subset of 314 wheat florets were pollinated with Wagga Wagga and Ganmain E. scaber pollen, and underwent ovule rescue at 24 hrs post pollination (Table 2). Using this technique a further 2 plants were recovered. Regression analysis was not possible on this data due to the small number of parents used in the attempt at hybridisation.

The PCR amplifications using four molecular markers failed to indicate the presence of the *E. scaber* DNA in the putative hybrid plants. All suspected hybrid plants amplified only the same DNA products as their wheat cultivar parents and gave no evidence of a hybrid origin. The two plants from ovule culture which survived to maturity appeared morphologically like their wheat parents and were fully fertile.

Discussion

The good initial seed development in the current study is significant. The high percentage of seed set (up to 94%) at around 14 days post pollination, is testament to the high crossability of the wheat cultivars used. Strong post-zygotic mechanisms are initiated early in wheat x *E. scaber* crosses, which cause embryo abortion. Early well developed seed illustrated that fertilisation was taking place. Florets in which no fertilisation had occurred, showed no seed development. Seeds which were left to mature on the wheat parent, demonstrated degradation of the endosperm. This highlights the need for early intervention to rescue embryos. In this regard, culturing ovules at 24 hours post pollination may be a way forward in developing hybrid embryos. If the number of attempts at this technique could have been increased, greater success in recovering hybrid embryos may have been achieved. Increasing the number of crossing attempts is the key to achieving hybrid plant recovery. Previous attempts at hybridisation between wheat and *E. scaber* had embryo recovery rates from 0 – 0.52% (Torabinejad *et al*. 1987; Ahmad and Comeau

Table 1. Predicted means for parentage seed set and plantlets cultured using wheat parent as the main effect from the regression model for results of experiment one.

Wheat Cultivar	Seed/pollinated floret (%)	Predicted plant recovery	E. scaber parent	Seed/pollinated floret (%)	Predicted plant recovery
Hexaploid wheats	Canowindra	78.50	1.57		
Chinese Spring	85.95	0.58	Ganmain	80.04	0.32
Alubuc	72.53	0.35	Holbrook	61.83	0.21
Star S	82.60	0.08	Panurara	65.29	0.10
Suzhoe 8	70.28	0.08	Wagga Wagga	64.26	0.40
Bacanora 88	89.33	0.08			
Long Mai 10	61.83	0.08			
Jimai 11	65.01	0.32			
Tetraploid wheats					
Altar 84	86.73	0.08			
Corcorit	93.75	0.04			
Numinus S	87.60	4.04			
Sacaba 81	91.42	0.08			
Gavia S	77.78	0.05			
Langdon 2843	68.91	0.04			
Langdon 10394	12.03	2.04			
Langdon 91334	4.11	0.19			
Langdon 95298	59.00	1.13			
P value	<0.001	<0.001		<0.001	<0.05
l.s.d	17.01	0.95		15.48	0.74

1991; Torabinejad and Mueller 1993) and is indicative of the difficulty in crossing the two species.

PCR markers used on the putative hybrid plants in this study failed to show any evidence of DNA from the *E. scaber* parent. It is possible that the plants formed were haploids in which all the paternal DNA was eliminated leaving the maternal DNA intact. Haploid formation is common among interspecific crosses between wheat and other grasses (Mochida and Tsujimoto 2001) and spontaneous chromosome doubling would explain the fully fertile plants recovered following ovule culture.

Conclusion

E. scaber has proven hard to hybridise with wheat. The high level of early seed formation is encouraging as it is an indication that *E. scaber* pollen can readily germinate on wheat pistils, grow to the ovule and trigger seed development. Formation of a hybrid zygote is implied, but not proved in this sequence. However, subsequent embryo development met with significant barriers. The formation of seed is a positive indicator of the potential to obtain a hybrid between the two species. The rescue procedures used in this study did show that embryos could be supported if they were found. However, it remains to be shown whether the embryos observed in this cross were due to self-pollination or doubled haploids following *Elymus* chromosome elimination. The use of heterozygous wheat parents would allow double haploids to be identified. Larger scale crossing attempts are required to surmount the post-zygotic barriers encountered.

Despite the difficulties in obtaining hybrid embryos, *E. scaber* may still prove to be useful in breeding perennial cereals for Australia. The species has the advantage of being the most widely distributed of all the perennial Triticeae across the southern wheat belt. In general, all wide hybridisation attempts between wheat and distant relatives have low rates of embryo recovery. Increasing the attempts at embryo rescue 24 hrs post pollination may deliver more wheat x *E. scaber* embryos and improve the possibility of delivering well adapted perennial grain germplasm.

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Table 2 Numbers of florets pollinated (FP), ovules cultured (OC) and plants recovered (PR) from attempts to hybridise a subset of E. scaber populations and wheat cultivars, using ovule culture media developed

Elymus source	**Wagga Wagga**			**Gamain**		
Wheat Cultivar	FP	OC(%)a	PR(%)b	FP	OC(%)a	PR(%)b
Chinese Spring	98	66(67)	0	-	-	-
Alubuc	16	12(75)	0	-	-	-
Suzhoe 8	40	36(90)	0	22	18(82)	0(0)
Long Mai 10	69	55(80)	2(4)	-	-	-
Jimai 11	43	41(95)	0	-	-	-
Sacaba 81	26	19(73)	0	-	-	-
Total	292	208(77)	2(0.96)	22	18(82)	0(0)
a as percent florets pollinated, b as percent ovules cultured						

The effect of sowing binary mixtures of perennial pastures on their establishment and density in the first two years

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Abstract: Sward establishment is a major investment for livestock producers, with plant density and survival after sowing determining future productivity under grazing. Newly sown swards often lack productivity in the first year after sowing, especially if establishment has been suboptimal. Maximising plant survival and density during this time can increase the amount of grazing achieved from the sward. A field experiment with two sites was conducted at the Department of Primary Industries research farm near Hamilton, Victoria, to evaluate the survival of a range of pasture species, sown as either monocultures or in binary mixtures, in the first 2-3 years following establishment. The two sites had contrasting soil types. At each site either eight or nine different pasture treatments were tested and replicated four times in a randomised complete block design. Pasture treatments were selected to suit the soil types, but three treatments were common to both sites to give a between-site comparison. Species frequency (% of 100 cells that contain herbage of the sown species) was measured annually in 2011 and 2012, following sward establishment in 2009/10. Results indicate that some of the binary mixtures maintained species frequency in the first 2-3 years following sowing. On well drained soils, lucerne sown in mixed swards with tall fescue, cocksfoot or chicory resulted in similar levels of frequency compared to the monoculture swards in 2011 and 2012. The suitability of sowing tall fescue in a mixed sward depended on the cultivar of tall fescue used; winter active tall fescue sown on a well drained soil had similar species frequency as the monoculture of tall fescue, but summer active tall fescue sown on a poorly drained soil had lower species frequency than the monoculture of tall fescue. Kikuyu established successfully and had relatively high species frequency counts in the first two years after establishment, when sown in a binary mixture with strawberry clover. This research has demonstrated that binary mixtures of some perennial pastures can persist in the 2-3 years after establishment to create a mixed sward.

Keywords: Persistence, seed mixes, companion species, grazing management, sowing, plant survival.

Introduction

Sowing pasture mixes is an area of increasing interest for livestock producers to improve the seasonal distribution of dry matter production and nutritive characteristics. The inclusion in swards of summer active species, in particular, could increase the grazing value of the sward over the key summer and autumn period when lamb producers are finishing prime lambs or preparing ewes for joining. Previous research has demonstrated the value of using mixed swards to increase livestock production (Golding *et al*. 2011). In their research, Golding *et al*. (2011) found that a mixed sward of the highly summer active species chicory and red clover, with the more winter/spring active species plantain and white clover, resulted in higher post-weaning lamb growth rates than perennial ryegrass with white clover or perennial ryegrass with plantain and white clover, both of which provide a much more winter/spring dominant pasture growth pattern. The chicory, plantain, red clover and white clover sward also increased the proportion of lambs that grew faster than 200 g/head.day and increased the final carcass weight of the lambs, relative to the more winter/spring dominant pasture mixes. However, sowing seed mixes increases between-species competition for space, light, moisture and nutrients, particularly in newly established pastures, where competition between seedlings will occur until equilibrium is reached given the available resources. Survey work has shown that where complex seed mixes are sown, generally only the two most persistent species persist (Virgona and Hildebrand 2006). Little is known of how various pasture mixes establish in southern Australia so a field experiment was conducted to evaluate a range of pasture combinations suitable for two contrasting soils.

Materials and methods

Site description: The experiment was conducted at the Department of Primary Industries research farm at Hamilton, Victoria, Australia (37°50'S, 142°04'E; altitude 200 m). The site has a temperate climate, with winter/spring dominant rainfall. The long term (1965-2012) average maximum and minimum daily air temperatures in the warmest month (February) are 26°C and 11°C and in the coolest month (July) are 12°C and 4°C. Mean soil temperatures range from 20°C in February to 8°C in July. The experiment had two sites about 2.5 km apart. Site one (referred to as 'wet site') was poorly drained and flooded with up to 3 cm of water for short periods during winter, but was able to retain soil moisture into summer. Site two ('dry site') was on a crest with good drainage and rarely experienced water logging. Soils at both sites were Brown Chromosols (Isbell 1996). Topsoil (0-10 cm) at the 'wet site' and 'dry site', respectively, had a mean Olsen P of 29 and 19 mg/kg, Colwell P of 79 and 64 mg/kg, Colwell K of 167 and 146 mg/kg and available S of 31 and 19 mg/kg.

Experimental design and treatments: The 'wet site' had eight treatments and the 'dry site' nine treatments, each sown in Randomised Complete Block designs with four replications (Table 1). Pasture species were matched to their optimum soil type/environment, with three treatments common to both sites (perennial ryegrass, phalaris and lucerne). Plots were 20 m by 15 m in size and were individually fenced enabling individual grazing management to be applied to each plot.

Site establishment and management: In the eight months prior to sowing, both sites were sprayed 3-4 times with 2 L/ha of a knockdown herbicide (540 g/L glyphosate, ~10% w/v surfactant with water comprising the balance); the 'wet site' was sprayed in April, August and October 2009 and the 'dry site' was sprayed out in January, April, August and September 2009. Both sites were power-harrowed prior to sowing to stimulate weed seed germination. Weeds were subsequently killed with the herbicide applications. The 'wet site' was power-harrowed in August and October 2009 and the 'dry site' in August and September 2009. Perennial species were sown on the 'wet site' on 22nd October 2009, with the balansa clover sown on 26th May 2010. The 'dry site' treatments were sown on 30th October 2009. Poor establishment in 2009 resulted in all perennial species being re-sown at both sites the following autumn ('wet site' on 26th May; 'dry site' on 27th May) using the same sowing rates. At the autumn 2010 sowing, all of the treatments, except those containing lucerne, balansa or strawberry clover, were sown with 7 kg/ha of subterranean clover (Trifolium subterraneum L. cv. Leura). Prior to re-sowing, the phalaris plots at the 'wet site', and all plots at the 'dry site' were sprayed out with 2 L/ha of a knockdown herbicide (540 g/L glyphosate, ~10% w/v surfactant with water comprising the balance). Plots were sown using a conventional disc drill with tine spacing of 15 cm and a sowing depth of 15 mm. All treatments were sown with 10 kg N/ha, 21.9 kg P/ha and 1.5 kg S/ha, applied as monoammonium phosphate (MAP). After sowing, the site was rolled with a rubber tyre roller to improve seed to soil contact. The plots that were to be sown with lucerne received 5 t/ha of surface applied lime in July 2009. The fertiliser regime included applying 18 kg P/ha, 1 kg S/ha and 13 kg Ca/ha as triple super phosphate to the 'wet site' in June 2010, applying 25 kg N/ha, 54 kg P/ha and 3.75 kg S/ha as MAP at both sites in December 2010 and applying 47 kg P/ha with 2.25 kg S/ha as superphosphate to all plots at both sites in March 2011.

Measurements: Species frequency (% of 100 cells that contain herbage of the sown species) was measured in 2011 and 2012 from four permanent quadrats in each plot (Brown 1954). A 1 m x 1 m square of weldmesh with 10 cm x 10 cm mesh was used in each fixed location, to give 100 cells.

Statistical analysis: Data was analysed using the method of restricted maximum likelihood (REML), where the model included year, treatment and pasture species within treatment. The two sites were analysed separately. Differences are discussed at the P=0.05 level. The statistical package used was GenStat® Release 12.1 VSN International, Hemel Hempstead, UK.

Results

Weather conditions: Annual rainfall in 2009, 2010, 2011 and 2012 was 660, 767, 981 and 641 mm, respectively, compared to the long term average of 692 mm. October and November 2009 had below average rainfall (37 mm and 46 mm, compared to long term averages of 62 mm and 54 mm). During the November 2010 to March 2011 period a strong La Nina cycle resulted in above average rainfall, with 490 mm of rain falling during this period compared to the long term average for this period which is 205 mm.

Species frequency: At the 'wet site', the species frequencies of the Avalon perennial ryegrass, phalaris and balansa clover monocultures increased between 2011 and 2012. There was no between year change in Banquet perennial ryegrass, tall fescue or lucerne. In the tall fescue/chicory mixture, both species had similar species frequency values in 2011, but both species experienced a significant decline in species frequency between 2011 and 2012, with species frequency declines of 44% in the tall fescue and 28% in the chicory. The tall fescue in the mixed sward had a lower species frequency than the tall fescue in the monoculture sward in both years, though their sowing rates were the same. The monoculture tall fescue did not experience a decline in species frequency between 2011 and 2012. In the kikuyu/strawberry clover mixture, the kikuyu had higher species frequency values than the strawberry clover in both years, but both species were relatively unchanged between 2011 and 2012. At the 'dry site' the Avalon perennial ryegrass and phalaris did not change significantly between 2011 and 2012. In fact, none of the monocultures at the 'dry site' changed significantly between years. In the cocksfoot/lucerne, tall fescue/lucerne and chicory/lucerne mixtures, the lucerne had a species frequency of 50% and its species frequency did not decline between 2011 and 2012. When sown in mixed swards, the lucerne, chicory, tall fescue and cocksfoot had similar species frequencies compared to their monoculture swards.

Discussion

On the poorly drained soils of the 'wet site', the tall fescue, when sown as a monoculture, had good post-establishment persistence, being present in 99 our of 100 cells in 2012. When it was sown in a mixture with chicory, however, the tall fescue had much lower levels of species frequency, being present in only 46 out of 100 cells in 2012. This was despite being sown at the same sowing rate as the monoculture tall fescue. These results are not surprising because summer active cultivars of tall fescue is relatively slow establishing (Hamilton-Manns *et al*. 1995; Hill *et al*. 1985) and sowing it with a rapidly emerging species like chicory gives it a competitive disadvantage. The winter active tall fescue sown at the 'dry site' was not adversely affected by being sown in a mixed sward, with the Resolute having similar species frequency levels as the monoculture Resolute swards. These results show the difference between the summer active cultivars (Quantum II) and the winter active cultivars (Resolute), with the summer active cultivars generally being slower establishing. The summer active cultivars may, therefore, be less suitable for inclusion in mixed swards than the more competitive winter active cultivars of tall fescue.

At the 'dry site' the lucerne sown in mixed swards had similar levels of species frequency as the lucerne sown in monocultures. Lucerne monocultures are inherently unstable in southern Australia as their relatively slow growth rates in winter coupled with the build up of fixed nitrogen can result in sward invasion by annual weeds, particularly capeweed and Poa spp. which may require regular herbicide control. The inclusion of a winter active grass species will utilise the fixed nitrogen and fill space in the sward, preventing weed invasion. This research has shown that either cocksfoot or tall fescue at the 'dry site' could be sown with lucerne without affecting the species frequency of either species, as evidenced by comparisons with the monoculture swards. Chicory is often sown with lucerne as a means of thickening the sward over the first 3-4 years of production. This research has shown that sowing lucerne and chicory together was not detrimental to the species frequency of either species.

The kikuyu in this experiment had species frequency values similar to those of the other grasses in the experiment. Kikuyu, being a subtropical grass, is generally not used in southern Victoria. Previous research at Hamilton found that in the two years after establishment, kikuyu had relatively good plant numbers, but after this period, the kikuyu plant numbers declined until the sward was almost completely devoid of kikuyu and annual grasses prevailed (EverGraze, unpublished data). The decline in plant numbers was attributed to the high level of subterranean clover in the sward, which resulted in a build up of nitrogen with no useful species to utilise this nitrogen over winter and spring, resulting in annual weed invasion when the kikuyu was dormant. This was exacerbated by the rotational grazing regime used which allowed a build up of annual weed herbage in the sward and prevented the kikuyu from resuming growth in summer due to smothering. It remains to be seen if the kikuyu/strawberry clover mixture tested in this research will continue to persist for more than two years following establishment, but early indicators suggests that if weed invasion can be prevented, the kikuyu could warrant further research.

Conclusion

This research has provided preliminary evidence that a range of binary perennial pasture mixes are suited to the soil types found in southern Victoria. In particular, three possible companion species for lucerne on well drained soils have been identified; cocksfoot, tall fescue and chicory. These combinations can be established together and maintain species frequency for at least the first two years after establishment. On poorly drained soils, summer active tall fescue did not maintain species frequency in mixed swards with chicory, but kikuyu is a pasture species that warrants further research. The results in this paper are preliminary and further research is needed to evaluate herbage yield, nutritive value and botanical composition over a longer period.

Table 1. Sowing rate (kg) and species frequency (% of 100 cells that contain herbage of the sown species) in 2011 and 2012. LSD's (P=0.05) indicate the three way interaction between year × treatment × pasture species for each site.

No.	Site	Treatment	Sowing rate (kg)	29 June 2011	4 July 2012
1	Wet site - Monocultures	Perennial ryegrass (cv. Avalon AR1)	18	65	84
2		Perennial ryegrass (cv. Banquet)	20	83	73
3		Phalaris (cv. Australian)	3	71	100
4		Tall fescue (cv. Quantum II)	18	91	99
5		Lucerne (cv. Sardi-7)	14	50	61
6		Balansa Clover (cv. Bolta)	6.5	40	85
7 8	Wet site - Mixtures	Tall fescue (cv. Quantum II) + Chicory (cv. Puna II) Kikuyu (cv Whittet)+ Strawberry clover (cv. Palestine)	18 4 1 1	69 60 88 64	46 43 96 57
		LSD (P=0.05)	17.24		
1	Dry site - Monocultures	Perennial ryegrass (cv. Avalon AR1)	18	90	77
2		Phalaris (cv. Australian)	3	83	95
3		Cocksfoot (cv. Porto)	4	82	94
4		Tall fescue (cv. Resolute)	15	97	99
5		Chicory (cv. Puna II)	4	63	52
6		Lucerne (cv. Sardi-7)	14	55	65
7	Dry Site - Mixtures	Cocksfoot (cv. Porto)+ Lucerne (cv. Stamina5)	4 8		87 53
8		Tall fescue (cv. Resolute)+ Lucerne (cv. Stamina5	15 8		99 56
9		Chicory (cv. Puna II)+ Lucerne (cv. Stamina5	4 8		57 56
.		LSD (P=0.05)	14.13		

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Persistence of perennial pastures subjected to rotational grazing and different livestock systems: results from a long term experiment

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Abstract: The liveweight gains, reproductive performance and enterprise resilience of sheep and beef grazing systems in southern Australia are underpinned by the productivity and persistence of pastures. The stress of grazing, together with environmental stresses, mean that poor pasture persistence is a major problem and is commonly cited by farmers in southern Australia as a key reason for not adopting modern perennial pasture cultivars. The 'EverGraze® – More livestock from perennials' paddock-scale field experiment was conducted at the Department of Primary Industries research farm near Hamilton, Victoria, to test the performance of two different pasture systems using three cultivars of perennial ryegrass with contrasting flowering dates (cvv. Banquet, Avalon and Fitzroy), tall fescue (cv. Quantum) and lucerne (cv. SARDI Seven), sown with subterranean clover. The pasture systems were rotationally grazed by three livestock treatments (sheep low grazing pressure, sheep high grazing pressure or cattle) and the persistence of the main sown species/cultivar monitored. The basal cover (% of 100 cross points that have contact with a plant base) and species frequency (% of 100 cells that contain herbage of the sown species) were measured annually following the autumn break. Basal cover and species frequency for Banquet and tall fescue remained relatively constant during the experiment. The cultivars Fitzroy and Avalon experienced a gradual decline in basal cover and species frequency, but in 2012 the swards still did not require renovation. The lucerne experienced between year variability, but through changes to grazing management recovered from declines in basal cover and species frequency and in its sixth year of production was still an effective sward. This research has shown that modern perennial pasture cultivars can persist in production systems for more than six years under high stocking rates, with rotational grazing and destocking during droughts.

Keywords: Pasture systems, pasture production, deep rooted, summer feed, establishment, drought.

Introduction

Perennial pastures offer a range of advantages for livestock producers, compared to annual pasture options, relating primarily to the cost and labour saving of not re-sowing and improved seasonal distribution of dry matter and nutritive characteristics. Economic analysis has shown that investment in the establishment of EverGraze perennial pasture systems can be profitable for farms in south-west Victoria even when pastures are re-sown in their sixth year (Lewis *et al*. 2013). However, if pastures fail to establish or persist, return on investment is reduced and the time taken to return to positive cashflow increases as livestock producers incur additional costs through lost livestock production, increased supplementary feeding and the cost of re-sowing (Panetta *et al*. 1993; Snaydon and Howe 1986). Many perennial pastures in southern Australia appear to lack persistence, with survey results indicating that many swards contain less than 20% perennial species (Quigley *et al*. 1992). Results from a long term large-scale grazing experiment conducted at Hamilton, Victoria, as part of the EverGraze project, were examined to assess the persistence of perennial pastures. It was hypothesised that the pastures would persist for at least 6 years under the implemented management regime that included rotational grazing and destocking during drought.

Materials and methods

Site description: The experiment was conducted at the Department of Primary Industries farm at Hamilton, Victoria, Australia (37°50'S, 142°04'E; altitude 200 m). The site has a temperate climate, with winter/spring dominant rainfall. Summer/early autumn are hot and dry, though large sporadic rainfall events may occur. The long term (1965-2012) average maximum and minimum daily air temperatures in the warmest month (February) are 26°C and 11°C and in the coolest month (July) are 12°C and 4°C. Mean soil temperatures range from 20°C in February to 8°C in July. The site has three main soil types that influence the water-holding capacity of the soil and, consequently, the pasture species that can be successfully grown on each (Newell 1962). The soil types are gravelly loam (hill crests), silty loam (slopes) and clay loam (valley floors).

Experimental design and treatments: Two pasture systems, replicated three times in a completely randomised design, were sown in 2004/2005. Each pasture system was comprised of three pastures species/cultivars, sown in adjacent 1 ha paddocks, one on each of the three soil types identified earlier. The first, termed the ryegrass system, was comprised of three cultivars of perennial ryegrass; early flowering Fitzroy on the hill crests, mid-flowering Avalon on the slopes and late flowering Banquet on the valley floors. The second pasture system, termed the triple system, was comprised of SARDI Seven lucerne on the hill crests, Avalon perennial ryegrass on the slopes and Quantum tall fescue on the valley floors. Superimposed over the pasture systems were three livestock system treatments. In 2006 and 2007, a Merino prime lamb system (~14-15 ewes/ha), where Centre Plus and Toland Merino ewes mated to a terminal sire were pregnancy scanned and separated to lamb in September as single bearers with 100% target weaning rate (sheep low grazing pressure) or twin bearers with 150% target weaning rate (sheep high grazing pressure). In 2008, 2009, 2010, the same Merino ewes (~15-16 ewes/ha) were lambed as a flock with dry ewes removed, but no separation of singles or twins (sheep low grazing pressure) and were compared with a Coopworth prime lamb system (~12 ewes/ha), where Coopworth ewes mated to a terminal sire (sheep high grazing pressure). Lambing occurred in September 2008, August 2009 and August 2010. In all years, the low and high grazing pressure sheep systems were designed to target weaning rates of 100 and 150% respectively and thus the difference in grazing pressure between treatments was primarily driven by the difference in nutritional demands of single and twin bearing ewes. In 2006-2009, a steer/heifer backgrounding treatment (3.5–4 head/ha) was tested, where the animals were stocked in autumn 2006, 2007 and 2008 and sold in December or January the following year. In 2009 and 2010 a cow-calf treatment (2 cows/ha) was imposed, where Angus and Charolais cross heifers (2009) and cows (2010) mated to Angus bulls were allocated in January each year for a September calving and the calves were grown to weaning in autumn 2010 and 2011. The sheep system treatments were ceased after lamb weaning in December 2010 and cattle systems at calf-weaning in 2011. From this point onwards all paddocks on the EverGraze paddocks were stocked periodically with various sheep mobs for grazing.

Grazing management: Each of the three paddocks in each pasture system was split in half with a movable electric fence creating six split paddocks for grazing management. The overall grazing management was aimed at grazing to the three leaf stage preferred by perennial ryegrass and tall fescue (Fulkerson and Donaghy 2001; Raeside *et al*. 2012). With the exception of lambing and calving, where livestock were set-stocked in two of the paddocks from each system, rotations were generally between 14-28 days in spring, 28-42 days in summer and autumn and 42-56 days in winter. This was modified at varying times of the year for animal management, seasonal pasture requirements and to avoid pugging at times of water-logging on the valley floors. Over the late summer/autumn period, paddocks were taken out of the rotation in a pasture system when the amount of herbage fell to 800 kg DM/ha or the proportion of bare ground exceeded 30%. Stock were then either grazed on other paddocks in the replicate or, if all paddocks in the replicate fell below this trigger point, the system replicate was destocked and supplemented off site. Stock were re-introduced to the replicate after the autumn-break when herbage exceeded 1000 kg DM/ha. In mid-2010, the six paddock rotational grazing system was discontinued across all livestock system treatments and replaced with a three paddock rotation and following weaning of lambs and calves in late 2010 and early 2011 paddocks on the EverGraze site were stocked periodically with various sheep mobs for grazing of the prevailing feed on offer with no strict rotations enforced other than to manage the build up of dry matter. From spring 2011 until late summer 2012, the site was grazed sporadically with sheep. During this time, the pasture accumulated 3–5 t DM/ha for the lucerne paddocks and 4–6 t DM/ha for the perennial grasses, which was left standing as dry feed.

Measurements: Basal cover (species presence as measured by contact with a plant base) was measured annually from four permanent quadrats in each paddock (Brown 1954). A 1 m x 1 m square of weldmesh with 10 cm x 10 cm mesh was used in each fixed location. The numbers of live plant bases directly under the 100 cross-points were counted. The same quadrats were used to measure species frequency at the same time as the basal cover measurements. The species frequency is the presence of species herbage in a unit area. By measuring both of these annually it is possible to determine the persistence of each of the sown perennial species.

Statistical analysis: Data was analysed using the method of restricted maximum likelihood (REML). Differences are discussed at the P=0.05 level. The statistical package used was GenStat® Release 12.1 VSN International, Hemel Hempstead, UK.

Results

Weather conditions: Annual rainfall was above average in 2007, 2010 and 2011, and below average in 2006 and 2008 (Table 1). Notable weather factors include the failure of October rainfall in each of 2006–2009 and the heavy rainfall in January 2007, November 2007, December 2008 and over the August 2010–April 2011 period. Summer temperatures were higher than the long-term average for each year except 2010/11. This was particularly noticeable in January–February 2009 with 9 days over 35°C and a peak of 44.1°C on 7 February 2008.

Basal cover: Lucerne basal cover dropped initially (2006-2007), but then slowly increased in subsequent years until 2010 (Figure 1). Tall fescue basal cover was essentially constant from 2006 to 2010 with a slight dip between 2006 and 2007. In 2012, there were large differences in basal cover. In the former cattle treatments, the lucerne basal cover declined, while all of the other species remained unchanged since 2011. In the sheep treatments, the basal cover of the

Banquet increased between 2011-2012, while the tall fescue, Fitzroy and Avalon remained unchanged and the lucerne declined.

Species frequency: Between 2006 and 2007 the grasses experienced a decline in species frequency, but between 2007 and 2011 their species frequency gradually increased and in 2011 the grasses had similar species frequencies to the 2006 levels (Figure 1). The lucerne generally remained unchanged between 2006 and 2011, but had sharp declines in species frequency in 2008, from which it recovered in subsequent years, and declines in 2012

Discussion

The lucerne persisted over a six year period (2006–2011) without significant losses in basal cover or species frequency. However, a decline in grazing control over 2011-12 lead to a decline in persistence. The EverGraze experiment was designed on the premise of 'right plant, right place, right management', with the successful adoption of these principles resulting in persistent lucerne swards over the period 2006–2011.

Right place refers to sowing species on their optimum soil type/location in the landscape. Although the landscape around Hamilton appears relatively flat, identifying subtle changes in soil type enabled lucerne to be sown in well drained areas where it was more likely to persist. Right management in this instance involved preparing a suitable seedbed where liming occurred to neutralise surface soil acidity, followed by regular fertiliser applications to maintain P, K and S at or above critical levels, and adopting a rotational grazing system and destocking once ground cover and feed on offer thresholds were reached. Where these principles were adhered to, the lucerne swards were able to survive under a range of difficult environmental conditions, including the failed spring rains over 2006–2009 and the heat wave in February 2009. However, when a lack of grazing control occurred over late spring 2011 to summer 2012, the lucerne experienced a sudden decline in frequency and basal cover. Over this 2011–12 period, a build up of dry matter (3–5 t DM/ha), of which annual grasses and summer growing weeds comprised a large portion, the lucerne thinned out substantially. In addition, as grazing treatments were discontinued during 2011 the change from cattle to sheep grazing of the lucerne in the cattle treatments may have exacerbated declines in frequency and basal cover as sheep are able to graze closer to the ground than cattle.

The tall fescue was sown on the poorly drained areas of the landscape, and with the lucerne on the well drained areas, combined to make an effective grazing system with increased summer activity with plant matched to place. Over the 2006–2011 period, the tall fescue swards were dense and persistent and had similar basal cover and species frequency levels as the perennial ryegrass swards. Basing grazing rotations on the three leaf stage for tall fescue, with a more intense period of grazing in spring, effectively maintained plant persistence. Following the period of lax grazing in the 2011–12, it was only the tall fescue in the sheep low grazing pressure treatment that experienced a significant decline in persistence. This may have been due to the lower grazing pressure experienced during this period when the treatments were discontinued as it is known grazing pressure stimulates tillering in grass swards. For the perennial ryegrass cultivars and system, despite initial reductions in frequency and basal cover from 2006 to 2007 following drought conditions in the spring of 2006, basal cover and frequency increased gradually through recruitment and increases in plant size. The larger increases in basal cover and frequency that occurred in 2011 and 2012 measures followed lighter grazing over 2011/12 and the wet 'La Nina' summer of 2010/11.

Conclusion

Modern perennial pasture cultivars can persist for more than six years under high stocking rates, with rotational grazing and destocking during droughts. The principles of 'right plant, right place, right management' can lead to persistent perennial pasture systems.

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Table 1. Annual rainfall during the experimental period, compared with the long-term (1965-2012) average

Year	2006	2007	2008	2009	2010	2011	2012	Average 1965-2012
Rainfall (mm)	493	800	628	660	767	981	641	692

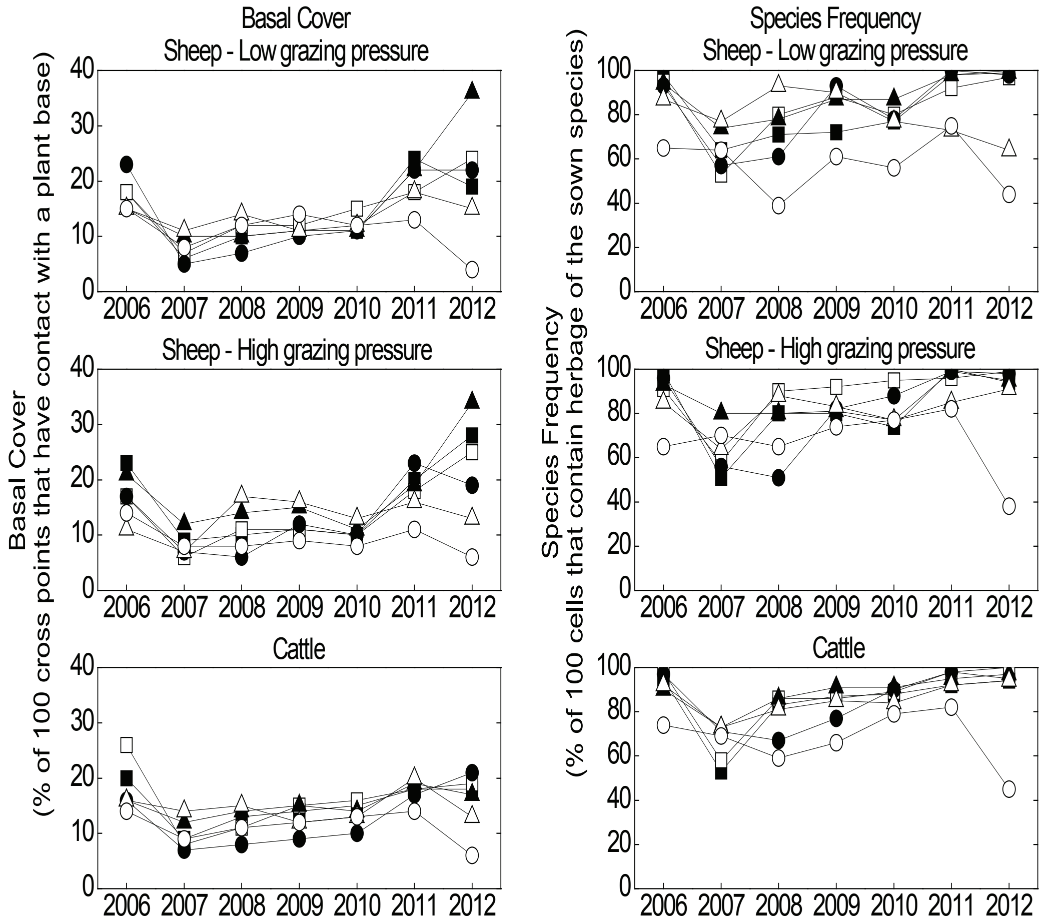

Figure 1. Basal cover and species frequency from the ryegrass system, Banquet (solid triangle), Avalon (solid square) and Fitzroy (solid circle), and the triple system, Quantum (open triangle), Avalon (open square) and SARDI Seven (open circle) under grazing by sheep low grazing pressure, sheep high grazing pressure and cattle. The LSD's (P=0.05) for the three way interaction between cultivar × livestock treatment × year are 7.60 for basal cover and 18.44 for species frequency.

Seasonal variation of the ergot-alkaloid, ergovaline, in perennial ryegrass (*Lolium perenne*) infected with standard and novel endophyte

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Abstract: Perennial ryegrass endophyte (*Neotyphodium lolii*) is a fungus that forms a symbiotic relationship with the host plant. The ryegrass plant often receives agronomic benefits from this symbiosis as the endophyte alkaloids confer greater resistance to biotic and abiotic stresses. Ergovaline is an end product of the ergot alkaloid pathway and can adversely affect animal health and productivity. However, ergovaline does not cause ryegrass staggers, and is effective at controlling ryegrass pests, even at lower concentrations than are present in the standard endophyte. As there are very few, if any, totally safe and effective endophytes available, it is desirable to improve our understanding of the variation in ergovaline concentrations throughout the year, either to facilitate the development of safer animal management practices or to measure progress for plant breeders. An experiment was conducted within an established field trial in central-west Victoria to monitor and record the ergovaline concentrations over 1 year. Concentrations were monitored in a breeding line infected with the standard, or 'wild type' endophyte ('PGWS1' SE), a commercial cultivar infected with the novel endophyte 'Endo5' ('BQTII' Endo5) and a cultivar with 'Endo 5' selected for even lower levels of ergovaline ('PGWS2' Endo5). Tiller samples were collected every 2 weeks over 1 year from each cultivar and analysed for ergovaline concentration. A further set of tillers were collected and dissected into leaf and pseudostem and analysed separately. The concentration of ergovaline was consistently higher in the pseudo stem than in the leaf in both endophytes. Ergovaline in the SE plants was consistently higher than in Endo5 plants throughout the year, and concentrations in the winter and spring were consistently low in the leaf (<0.4 mg/kg) for both endophytes. Their unexpected increase in concentration in both leaf and stem, more pronounced in the SE plants, during the months of April and May, was not associated with increased temperature, but was probably linked with the presence of insect pests following the autumn growth. This study has shown: a) two distinct peaks in ergovaline production in the late-summer and autumn periods, more apparent in the pseudostem and in the SE line; b) Endo5 in the plant germplasm used in this experiment produces lower levels of ergovaline than line SE throughout the year; c) ergovaline in the Endo5 endophyte cultivars was predominately in the crown of the plants; and d), that further selection for reduced ergovaline has been achieved in 'PGWS2' Endo5.

Introduction

Perennial ryegrass (*Lolium perenne*) is a valuable pasture species, which normally contain a fungal endophyte in Australia in the high rainfall and irrigation zones of southern Australia. Ryegrass endophytes (*Neotyphodium lolii*) are fungi that form a symbiotic relationship within the host ryegrass plant. These interactions are widely studied as the hosts often receive agronomic benefits through greater resistance to biotic and abiotic stresses (Rolston *et al*. 2002; Easton 2007) and because livestock toxicosis has often been attributed to endophytes, with symptoms including increased occurrence of perennial ryegrass staggers (Fletcher and Harvey 1981) and heat stress (Fletcher 1993). Standard Endophyte (SE), otherwise known as 'wild-type' endophyte, is widespread in Australia with 90% of established ryegrass pastures infected with SE (Quigley and Reed 1999). While considerable progress has been made in identifying and releasing new strains of 'novel' endophytes that express alkaloid profiles that provide protection against many ryegrass pests with a greatly reduced risk of toxicosis, the perfect endophyte has not yet been found (Fletcher 2012).

Ergovaline is considered responsible for many of the animal health problems associated with endophyte (Lane 1999). It is an ergot-alkaloid class of compound, and is the end product in this biosynthetic pathway (Schardl *et al*. 2012). Erogovaline acts as a vasoconstrictor, and is implicated in thermo-regulatory dysfunction and contributes to poor animal performance (Cosgrove and Hume 2005). However, ergovaline does not cause ryegrass staggers, and endophytes, such as Endo5, are available which produce ergovaline and peramine, but none of the ryegrass stagger causing alkaloids. An experiment by (Reed *et al*. 2011b) concludes that a possible synergistic effect of toxins, such as lolitrem B and ergovaline cannot be ignored in regard to animal health and performance. As there is an interaction between ergovaline and Lolitrem B, such enodphytes are likely to be less toxic to animals even with equivalent ergovaline concentrations found in new, selected, cultivars. Various studies have shown that ergovaline can be very effective in protecting the plant against attack from common pasture pests including the African black beetle (*Heteronychus arator*) (Wheatley *et al*. 2003; Hume *et al*. 2007) and root aphid (*Aploneura lentisci*) (Popay and Gerard 2007) in both Australia and New Zealand.

The concentration of ergovaline in the ryegrass plant fluctuates throughout the year and it has been shown to be more concentrated in the pseudostem than in the leaf blade (Watson *et al*. 1999; Speiring *et al*. 2002). Increases in concentration are most likely to occur during summer and early autumn because of a number of contributing factors including higher temperatures, increased dry matter and response to rainfall (Reed *et al*. 2011).

It is possible to select for reduced levels of ergovaline

(Adcock *et al*. 1997; Hill *et al.* 2002) and this trait has been shown to be highly heritable (Easton *et al*. 2002). A late maturing tetraploid cultivar, 'Banquet II Endo5', was selected for reduced levels of ergovaline (M.Norriss, pers. comm.) and several thousand tonnes of seed of this cultivar have been sold in Australia over the past 7 years (PGWS unpublished data). It has proven to be effective against pests and no adverse animal health effects have been reported, but there are few studies on the ergovaline levels produced throughout the year by the Endo5 endophyte. This study aims to correct this shortfall. Therefore, samples were collected from an existing replicated perennial ryegrass trial to determine how ergovaline concentrations vary over a 12-month period, and to establish if there are any differences between seasonal concentrations in SE and the Endo5 commercial endophyte and to report on progress being made in selection for reduced ergovaline in a new Endo5 infected cultivar.

Methods

An experiment was conducted on a pre-existing, small plot, perennial ryegrass agronomic trial at the PGG Wrightson Seeds Leigh Creek Research Station, Ballarat, in central-west Victoria. A randomised complete block trial was machine sown on the 22nd of April 2010, into a prepared seed bed of red volcanic-loam soil. Fertiliser (NPKS) was applied throughout the trial period to ensure no nutrients were limiting. Three different cultivars/selections were sampled, a mid-maturing diploid ryegrass breeding line containing standard, or wild type, endophyte ('PGWS 1' SE), a commercially available late maturing tetraploid containing the 'Endo5' endophyte ('Banquet II' –' BQTII Endo5'), and a late maturing tetraploid breeding line also containing 'Endo5' but also selected for reduced ergovaline levels, referred to as 'PGWS2 Endo5'.

The trial was rotationally harvested for dry matter yields when the sward was between the 2.5 to 3-leaf stage, and when the herbage mass reached between 2500 and 3500 kg DM/ha. The trial was also grazed at specific intervals following harvest. The trial received two irrigations from a travelling overhead boom throughout the trial period, 32 mm was applied on 20/1/2012 and 40 mm applied on the 20/1/2012. Rainfall throughout the period was measured on-site (Leigh Creek research station). Temperature readings were taken from a Bureau of Meteorology station located 4.2 km away (Kirks Reservoir BOM #087014) from the field trial. Both temperature and rainfall throughout the experiment were near average expected for this location. Tillers were collected for ergovaline analysis at the mid-week stage irrespective of growth stage, every fortnight for 12 months from November 2011 to September 2011. Two separate samples were collected from each cultivar on one replicate. The first consisted of 50 tillers, which were randomly selected and cut at ground level using a scalpel and further dissected into leaf and pseudostem. Emerging reproductive stems were avoiding during sampling. The second sample was collected from 30 plants at 30 mm above ground level representing the plant material available to sheep in a typical rotational grazing system. All samples were frozen immediately and remained frozen until analysed. Endophyte infection of each cultivar line was determined by immune-blotting 100 tillers on 29/12/12.

Analysis of ergovaline and ergovalinine levels was performed by HPLC, using a modification of a published procedure (Shelby and Flieger 1997; Shelby *et al.* 1997). Details of the modifications are discussed in Reed *et al*. (2004). Briefly, an acetic acid extract of the plant tissue is taken & cleaned on a mixed-mode SPE cartridge then eluted in methanol prior to HPLC with fluorescence detection.

All three lines tested positive for endophyte infection, with levels in 'PGWS1 SE' at 93%, 'BQTII Endo5' at 94% and 'PGWS2 Endo5' at 97%. The concentration of ergovaline varied throughout the year with two distinct peaks in summer and late autumn. At other times of the year concentrations in SE were low and in the two Endo5 endophytes, very low. There was approximately four times more ergovaline in the pseudostem than in the leaf during the peak periods with the whole plant being approximately halfway between the two (Figure 1).

The highest levels of ergovaline were recorded at the first peak (13/1/12 and 31/1/12) in the SE whole plant and SE pseudostem samples, which produced over twice the concentration of ergovaline than the Endo5 samples. At the next sample date, (20/2/12) the ergovaline concentration had halved for all treatments to be less than 1.0 mg/kg in the pseudostem. The second peak occurred on 18/4/12 and 1/5/12. The relative differences in whole plant concentration between the three cultivars were not as great as the first peak in the earlier January period, however ergovaline concentration was still greater in PGWS1 SE > BQTII Endo5 > PGWS2 Endo5(2). The ergovaline concentration in the leaf of the SE was double that in the Endo5 samples, and almost 1.0 mg/kg greater in the pseudostem. A small rise in ergovaline concentration was observed in the late October-November period in the pseudostem and whole tiller.

Ergovaline concentration did not rise above 1.0 mg/kg, regardless of endophyte type or plant segment from late autumn (16/5/12) through to mid-spring (18/10/12). Ergovaline was still detectable in the SE through most of this period and it was consistently higher than in both the Endo5 lines. In many of these assessments, the ergovaline concentration in the two Endo5 samples was less than 0.05 mg/kg, with at the limit of detection. There was a trend for the PGWS2 Endo5 to be lower than BQTII Endo5 throughout this period.

The SE samples recorded the highest concentration of ergovaline at almost every sample date. Some exceptions occurred on: 8/11/11 and 30/11/11 where SE was slightly higher than Endo5 and Endo5(2). On the 13/1/12 Endo5(2) was higher in the stem than SE; on 20/2/12 Endo5 was higher than SE in the stem and leaf; and on 29/12/11 the whole plant analysis for SE showed higher levels than both Endo5 and Endo5(2). Endo5(2) generally had lower levels of ergovaline than Endo5, however, this was observed in the leaf, but not necessarily in the pseudo-stem where on some occasions Endo5(2) had higher levels than Endo5. Whole plant concentrations in Endo5 (2) were lower than Endo5 except for four sample dates.

Regression analysis has shown that the highest correlation between ergovaline and temperature was observed in the pseudostem of Endo5 ($R^2 = 0.53$). The relationship between temperature and ergovaline expression is quite apparent in the summer months where the highest peaks were observed at the highest temperatures, and throughout the cooler months, when maximum temperatures dropped below 15°C and ergovaline levels remained less than 0.5 mg/kg. Little correlation between irrigation or rainfall was observed between ergovaline expression and cultivar/endophyte type or plant segment. However, soil moisture content measurements were not made.

Discussion

This study has demonstrated that concentrations of the ergovaline alkaloid, regardless of endophyte type, vary throughout the year. This is consistent with work reported by Reed *et al*. (2011) who measured similar fluctuations of ergovaline in SE infected perennial ryegrass. Reed *et al*. (2011) also recorded ergovaline concentrations peaking twice during the sampling period at similar times to this study. The first peak was in November-December, which Reed *et al*. (2011) attributed to seed head emergence and development. Although this peak was observed earlier in the season than our study, this could be explained, firstly, by the fact that we had used later maturing cultivars, and secondly, our specific location has a longer growing season in a milder environment. The second peak reported by Reed *et al*. (2011) was in mid-autumn, consistent with our results, which they attributed to new re-growth stimulated by successive rainfall events. A study in New Zealand showed similar seasonal trends in the cultivar 'Nui' containing standard endophyte (SE), where concentrations of ergovaline were greater, recording 0.9 mg/kg in winter and reaching 1.4-1.8 mg/kg in summer (Fletcher *et al*. 2001).

Critical thresholds have been developed for adverse animal health effects for different livestock classes. Aldrich-Markham and Pirelli (1995) state that this threshold is 0.4 – 0.75 mg/kg for cattle, although subclinical effects can occur at lower levels of 0.2 – 0.3 mg/kg. For sheep, the threshold these workers suggest between 0.8 – 1.2 mg/kg, which is consistent with Tor-Agbidye et al. (2001) who suggests 0.8 mg/kg. In the leaf component of the two selected Endo5 linesendophytes (BQTII and PGWS2), ergovaline concentration was less than 0.3 mg/kg in 14 of the 23 samples (Figure 1A) and never exceeded 0.5 mg/kg. Concentrations in the pseudostem were generally much greater than in the leaf for all three cultivars (Figure 2B). SE reached 3.5 mg/kg in the summer peak, and remained generally higher than the Endo5 lines throughout the year. In a New Zealand study, Watson et al. (1999) showed similar results in a 'Nui' SE sward, where concentrations were significantly lower in the leaf blade than leaf sheath during the peak periods of January to May. Pseudo stem levels remain high while leaf levels are much lower than in the SE in the Endo5, which is a good compromise for both persistence and animal performance. Rotationally grazed ryegrass will have an elongated pseudostem which could be accessible to sheep if grazed very hard whereas set-stocked ryegrass will have very little accessible pseudostem. In the whole plant analysis, which were sampled at a perceived grazing height (30 mm), ergovaline concentrations were < 0.3 mg/kg at 14 and 16 occasions of the 23 samples for the BQT II Endo5 and PGWS2 Endo5 respectively, and concentrations were always less than in the SE samples. In the SE, whole plant concentrations were only < 0.3 mg/kg on 10 occasions out of the 23 samples, with the majority (8 samples) of these occurring during the late winter-spring period.

Summer management is reported to influence ergovaline concentration. Reed *et al*. (2011) showed that where growth was allowed to continue through summer (i.e. low grazing pressure systems), ergovaline concentration was relatively low (<0.7 mg/kg) but in hard-grazed pasture (sward height 30 mm), ergovaline concentration was greater (up to 1.1 mg/kg). This perhaps reflects the higher percentage of pseudostem in the samples or a plant/endophyte response to stress. A limiting factor in this study was the lack of measurements of sward height, or feed availability at each sample date, which would have provided information that is more robust. Reed *et al*. (2011) also explains that alkaloid concentration is influenced by a number of other factors including physiological status of the plant, genetics of both the host grass and endophyte and use of nitrogen fertiliser. For example, a field trial in New Zealand found that an application of nitrogen fertiliser doubled the production of ergovaline, particularly in non-irrigated stands (Lane *et al*. 1997). Nitrogen levels were maintained to promote optimum growth in our study and, although we can assume this may have led to higher ergovaline than in most pastures. We did not observe any correlation between rainfall or irrigation and ergovaline levels. Easton *et al*. (2002) found that the variation in toxin concentration was a result of the mycelial mass of the endophyte, again not measured in our study. Reed *et al*. (2011b) showed this accounted for 19-20% of the variation (of ergovaline and lolitrem B) in ryegrass.

There have been a number of studies, which investigated the effect of environment on alkaloid concentration, specifically focussing on lolitrem B and ergovaline (Watson et al. 1999; Fletcher *et al*. 2001; Reed *et al*. 2011; Reed *et al*. 2011b). For example, Fletcher *et al*. (2001) examined alkaloid concentration and climatic data and used a statistical modelling approach which accounted for 42% of the variation in ergovaline in both SE and AR6 (ergovaline producing) endophytes. Although measuring the environmental effects was beyond the scope of our study, Figure 2 does show a relationship between temperature and ergovaline concentration. Regression analysis shows this to be 0.53 (R2 = 0.53), which could explain around 28% of the variation. Other triggers for the peaks in concentration in our study could possibly be linked to rainfall, temperature, resultant plant growth and stress of other biotic factors. Reed *et al*. (2011) outlined that grazing effects on pasture mass and composition must be considered for their influence on toxin concentration suggests that good pasture-growing conditions appear to be positively related to high toxin concentrations. This appears consistent with the second peak of ergovaline in the April-May period where rainfall and high temperatures resulted in increased pasture growth rates of the pastures, with early April dry matter harvests suggesting this (Figure 3). It could also be suggested that an increase in biotic stresses such as root aphid, which have previously been reported in high numbers at this site (D.Hume, A.Popay and J.Sewell unpublished data), could possibly be a contributing factor to the increases of alkaloid. Ryegrass infected with the Endo5 endophyte strain has no lolitrem B and therefore will not cause ryegrass staggers, and given the possible interaction between ergovaline and lolitrem B (Brown *et al.* 1999), whereby lolitrem B could be exacerbating ergovaline effects, the lower levels of ergovaline in Endo5 infected ryegrasses should result in a lower risk of animal toxicosis. However, the total amount of pasture available to the animal is also important, as outlined previously. As the PGWS1 SE cultivar is predominantly a summer dormant type, and the two Endo5 cultivars (BQTII and PGWS2) are both late maturating and more summer active (Figure 3), they are likely to have more high quality feed available over summer. This may increase animal intake during the high-risk period, especially if these pastures provide the only green feed on the farm. It has also been shown in controlled studies that increasing ambient temperature increases the effects on lambs of ergovaline, and differences were more apparent at higher temperatures (Fletcher 1993) and it therefore could be postulated that summer active cultivars pose a higher risk under some circumstances. Further to this, as Reed and Mace (2013) highlight, the relatively high solar radiation and temperature expected in Victoria, compared with other countries such as New Zealand, will result in more severe heat stress on ruminants and therefore this may aggravate the effect of the ergot-alkaloids and ergovaline.

The elimination of ryegrass staggers through the Endo5 endophyte, coupled with the reduced ergovaline levels, does provide some confidence to farmers that Endo5 is a significant improvement on the old wild type or SE endophyte strain.

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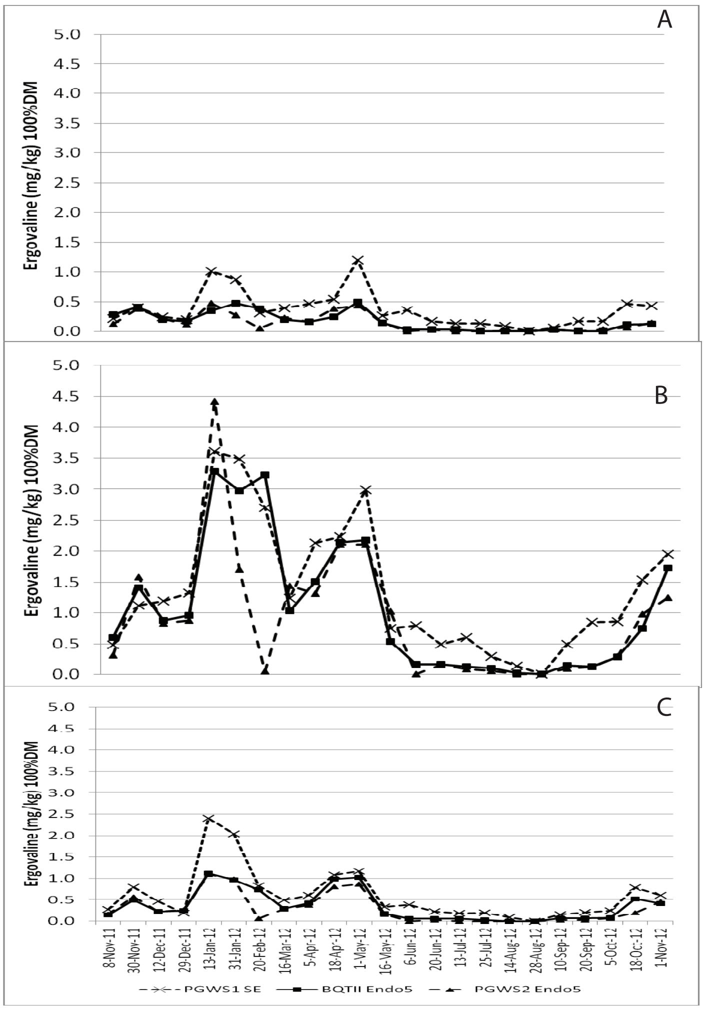

Figure 1. Ergovaline concentration (mg/kg) over time in leaf (A), pseudostem (B) and whole plant (C) for the standard endophyte cultivar (PGWS1), Banquet II with Endo5 (BQTII Endo5) and a breeding line cultivar with an Endo5 selection (PGWS2 Endo5).

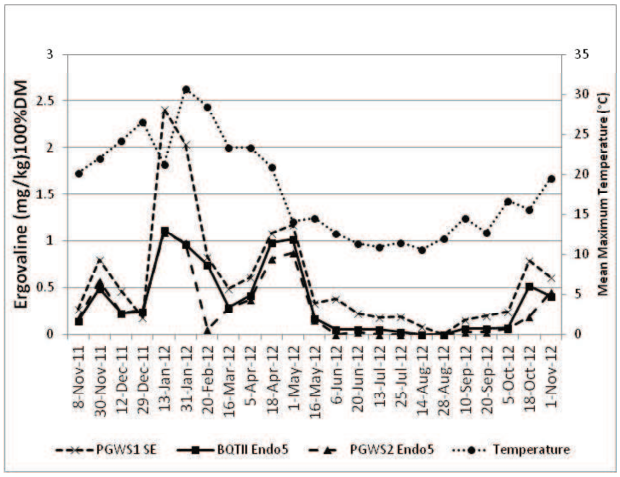

Figure 2. Whole plant ergovaline concentration and mean maximum daily temperature averaged over the sampling period, for PGWS1 SE, BQTII Endo5 and PGWS2 Endo5.

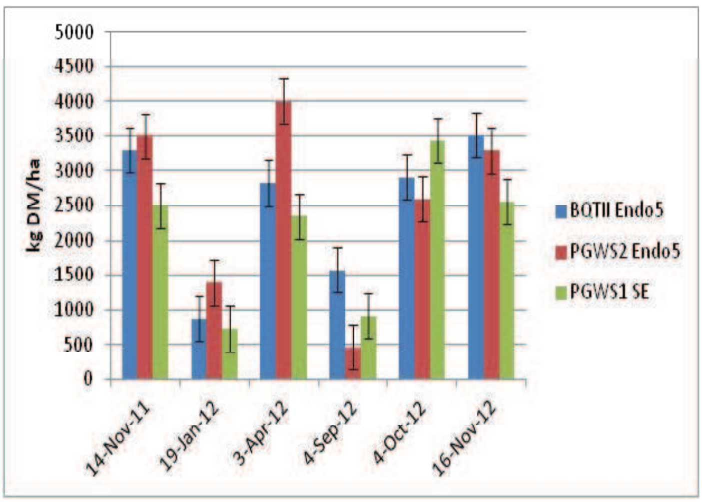

Figure 3. Dry matter production (kg DM/ha) throughout the sampling period for PGWS1 SE, BQTII Endo5, PGWS2 Endo5.

An improved method for recovering and quantifying neurotoxic alkaloids from endophyte-infected ryegrass

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Abstract: Most published methods for quantifying neurotoxic alkaloids such as lolitrem B from perennial ryegrass rely on separation using normal phase HPLC followed by fluorescence detection. We report here a method based on reversed-phase HPLC using a C18 column followed by electrospray ionisation liquid chromatography/mass spectrometry (ESI LC-MS). Lolitrem B was easily quantified with this method at very low levels of abundance (limit of detection = 1 pg injected on column). Extraction of dried ryegrass foliage with various solvents showed dichloromethane to be most efficient at recovering lolitrem B, but acetonitrile recovered 75% of the amount extracted by dichloromethane and has numerous advantages over dichlormethane as an extraction solvent. This method should prove to be very useful for quantitating lolitrem B in perennial ryegrass samples because it offers great sensitivity and selectivity and relies on commonly used chromatographic columns and solvents.

Key words: analytical chemistry, livestock production, ryegrass staggers

Introduction

Endophytic fungi produce a variety of compounds which confer advantageous properties to the plants they inhabit including drought tolerance and resistance to insects and weeds (Schardl *et al*. 2007). However, these compounds can also have a devastating effect on livestock grazing on them. One such example is the potent endophytic neurotoxin lolitrem B which is found in the forage species *Lolium perenne* L. (perennial ryegrass, Gallagher *et al*. 1981). Lolitrems, a family of closely related ergopeptine alkaloids produced by *L. perenne* infested with the endophyte *Neotyphodium lolii*, cause tremors in livestock and result in decreased production and increased mortality (Parton and Chambers 2001). The lolitrems are active at levels as low as 5 ppm (Gallagher *et al.* 1985), so being able to quantify these compounds in perennial ryegrass tissues and seed is of importance to the livestock industry.

To date, most reports quantitating lolitrems in ryegrass have relied on the original method reported by Gallagher *et al.* (1985). This method requires the use of normal phase HPLC, halogenated solvents, and fluorescence detection. Since then, significant advances have been made in analytical techniques for separation of compounds in mixtures, particularly the widespread use of reversed phase columns, and use of ESI LC/MS (liquid chromatography coupled with mass spectrometry using electrospray ionisation to introduce analytes into the mass analyser). These advances have the potential to increase the sensitivity of methods for quantitating lolitrems from ryegrass while decreasing reliance on halogenated solvents, which are potent carcinogens.

The objectives of this study were to apply reversed phase HPLC coupled with ESI LC/MS to quantify lolitrem B in perennial ryegrass samples, and to compare extraction efficiency of lolitrem B using a variety of organic solvents. The overall goal was to develop a method for detecting lolitrem B at very low levels using commonly available analytical chemistry techniques.

Methods

Sample preparation: Dried samples of perennial ryegrass were weighed (100 mg) and extracted with 2 mL of solvent. The following solvents were compared: acetonitrile, dichloromethane, hexane, methanol and dichloromethane:methanol (4:1). Samples were vortexed for 30 sec, then gently shaken on an orbital shaker for 24 h and centrifuged at 8,000 rpm for 5 min. The supernatant was drawn off with a pipette and filtered through a 0.22 µm filter (dichloromethane extracts could not be filtered because the filter membrane and housing was not compatible with halogenated solvents). Solvent was removed under a stream of nitrogen while warming the samples on a heat block; after weighing the samples, they were dissolved in dichloromethane (2 mL) and subjected to clean-up on silica gel solid phase extraction (SPE) cartridges (Agilent SimpliQ, 3 mL). SPE cartridges were preconditioned with methanol followed by dichloromethane, and samples were eluted with dichloromethane (3 mL, to waste) followed by 4:1 dichloromethane:methanol (2 mL).

Quantitation of lolitrem B: Samples were analysed using an Agilent 1200 HPLC coupled with an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray source. HPLC solvents were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Samples were eluted with a solvent gradient from 50:50 A:B, increasing to 100% B over 8 min at a flow rate of 0.4 mL/min. After reaching 100% B, the column was held at 100% B for 2 min and returned to 50:50 A:B for 3 min before the next injection. The column was C18 (Zorbax Eclipse XDB-C18, 4.6 x 50 mm, Agilent). Samples were introduced into the mass analyser via electrospray ionisation using a drying gas flow of 10 L/min and drying temperature of 350°C. Preliminary analyses were run in full scan mode with a fragmentation voltage of 135V, and quantitation was conducted using multiple reaction mode, filtering on a precursor ion of m/z 686 and a product ion of m/z 629, based on optimisation results (fragmentation voltage = 180V, collision energy = 35). Purified lolitrem B was purchased from AgResearch Ltd., NZ.

Results and discussion

Lolitrem B was readily detected using the chromatographic and mass spectroscopic techniques described above. In addition to lolitrem B, lolitrem F was often detected in samples (Figure 1). Lolitrem F is a stereoisomer of lolitrem B, differing only in the orientation of the hydrogen atom on carbon 35 (Munday-Finch *et al*. 1996). Despite this subtle difference, these two molecules were eluted with complete baseline separation, differing by 10-15 sec in retention time. Dilution series analysis of lolitrem B revealed an apparent limit of detection (LOD) of ca. 103 fg (1 pg) (Fig. 2). This is significantly more sensitive than previous studies utilising fluorescence detection, which has a LOD of 0.5 ng (Gallagher *et al.* 1985). Based on our sample size of 100 mg of dried tissue, our detection limit of lolitrem B in dried perennial ryegrass foliage is equivalent to 10 ng/kg, which is also a dramatic improvement over detection relying on fluorescence detection, reported by Moyano *et al*. (2009) as 0.05 mg/kg (50 ug/kg). This represents a 5000-fold increase in sensitivity.

Another significant advantage of ESI LC/MS is the exceptional selectivity when operating in multiple reaction mode. In this mode, ionised molecules entering the system are first filtered to exclude all except those with a molecular weight of the compound of interest, and then only characteristic product ions resulting from fragmentation of the parent molecule are quantitated. Combined with knowledge of the retention time of the compound eluting from HPLC, it is a virtual certainty that ions detected at specified points in time can result only from the presence of the parent molecule in a mixture. This selectivity contributes to the high signal-to-noise ratio, making it possible to quantify molecules at extremely low levels.

Comparison of extraction of lolitrem B with a range of solvents revealed that dichlormethane was the most efficient solvent (Table 1). Acetonitrile and hexane extracted about 75% of the amount extracted by dichloromethane, but the least efficient solvent for extracting lolitrem B was 4:1 dichloromethane:methanol, which extracted only 30% of the lolitrem B recovered by dichloromethane. Dichloromethane also extracted much more material (a wider range of compounds other than lolitrem B from grass samples) compared to hexane and acetonitrile (Table 1). This is in contrast to extraction of the ergot alkaloid ergovaline from tall fescue seed; in this case, methanol provided highest extraction efficiency of ergopeptine-related alkaloids (Garner et *al*. 1993). This difference is no doubt explained by the high lipophicity of lolitrem B and the relatively more polar nature of the ergopeptine alkaloids.

Acetonitrile may therefore be the solvent of choice when quantifying lolitrem B in perennial ryegrass samples because it extracted a high percentage of lolitrem B (ca. 75%) without significant amounts of irrelevant and potentially interfering compounds. Acetonitrile has other advantages over dichloromethane: 1) it is compatible with plastics commonly used in disposable sample filtration units, 2) it is fully miscible with solvents used for reversed-phase HPLC (e.g. water and acetonitrile), 3) it is less toxic than halogenated solvents such as dichloromethane and 4) wastes do not require the same degree of special handling as halogenated solvents.

The extraction procedure described above could potentially be simplified for routine extraction of lolitrem B from perennial ryegrass samples. Owing to the lower limit of detection, smaller quantities of plant material could likely be used, and vortexing samples with solvent might obviate the need for an orbital shaker.

Conclusions

This novel method for quantitating lolitrem B in perennial ryegrass offers significant advantages over previously published methods that are widely cited in the literature. HPLC methods that are currently used in analytical laboratories (e.g. reversed phase separation using acetonitrile and water in the mobile phase) are well suited to analysis of lolitrem B, and the use of ESI LC-MS offers extremely high sensitivity and selectivity (Callahan et al. 2009). Further refinements to the extraction procedure are likely to result in a greatly simplified and rapid method for analysing this environmentally important molecule.

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Table 1. Relative recovery of lolitrem B and total weight of material extracted from a perennial ryegrass sample extracted with various solvents. Recoveries are expressed as a percentage of the amount of lolitrem B extracted by dichloromethane.

Solvent	Relative recovery of Lolitrem B (%)	Total weight of extract (mg)
Dichloromethane	100.0	10.0
Hexane	77.0	2.9
Acetonitrile	73.1	2.1
Methanol	52.0	22.3
Dichloromethane plus methanol (4:1)	30.5	16.2

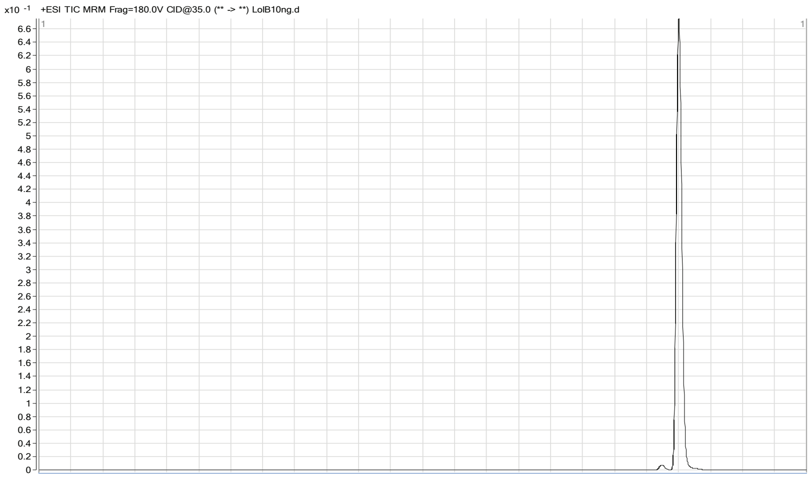

Figure 1. Total ion chromatogram of authentic standard of lolitrem B as separated via reversed-phase HPLC and detected via ESI LC-MS in multiple reaction mode (precursor ion=686 m/z, product ion=629 m/z). The small peak preceding the lolitrem B peak (the large peak at RT=10 min) is lolitrem F.

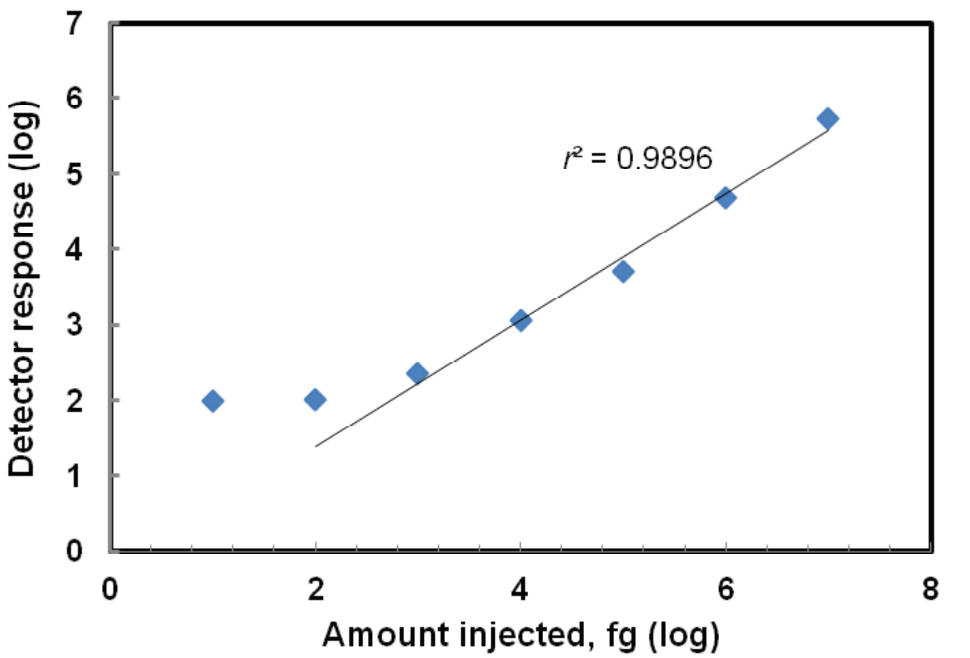

Figure 2. Dose/response of lolitrem B as detected by ESI LC-MS in multiple reaction mode. Below 103 (1 pg) of on-column injection, lolitrem B was not accurately quantitated. In the range of 103 to 107 g of compound injected, the detector response was well correlated with quantity injected (r2 = 0.9896).

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