final report

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Multi-purpose 'healthy' grazing systems using perennial shrubs

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Enrich

Multi-purpose 'healthy' grazing systems using perennial shrubs

Final Report
November 2008

Sheep grazing mixed forage shrubs at the Badgingarra experimental site (March 2008)

Dean Revell, Phil Vercoe, Mike Bennell, Jason Emms, Steve Hughes, Zoey Durmic, Marta Monjardino, Felicity Byrne, Andrew Kotze, Andrew Toovey, John O'Grady, Peter Jessop, Tim Wiley

Overarching statement

The dry margins of southern Australia's dryland agricultural regions are at risk of becoming economically and environmentally unsustainable. Prolonged drought and trends towards ongoing change in rainfall patterns are making landuse dominated by cropping and annual pastures unsustainable. This represents an opportunity for large scale change to livestock industries, where blending feed production from woody and herbaceous perennials with traditional pastures become the preferred productive landuse in the future. Through the Enrich project we have investigated new options for sustainable grazing systems incorporating Australian perennial shrubs. Shrubs can assist with profit (e.g., provision of out-of-season feed, improving animal health & welfare) and natural resource management (e.g., managing salinity, erosion, hostile soils & biodiversity) because of their ability to grow and persist under difficult environmental conditions and their resilience in our challenging environment.

Research team

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Summary of accomplishments of the project team

We **interviewed 40 producers** in four States who have been using shrubs as part of their forage system, and catalogued some of their experiences, ideas and production systems. We have coupled this with **whole-farm economic modelling** (using the MIDAS model) to test a range of scenarios that impact on the optimal scale and the profitability of using forage shrubs. For example, under a 'typical' central wheatbelt mixed farm, the modelling output indicated that about 10% of a farm allocated to perennial shrubs would increase whole-farm profit in the order of 20%. This conclusion held across a range of scenarios, including different commodity prices, but of course depends on the mix of soil types on a farm and the opportunity costs associated with forage shrubs.

We have identified over 100 species of shrub with potential for use as part of a forage system. We have **50 species well established at an evaluation site** in Monarto, near Murray Bridge, **SA** (planted in 2006). As from September 2008, we now have this replicated in Condobolin, **NSW** and Merredin, **WA**. We have also collaborated with **nine regional groups** across WA, SA, Victoria and NSW and have 15-20 species planted across a range of environments.

We have developed a **unique screening approach** to assess, as widely as possible, the potential benefits that forage shrubs may provide. This strategy was designed for two reasons. First, to consider a **broader set of traits than has previously been considered**, because any one species will have limitations – typically productivity, 'palatability' or nutritional profile. Second, because the plants have not been through any plant breeding program, there is potential for **plant secondary compounds in shrub species to affect (positively or negatively) livestock production**.

A range of Australian native shrubs have **fermentation characterisitcs** (gas production, and/or VFA) comparable to a common supplementary fodder, such as oaten chaff. Within these, there were plants that had **rumen-modulating properties** such as reduced methane production. There were also plants that had more pronounced effect on these properties, but also affected rumen fermentation. We have also identified that there is **significant anthelmintic activity** in many of the shrub species examined. This suggests that there is potential for some shrubs to provide a degree of activity against worms in grazing systems.

The successful use of mixed plant assemblies, including multiple shrub species and herbaceous plants should be underpinned by an understanding of grazing behaviour and the influence of management on diet selection and feed intake. Ultimately, systems will need to be designed and managed over time and space, which must take into account the dynamics between plants, animals and the environment. A grazing research site has been established at Badgingarra, WA, where 7 woody perennial species are on offer to livestock, together with perennial and annual pastures. Sheep **maintained weight without supplementary feeding over autumn** (when liveweight loss or supplementary feeding would normally be expected) when the **shrubs constituted 5-20% of their DM intake** (depending on grazing pressure). We have investigated **diet selection under different grazing pressures**, the **influence of animal experiences on selection**, and the potential for one of the shrub species to **control intestinal parasites**. The dynamic between selectivity, amount eaten and production can be manipulated over time using grazing management.

Reporting against project deliverables

Prospect statements for shrubs including a shortlist, based on the broad screening process, that warrant further investigation in field experiments and targets for domestication/plant improvement

Forage shrubs at about 10% of a typical crop-livestock farm boost profit in the order of 20%. This occurs through direct effects of reduced supplementary feeding over summer/autumn, and deferred grazing of annual pastures that allows an increase in annual stocking rates. This finding held across a wide range of scenarios such as grain or livestock commodity prices, soil types and carbon prices, although the absolute farm profit varied as would be expected. The allocation of shrubs to land that is marginal for cropping is the most likely scenario, where the opportunity cost of competing enterprises or technologies is minimal.

Additional benefits of shrubs may also occur. Based on interviews with farmers, shrubs are valued for their positive effects on (i) reducing the risk of wind erosion on vulnerable soil types; (ii) reducing the risk of dryland salinity; (iii) increasing confidence in feed supply at a time when a 'feed gap' is typically experienced; (iv) reducing labour demands at the break-of-season when cropping activities are in progress and when hand-feeding livestock competes with labour and management; and (v) provision of shade and shelter for livestock. Furthermore, our *in vitro* screening of plant species for 'bioactive' properties suggest that particular species have potential enhance livestock performance by altering rumen fermentation and/or reducing gut parasite burdens.

A shortlist of plants warranting further investigation has been developed (Table 1), but we are keen to state that further evaluation of plant performance is required across more than one site (Monarto, SA). This will be possible in the next phase of research with two new research sites now established (at Condobolin, NSW and Merredin, WA), each with about 50 species under evlaution. In addition, we will have 14 sites, each with 15 species, across southern Australia in partnership with a range of regional groups. Eleven sites have been planted and at least three more are planned for planting in 2009.

Detailed information on shrub productivity and adaptability to cultivation in the wheat-sheep zone.

About 50 species of shrub were successfully propagated and established at the Monarto evaluation site. Productivity of each of these species is quantified in the following Section 2.

Risk reduction practices associated with shrub establishment and their profitable use

The key issues that will impact on the profitable use of forage shrubs were quantified through bioeconomic modelling. We tested a range of scenarios, including commodity prices, establishment costs, shrub productivity and nutritive value, combinations of land classes (soil types), and carbon pricing. We focussed our work on the central wheatbelt MIDAS model rather than covering multiple regions. This allowed greater depth of scenario testing in the modelling given time and personnel constraints. Reliable data on shrub performance across difference environment is not available, and was beyond the scope of this project. Instead, the modelling approach dealt with the broad issues relevant to the profitable use of perennial plants in grazing systems.

Table 1. Summary of various shrub species possessing different traits of interest.

Species with significant biomass production:	**Species with significant biomass production & high VFA production from rumen fermentation:**
Atriplex nummularia *Acacia saligna (WA)* *Atriplex rhagodioides* *Atriplex semibaccata* *Chenopodium nitrariaceum* *Enchylaena tomentosa* *Maireana tomentosa*	*Atriplex amnicola* *Atriplex cinerea* *Chenopodium auricomum* *Medicago strasseri*
Species with significant biomass production & anthelmintic potential:	**Species with anthelmintic potential:**
Rhagdoia preissii *Rhagodia parabolica*	*Eremophila glabra* *Eremophila maculate*
Species with potential to reduce methane, but not necessarily with high biomass production:	**Species with potential to reduce methane and with anthelmintic properties but of low biomass:**
Abutilon octocarpum *Acacia myrtifolia* *Atriplex isatidea* *Atriplex paludosa* *Convolvulus remotus* *Cullen australasicum* *Enchylaena tomentose* *Lotus australis* *Maireana brevifolia* *Maireane georgei*	*Kennedia eximia* *Kennedia prorepens* *Kennedia rubicunda*
Species with significant biomass production & high VFA production & potential to reduce methane:	**Species with significant biomass production, high VFA, potential to reduce methane & anthelmintic potential:**
Convolvulus remotus *Rhagodia spinecsens*	*Rhagodia crassifolia*

Description of what animals choose to eat and why, when offered spatially and temporally diverse mixtures

Data on diet selection is still preliminary at this stage. The main shrub site at Monarto (SA) has been grazed and observations of plant palatability are documented. A mix of shrub species have undergone repeated grazing at part of the stage 2 work at Monarto. In addition, a series of three grazing experiments have been completed at the Badgingarra Research Station where seven shrub species have been established, together with perennial grasses and annual inter-row pasture.

Information on shrub preferences can be related to a comprehensive data set on nutritive value traits (ADF, NDF, crude protein and mineral composition), and *in vitro* effects on rumen fermentation and intestinal parasites. We have not quantified antinutritional factors, as this would be an enormous and nearly impossible task for such a large number of species, especially as for most there is very little information on what classes of secondary compounds are likely to be present. Once we reduce the list of candidate shrubs species to <10, we will need to carefully consider their profile of secondary compounds, and relate this to specifically to diet selection and feed intake.

Design principles for changing farmscapes with diverse plant mixtures in the feedbase for livestock.
In this context, 'design principles' encompass (i) identification of the optimal area of a farm established to forage shrubs (modelled), (ii) time of grazing for optimal use (modelled), (iii) scale and layout (farmer interviews), (iv) plant establishment and productivity (quantified for over 50 species), (v) nutritive value traits (quantified for over 50 species) and (vi) bioactivity (assessed through in vitro screening that were successfully established in this project.

Contents

Section 1

Current practices with forage shrubs and economic modelling predictions

Principles to the successful incorporation of forage shrubs into mixed production systems for profitable and resilient farming

[A draft of booklet that is currently being modified into a 'prospects statement']

Aim

This booklet provides an overview of the main principles the will determine how well forage shrubs can be incorporated into a mixed farming system. It discusses the issues and decisions that need to be addressed by landholders, ranging from broad questions on why forage shrubs may have a valuable role in a farming system, through to more specific information on managing forage shrubs. The booklet is not a step-by-step manual, as specific details depend on the farming system, goals of the land manager, soil types, climatic zone and shrubs species. Nevertheless, the booklet should provide a useful starting point by discussing many of the pros and cons of establishing, using and managing forage shrubs. This background knowledge will help build the confidence of landholders and advisors to modify existing systems and management practices. We envisage that the booklet could be used by farmers, extension officers, regional catchment or NRM groups, and others interested in profitable and sustainable land management.

Agricultural systems in the next decade will need to be responsive to:

- A continuing long-term decline in the terms of trade, despite shorter term fluctuations in commodity prices
- Emerging consumer demands for healthy food produced with a minimal chemical inputs and livestock systems that have high welfare standards
- Expectations from markets (both local and export) for production systems that have a reduced ecological footprint
- Producers who have precise and real-time knowledge about the various land capability classes on their properties
- Increasing costs of inputs, especially those based on fossil fuels (diesel and fertilisers)
- A shortage of labour in regional areas due to population shifts to coastal cities and towns and competition for labour in other sectors
- Increasing pressure to reduce greenhouse gas emissions and sequester carbon in the soil, driven by legislative changes and/or market signals
- An attitude that embraces and rewards land stewardship

These demands will require our production systems to:

- Be more in tune with the long-term capacity of the natural resources
- Look more closely at reducing input costs, with strategies informed by understanding natural processes; i.e., capitalise on the inherent biological system, rather than relying on external inputs.

Box 1 – Big statements will need to be followed with big action

Garnaut Report
"The challenge of climate change will be severe for Australia, but we take some assets into the response. Australia has a high level of adaptive capacity. We have adjusted to living in a highly variable climate. We have a well developed economy that is socialised to structural change. An exceptional human resource base in engineering and science and well developed disaster mitigation strategies and biosecurity management capacity will have high value. Adaptation issues will feature prominently in the final reports. The Review will take the local and regional nature of the challenge fully into account in its discussion of the key roles of State and local Governments in developing optimal adaptive responses."

2020 Summit Outcome: sustainability within Australia's resource constraints
The outcome sought is seamless integration of agricultural production and natural resource management practices and policies. This was expressed by one of the participants: 'Our 2020 farm would be highly productive and produce a variety of products; the producer would also be paid for wetland conservation and carbon sequestration; it would be resilient to climate change; and it would be supplying energy to the national grid'. Investing in integrated research and development is crucial to achieving this.

Government statements
Climatic shifts and potential decline in exports: ABC Radio "PM" program, 7 December 2007

The Australian Bureau of Agricultural and Resource Economics released a paper, which projected the effects of climate change on agriculture. Prime Minister Kevin Rudd said "By 2030, we face the possibility of a 10 per cent decline in agricultural production. By 2050, the possibility of a 20 per cent decline in agricultural production against a no-change basis. Then, up to a 63 per cent decline in Australian rural exports by 2030, and up to a 79 per cent decline by 2050."

Three underlying premises that have informed our work:

- *Shrubs alone will not provide sufficient edible biomass to sufficiently 'fuel' productive livestock systems.*
  This meant that our research has always been in the context of how shrubs could be incorporated into forage systems for multiple benefits. Consequently, we are interested in developing a diverse palette of plants (spatially and temporally) to provide new options and flexibility in farming systems.

- *Animals have a capacity to learn about the positive and negative consequences of eating particular plants, and modify their grazing behaviour accordingly*
  This is an important point, because it makes feasible the concept of managing an assembly of plants for the benefit of the landscape and productive grazing animals. It also means that we need to keep an open mind of how animals can best mix and match plant combinations, and how we might need to modify our management practices to capitalise on this capacity.

- *Potential attributes of Australian native shrubs have not been fully explored or valued.*
  In our work, these potential attributes are the presence of plant secondary metabolites (PSM) that interact with the animals that consume them. We have been interested in

their effects on ruminal fermentation and potential anti-parasite effects. Previously, PSM have been viewed as being anti-nutritional, but the effects of PSM depend on the dose (concentration) in an animal's diet. In considering the role of shrubs in mixtures (i.e., our first premise), we create the potential for animals to consume amounts of 'bioactive' plants that are not detrimental, and in fact, beneficial to their gut health.

Why Australian plants?

Our work in Enrich phase 1 has been designed to develop principles for incorporating shrubs into forage systems. To this end, many of our findings are applicable across different shrub species. However, in screening for candidate species of forage shrub, we focussed mostly (but not exclusively) on Australian natives for two main reasons:

- The first is that Australian native species have evolved in the environmental conditions in which we want them to be used. Historically, we have tended to select exotic plant species with high production potential and then develop management packages to try and exploit that potential in our environment. In the Enrich project, we have taken the alternative approach of identifying plants already adapted to the environment and then explore innovative ways to capitalise on their traits in production systems.

- The second is that Australian plants offer exciting prospects for identifying bioactive compounds [a point discussed in detail by Revell, Durmic, Bennell, and Vercoe (2008). The *in situ* use of plant mixtures including native shrubs in Australian grazing systems: the potential to capitalise on plant diversity for livestock health and productivity. British Society of Animal Science Occasional Publication].

Profitability with natural resource management

The view that we need to 'find a balance' between profitable land use on the one hand and improving our management of natural resources on the other, carries the implicit message that the two goals are not compatible. But of course, production systems are built on the natural resources and any decline – insidious or overt – in the quality or abundance of natural resources will impact on the bottom line. In agriculture, our industries have been faced with a long-term decline in the terms of trade (c. 2% p.a.) and, overall, a decline in the natural resource base (c. 1% p.a.). Consequently, an increase in productivity of at least 3% p.a. is required to maintain the status quo. Strategies to improve profitability that exacerbate the decline in natural resources can, at best, be judged as successful only in the short-term, because eventually the natural resource base will not be able to sustain the level of productivity that is required. Similarly, strategies to improve NRM that do not help to increase the rate of return on investment will not be adopted on a broad scale and the attention of land managers will be focussed on short-term productivity targets to meet immediate financial commitments. Our goal is to develop strategies that can provide improvements in both profitability and natural resource management simultaneously. This needs to be more than an aspirational statement; it is an imperative for attracting long-term investment in the financial and human capital associated with the agricultural industries.

Risk management requires flexibility

One of the strongest certainties facing land managers is variability. Some of this variability can be quantified, such as the distribution of different soil types (or land management units) across the landscape. But others, such as rainfall abundance and timing cannot be predicted with accuracy. No single production system is capable of maximising return on investment under all scenarios. Risk management is about maintaining a degree of flexibility to respond to any set of conditions. Recent experiences across southern Australia and many climate predictions suggest that climate variability is increasing.

The inclusion of deep-rooted plants in farmscapes offer one opportunity to provide flexibility to land managers if the vegetation possesses one or more of the following attributes:

- relatively tolerant of prolonged dry periods by accessing deeper ground water
- capacity to provide ground cover and/or reduce the risk of wind erosion, thereby reducing the short- and long-term negative impacts of soil loss
- can perform on marginal soil types where other enterprises are becoming uneconomic
- can improve soil health
- can provide out-of-season feed for livestock
- can improve livestock health and welfare

In the Enrich phase 1 project we took a 'three-pronged approach' in evaluating forage shrubs for mixed, healthy production systems.

1. We broadened the search for candidates beyond previous studies by including about 70 species of Australian native shrubs.

2. We considered the role of shrubs in mixtures (not monoculture or plantations on their own) and assessed the value of shrubs in a whole-farm context. We were not looking for a new 'silver bullet' plant species, but aimed to provide more options for landholders in terms of the shrub species that may be suitable. We also assessed the impact of using forage shrubs on the whole-farm profit rather than isolating the shrub system from the rest of the farm.

3. We took a new approach to evaluating the potential contributions of shrubs to include impacts on gut health, both ruminal fermentation profiles and effects on intestinal parasites. This was because we were not expecting to find a shrub species that single-handedly met all requirements of an ideal forage plant. Indeed, based on previous work, we expected many species to be relatively low in edible biomass production and possibly only moderate in terms of nutritive value. Therefore, for the shrubs to be competitive with other plant options as part of the feed base, they would probably need to have other attributes, such as beneficial effects on livestock health or natural resource management.

In a mixed forage system, with different plants providing different attributes, there was a need to take a more innovative view of how grazing livestock interact with plant diversity. The inherent and learnt grazing behaviours and diet selection of animals offer a means to convert the concept of 'mixed plant assemblies' into practical system. The grazing animals can help to manage the plant diversity, for the benefit of themselves and, with our management, for the benefit of the whole-farm system.

Box 2 - Biological complexity with managerial simplicity

Our goal is to promote and capitalise on biological complexity whilst striving for managerial simplicity. Diversity in the feed base is important because it provides resilience to farming systems that must operate in the face of uncertainties and variability. However, in the past we have perceived that biological complexity must carry a penalty of being more difficult to manage than simple systems. But simple systems are incomplete systems, which inevitably require more inputs over time to replace the components that are missing. With diverse and complex systems, managers do not have to 'control' the myriad of interactions. Instead, we should aim to create the opportunity for the components of the biological system to interact with each other in a way that maximises the capacity of the system to manage itself. In the context of the Enrich project, this implies allowing different plants to perform in different parts of the farm landscape, and allowing grazing animals to interact with the mixture through inherent and/or learnt grazing behaviours.

Our roles as researchers and land managers is to ensure the new system is economic by: selecting the plants to use in the mixture based on cost of establishment and environmental constraints (eg, soil type); allocating the plants to parts of the farm landscape that optimises the interactions with other parts of the whole-farm system; and assembling combinations of plants that take into account the interactions between plant and animal biochemistry. We've made a start on these issues, but we all have more to learn yet.

Producer interviews and bio-economic modelling

As part of the 'Enrich' project, a series of face-to-face farmer interviews were held to quantify why particular systems that have incorporated shrubs have been successful (e.g. saltbush in western NSW, tagasaste in the northern WA wheatbelt). Forty farmer interviews (by Andrew Toovey, CSIRO) were completed across seven agricultural regions in southern Australia (Figure 1).

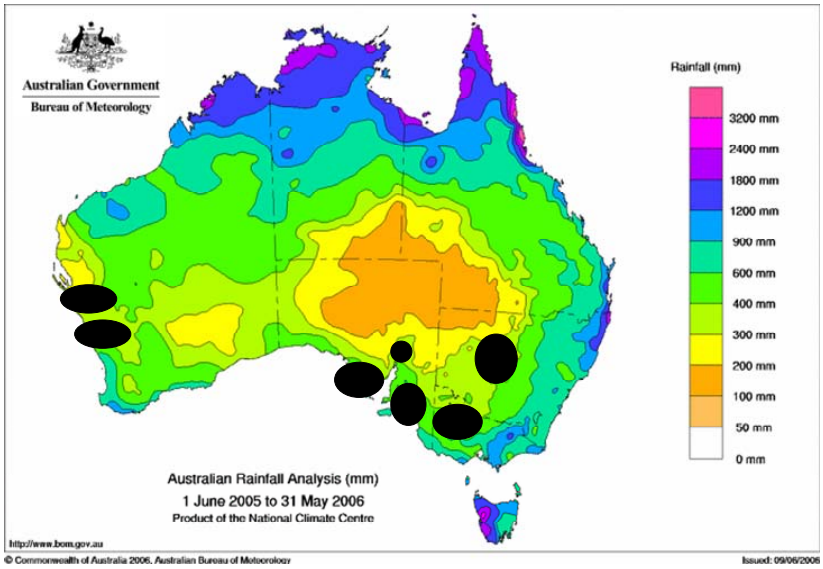


Figure 1. Map showing the regions represented by the 40 farms of the producers who were interviewed.

The following information is based on details from producers with practical experiences. We have combined that knowledge with economic modelling to investigate a range of scenarios and broaden the set of conditions under which a forage shrub system might be exposed. The modelling approach was to use a whole-farm optimisation model, called MIDAS. We based to modelling on a 'typical' central wheatbelt farm (WA), but the main messages that emerge from that work have broad applicability. The details and inputs used in the MIDAS modelling were provided in the previous milestone report. .

Why are some farmers interested in forage shrubs?
The interest in forage shrubs is currently largely driven by three main aims:

(i) develop productive use of soil types that are becoming unsuitable for profitable grain production

(ii) reduce the risk of salinity and/or soil erosion

(iii) provide out-of-season feed and thus contribute to whole-farm profitability.

The survey highlighted opportunities for broader scale improvements in the strategic use of forage shrubs. It is very clear that well managed systems are providing significant contributions to whole-farm profitability whilst also achieving land management objectives. But the key words here are "well managed". Planting shrubs without incorporating them into the annual farm plan will almost inevitably lead to a net cost to the land holder. But on the contrary, incorporating forage shrubs in the farm plan can boost whole-farm profits and provide added flexibility. These will be teased out on the following pages.

What is already being used and where?

West Midlands WA (Lancelin, Badgingarra, Mingenew, Geraldton, Dongara, Binnu)

The farmers interviewed throughout this region have farming enterprises that are still predominately focused on cereal and legume cropping. The use of forage shrubs on their properties is generally restricted to areas of the farm where they are currently unable to produce an economic return from cropping. Hence, forage shrubs are only located on the soil types where cropping is vulnerable. The predominant forage shrub currently being grown in this region is tagasaste (*Chamaecytisus proliferus*), with small areas of old man saltbush (*Atriplex nummularia*) and golden wreath wattle (*Acacia saligna*). Increasing areas are being planted to Rhagodia (Rhagodia (*Rhagodia preisii*).

An enormous research effort into tagasaste on the deep pale sands of this region over about 25 years has led to the development of effective cattle grazing systems on areas not suited to cropping. Typically tagasaste only represents a small proportion of each farmer's property (3-5 %), although there are exceptions where this is much higher, even up to 50%. Farms with a higher proportion allocated to tagasaste have much larger areas of deep, low fertility, non-wetting sand.

The major reasons for only small areas being established are:
1. Only have small areas of suitable soil type on the property
2. Cropping is still considered to be the core business and large areas where cropping is still considered to be viable

3. Farmers not being sure of the extra value of planting more, and wanting to avoid planting too much (especially given the cost of removing it)
4. Concern about on-going costs of mechanical trimming of the plants, at least for tagasaste.

Initial plantings of tagasaste in the mid 1980s were in the higher rainfall (western coastal area) of the region, where average annual rainfall is around 600mm. However in recent years tagasaste has been established further north and inland (e.g., Binnu) than was previously thought possible. Although suitable soil types are present in this area, the rainfall is considerably less (300mm) than was previously considered necessary for tagasaste to be reasonably productive. Initial indications seven years after initial establishment are that these stands are performing very well with significant increases in the stocking rates when compared to the annual pasture systems on the same properties.

As in many parts of WA, the presence of salinity on many farmers property has lead to a decline in profitability of cereal crops on portions of their property. Several farmers we interviewed have established old man saltbush on their property with the aim of slowing the spread of salinity problems, coupled with the ability to provide an additional 'out-of-season' feed source for their livestock.

Four of the farmers interviewed have also recently planted Rhagodia on their properties. The earliest plantings were in 2000. One site east of Binnu (WA) was planted in spring 2006 by Don Nairn and Ian Pullbrook, and rainfall was just 125mm in that year, yet the establishment rate remained high at 95%. There seems good potential for the plant to establish and grow, even during a particularly dry year. Details of the grazing capacity, palatability to livestock and shrub biomass production are being currently collected from a number of projects. More if this information is required before the real value of the plant can be gauged.

Box 3. Site preparation is important

Rowan Ford's property (East Binnu) gives a clear indication of the influence that previous soil management can have on the establishment and productivity of a tagasaste stand. The photograph below shows a stand of tagasaste that was planted in 2004. The area on the left hand side of the picture, where there was ~90% establishment was planted into soil that had been under a continuous cropping cycle for 5 years longer than the area on the right hand side of the picture. The area on the right hand side had only a 60% establishment success. Rowan attributed this to weed control in the area on the left, which allowed the tagasaste seedlings to establish well with minimal competition for water.

South East SA (Keith, Loxton, Waikerie)
Six farmers were interviewed in this region. The predominant forage shrub currently being used is old man saltbush (*Atriplex nummularia*) with small areas of tagasaste (*Chamaecytisus proliferus*) present in the southern area of this region.

As with most other regions represented in our interviews, only small proportions of a farmers property has been planted to fodder shrubs. Much of the saltbush that is being planted in this region is Eyres Green Giant.

Farmer interviews confirmed the findings of Stokes and ?? (2006??) who found that the area of tagasaste being planted throughout this region reached a peak of adoption of ~100ha p.a. but it has declined to <50ha/year). There are two major reasons for this decline in tagasaste expansion in this area:

1. The regional PIRSA advisor, who was an excellent source of information and advice for farmers on the establishment and management of tagasaste left the area.
2. Recent improvements clay-spreading technology to improve the use of non-wetting soils have increased its economic competitiveness with tagasaste. This is good example of how a 'competing' technology can alter the real or perceived viability of establishing forage shrubs.

Central North and Eyre Peninsula SA (Carrieton, Tumby Bay, Arno Bay, Port Neill)

Four farmers were interviewed across these regions. A producer at Carrieton has established 10% of his property to old man saltbush (*Atriplex nummularia*), which is a higher proportion than most other interviewees. The saltbush has been planted onto rangeland soils that are situated north of the Goyder Line.

The predominant forage shrub currently being used in the Eyre Peninsula is old man saltbush (*Atriplex nummularia*) with small areas of tagasaste (*Chamaecytisus proliferus*) present in the south western area of this region. Similarly to all other regions throughout southern Australia only small proportions of a farmers property has been planted to forage shrubs.

Much of the saltbush that has being planted in this region is the same clonal variety of Eyres Green Giant that is also being planted in the south east region of SA. Many of the areas in this region are facing significant soil salinity problems with OMSB currently considered to be the best option for animal production from what would otherwise be non-productive portions of their properties.

Tagasaste has been planted in this region since the mid 1980s. Only small portions of farmers properties have been planted to tagasaste, limited to pockets of suitable soil for the successful utilisation of tagasaste.

Central west NSW
Nine farmers were interviewed throughout central and western New South Wales. Again the predominant forage shrub currently being used is old man saltbush (*Atriplex nummularia*).

North western Victoria

Nine farmers were interviewed throughout this region with the predominant fodder shrub currently being used in the Eyre Peninsula is old man saltbush (*Atriplex nummularia*). Several significantly different characteristics emerged from discussions with these farmers:

1. Often established on what the farmers consider average to good soils. This was a pattern that emerged for many of the eastern state farmers when compared to farmers in Western Australia and South Australia.
2. Almost without exception these plantings have occurred into non-saline soils.

What the survey told us:

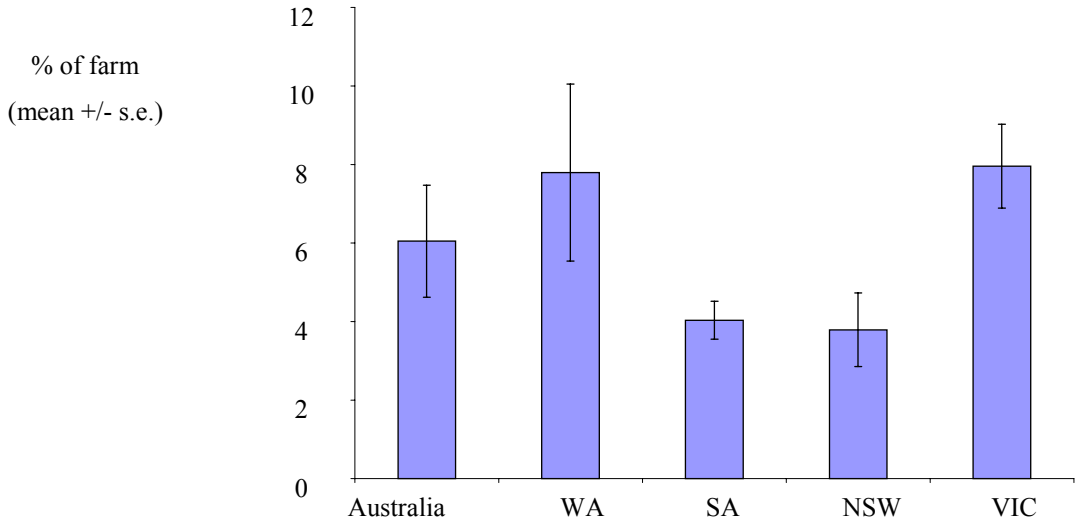


Figure 2. The area of forage shrubs on each farm, as a percentage of farm area.

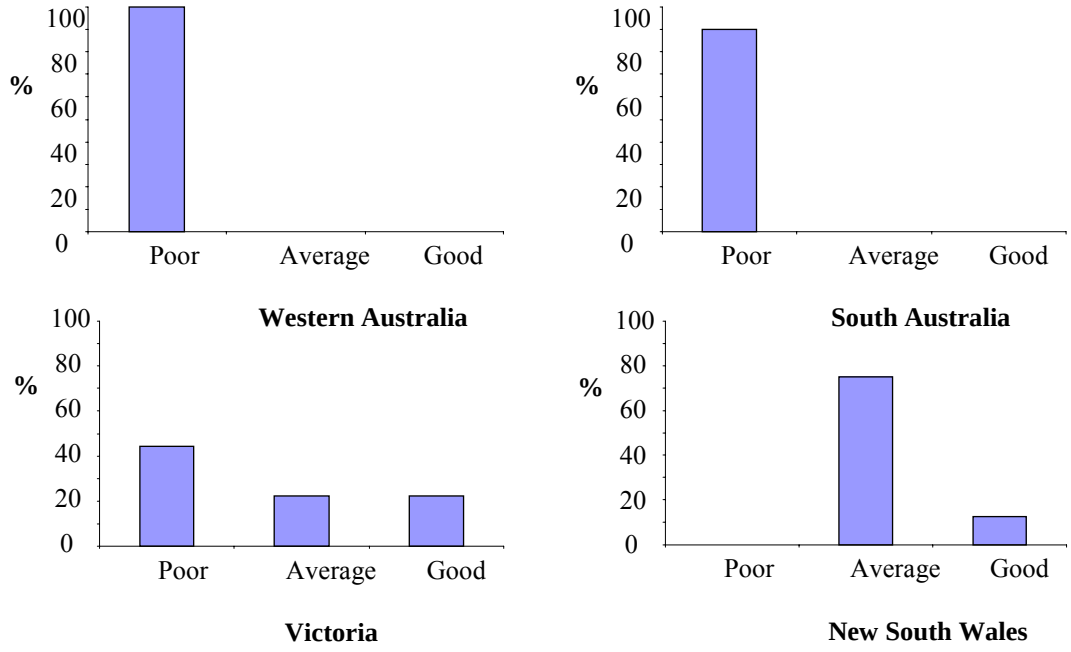


Figure 3. The types of soils that are planted to forage shrubs in each state.

All farmers interviewed in WA and SA indicated that they are attempting to get a rate of return on the least profitable portions of their farm that has recently been only producing negative returns with conventional approaches (annual pastures and/or cropping). These poor soils have been identified through a variety of methods including yield maps, soil maps, and visual assessment. This factor is reinforced by the suitability of tagasaste to pale deep sands that are traditionally unsuitable to annual cropping due to their high requirement for fertilizer and the susceptibility to wind and to a lesser degree water erosion.

Many more farmers in NSW and Victoria are planting fodder shrubs on what they consider to be some of their average or good soils, rather than focussing only on the poorer soils. The reasons behind this are probably that:

a) these farmers have not recently been cropping a significant proportion of their property (if at all) and therefore they are not forced to take out soils that would have been suitable for cropping, and
b) many of these producers have been achieving four-fold increases in stocking rates with the establishment of old man saltbush. So to achieve the greatest possible return for the investment of planting OMSB they are planting it in soils that were previously 1DSE as opposed to 0.7DSE.

What the economic model tells us:

Land classes

The optimal area of a farm allocated to forage shrubs will depend on the proportions of each land class, or soil type, on a farm as this directly determines the other enterprises (e.g., cropping) that compete for land use. As the producer feedback from the Enrich surveys clearly shows the benefits of forage shrubs on making better use of marginal soils where there is a low opportunity cost from lost cropping. In order to test further, we have investigated the whole-farm impact of increasing the relative proportions of the poorest LMUs in the model. In the following example, we have increased the area of the LMU 1 (the poorest soil type) from 7 to 11.5 to 15%, with the others LMUs (2 to 8) changing accordingly.

The modelling results show, not surprisingly, that whole-farm profit drops with higher proportions of poor soils. However, they also confirm that a farm with more marginal soils has a larger optimal area of shrubs than a farm with a smaller proportion of marginal soils. For a farm with 7% of its area as LMU class 1, the optimal area to allocate to forage shrubs was 10-120%, but this increased to up to 30% for a farm with 15% of its area as LMU class 1 (Figure 4). This means there is particular potential for shrubs to boost economic returns in drier regions or in areas with low fertility soils.

Another key finding was that profit increased considerably as forage shrubs increased from 0 to 10% of farm area, and that this increase was consistent across each scenario. The precise optimal area of shrubs varied, but it was always in the 10-30% range. Higher shrub areas were associated with a marked decline in profitability. However, shrubs caused a net loss - compared with the situation with no shrubs at all - only if more than 40% of a farm's area was dedicated to them.

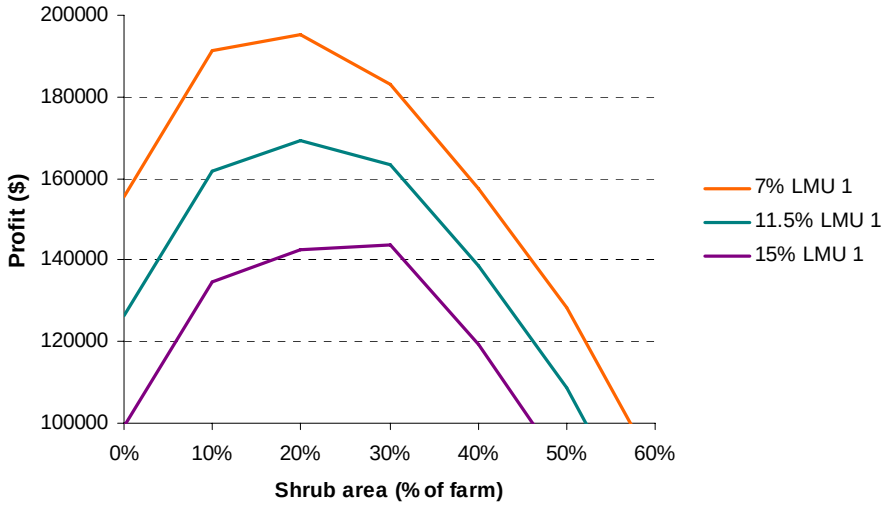


Figure 4. Impact of changing the relative proportions of land management units (LMUs) on whole-farm profit across a range of shrub areas.

The effect of biomass production and nutritive value on optimal planting areas

Another factor affecting the optimal area allocated to shrubs is the nutritional quality and quantity of the plant material. This analysis looked at the impact of three different levels of nutritive value (7, 8, 9 MJ ME/kg edible DM) land three levels of edible biomass (1, 2, 3 kg edible DM/plant) on whole-farm profit. The middle values for each were chosen to represent the current situation, but current research is aiming to improve plant production and nutritive value. The two figures below (Figures 5 and 6) show that, under typical farm conditions, about 10% of the farm area allocated to shrubs maximizes whole-farm profit. They also show that the penalty for planting more than this area is reduced if the quality or quantity produced is increased. This is a clear message for researchers – there will be real benefits in improving forage shrubs, and it is probably better to aim for higher nutritive value than more biomass if we can't get both.

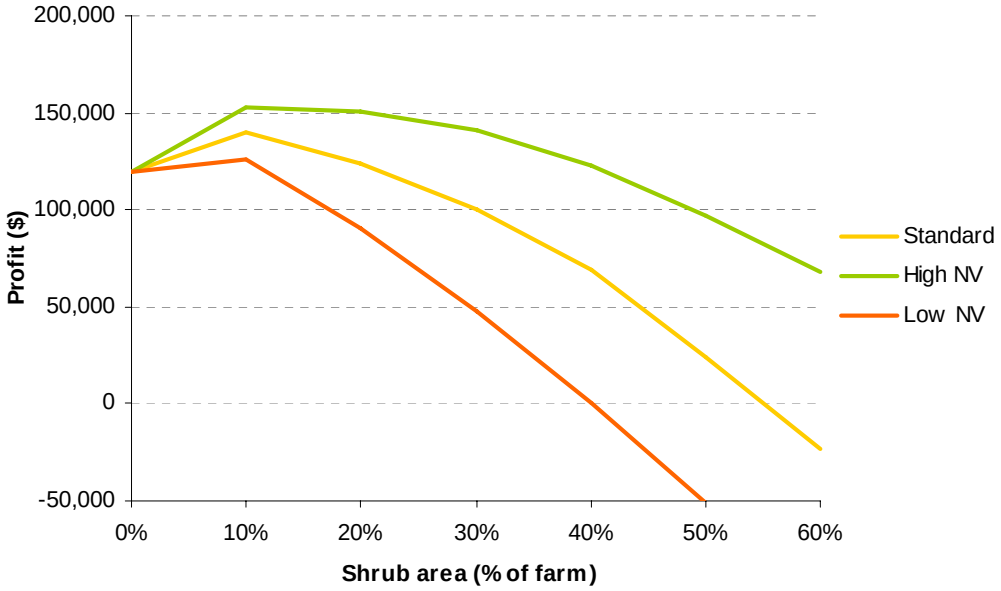


Figure 5. Whole-farm profit against shrub area for a standard, high and low nutritive value.

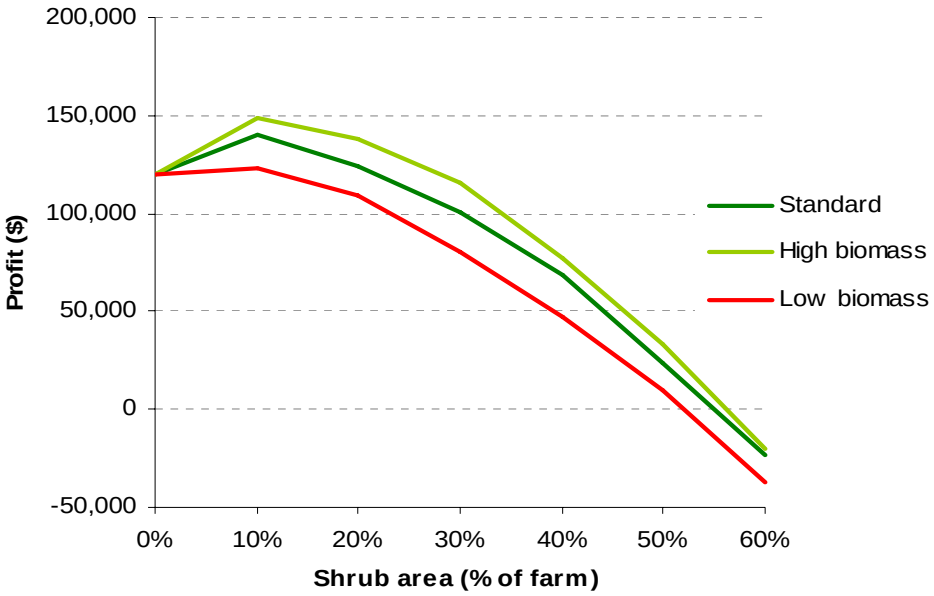


Figure 5. Whole-farm profit against shrub area for a standard, high and low biomass production.

Why plant forage shrubs?

The most common reason is because farmers want to increase the amount of feed available during the summer/autumn 'feed gap' that is a feature of the Mediterranean climate that the majority of farmers throughout southern Australia face each year. Twenty three of the 40 farmers interviewed stated that the most important reason as to why they established a fodder shrub on their property was because they wanted to be able to increase the stocking rate on their property. Initial thoughts were that forage shrubs, and in particular OMSB, could be viewed as a 'living haystack' that would be an available feed reserve for particularly dry years. However this view has changed so that many farmers now consider that they must graze their OMSB at least one a year to achieve the maximum production possible. Forage shrubs are now being viewed as an integral part of the overall grazing system and not just as an additional back-up feed supply to be utilised during difficult/dry years. Many farmers have found that an area of forage shrubs can be a suitable feed source during the autumn feed gap, significantly reducing supplementary feed costs.

Sowing density and spatial arrangement

Early planting of OMSB (1980's) were generally done at a density of 2400-2700 plant/ha in a strip formation with spacings of 1.2m within the row and 3-3.5m between rows. Several reasons have lead to this arrangement only rarely being planted in recent years:
1. Row spacings of 3-3.5m become very difficult to drive a vehicle through once the shrubs become established,
2 . Mustering of stock is also very difficult in these blocks, and
3. At this density very little inter-row pasture is present which means that the only available feed source for livestock in these blocks is OMSB.

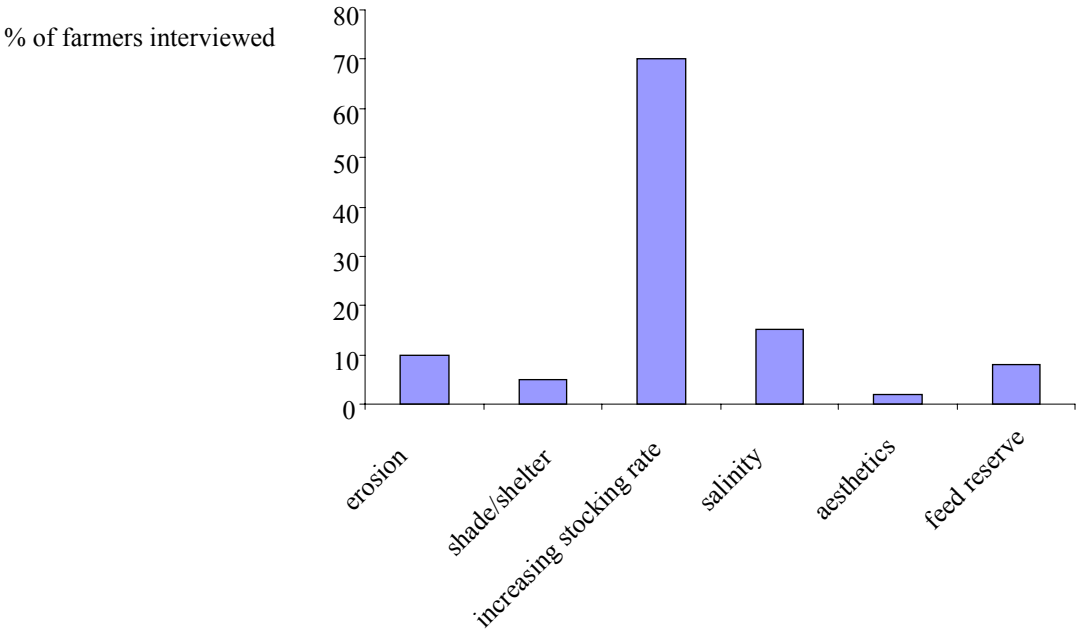


Figure 6. Reasons that producers have established forage shrubs on their properties

Recent plantings of OMSB have more typically consisted of a double or triple row (1.2m * 2 or 3m) with anything from a 4m up to a 200m inter-row gap. These wider row spacings represent an alley farming system with the inter-row generally being spaced at an interval that suits the farmers spraying or cultivation equipment. Several farmers in NSW are spacing there inter-row at ~20m to gain the maximum amount of benefit from the wind break effect to increase the amount of pasture production.

Figure 7. Lack of interrow in narrowly spaced shrub rows.

Tagasaste was initially established during the 1980's as single rows with 6m inter-row. Unless this arrangement is strictly managed and not allowed to overgrow it can become very difficult to maintain access for both livestock and vehicles. To avoid this problem recent plantings are being done as double rows 2m apart with a minimum of an 8m inter-row. In addition several farmers are leaving a 3-4m gap within each of the double rows at staggered intervals of 1-200m to improve vehicle access to make stock mustering easier. In addition to this several farmers are allowing every 10th double row to grow to its full height to ensure that livestock have access to adequate shelter within these areas.

Figure 8. Tagasaste rows with wide interrow spacing in between double rows of the shrub.

The potential for forage shrubs to offer additional benefits is an important aspect that has been considered by producers. Significant benefits can be gained from the strategic placement of forage shrubs throughout a farm. One WA farmer who has tagasaste set up in double rows with an 8m inter-row has strategically chosen rows at regular intervals to act as wind breaks and shelter belts. These tagasaste rows are allowed to grow to their full height (3-4m) and provide a significant amount of shelter for his cattle. A number of farmers are aligning their double rows as either curves or overlapping corners to ensure that if the wind blows from any direction it will not be able to blow directly down amongst the rows. The spatial arrangement impacts on the capacity to manage pastures in between the shrub rows and well as the shelter the shrubs can afford to the animals.

Figure 9. Livestock seek out shade, as shown here on a property in NSW.

Figure 10. Example of saltbush in NSW being planted in curves to reduce wind tunnelling effects

Perhaps one of the most pressing needs for 'additional' benefits has the control of wind erosion of fragile paddocks left with low ground cover after a number of poor seasons. Producers with light sandy soils have been particularly motivated to establish rows of shrubs on vulnerable parts of their paddocks (e.g., ridge tops) to minimise wind erosion over summer, to great effect.

Figure 11. Adjacent paddocks at a property north of Perth, WA, demonstrating he benefits of controlling wind erosion.

What the economic model tells us:

The economic analyses showed that including shrubs on the farm has the potential to:

- Increase overall farm profit by an average of 16% at an optimal area of shrub-pasture sward of around 10% of the farm
- Profitably use and improve marginal soils

The main economic value of shrubs results from:

- Provision of valuable feed after the break of the season (feed gap)
- Deferment of pasture to other paddocks (ie. higher pasture growth)

- A combination of environmental benefits reduced soil erosion and reduced deep flow of water (i.e., reduced recharge)

We have attempted to demonstrate the consequences of forage shrubs on ground water recharge and soil erosion. Farming systems based on annual crops and pastures use insufficient water from incident rainfall, and rarely use from the deeper layers of the soil (below the shallow root zone). As a result of this, the raising water tables carry accumulated salts to the soil surface leading to (secondary dryland) salinity. Including perennials in the system with their longer roots and increased water use has the potential to significantly reduce this risk. In the CWM we have represented ground water recharge (or deep drainage or deep flow) for shrubs as a 20-year average for every LMU. An increase in the farm area under shrubs (or any other perennials such as lucerne and trees) is likely to decrease deep flow and have a positive impact on the sustainability of the farm. Directly converting this reduction in deep flow to a reduction in dryland salinity is beyond the scope of this project as depends on other factors such as rainfall (Pannell *et al.*, 2004; Sanford and Young, 2005; Bathgate *et al.*, 2007).

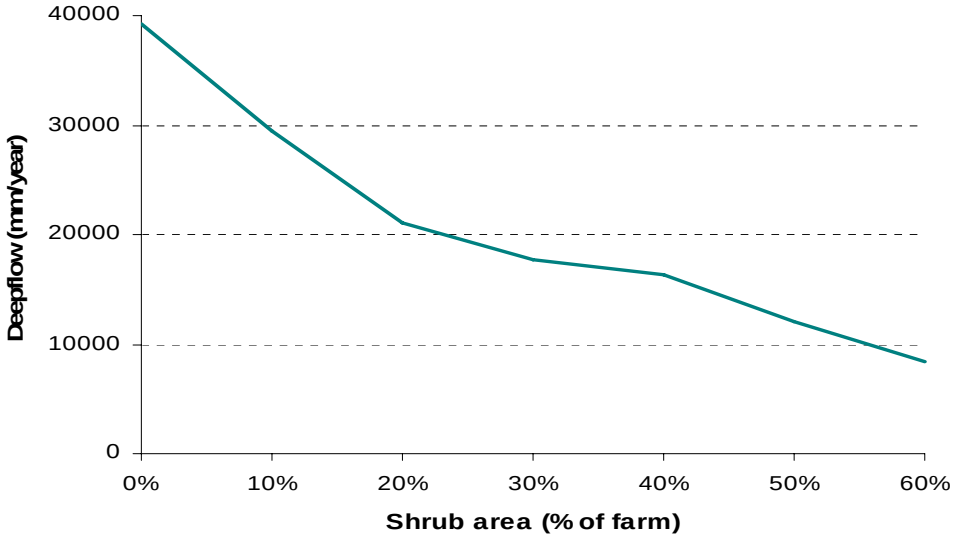


Figure 12. Deep flow trends against shrub area.

As expected, farm deep flow is inversely proportional to shrub area on the farm due to increased water use by deep-rooted species. An important result is that the biggest response in reduced deep drainage occurs with the first 10-20% of the farm being allocated to shrubs (Figure 12). However, the positive impact of reduced deep flow by shrubs does not affect whole-farm profit on its own.

Soil erosion by wind occurs when a sandy soil surface is exposed to strong wind (> 30 km/h), with small particles (< 0.5 mm) moving by rolling or bouncing across the land surface, and as dust lifted into the air. On the other hand, soils which contain more than 15% clay are normally aggregated into particles too large to be moved at all, although aggressive cultivation, burning and overgrazing can degrade such aggregation to finer particles which may then be susceptible to removal by wind. Susceptibility to wind erosion decreases with higher annual rainfall because water surface tension in wet soils resists wind erosion and higher rainfall usually leads to greater surface cover and surface protection anyway (Moore *et al*., 1998). Generally, if there is adequate groundcover (e.g. gravel, stubble, pasture, perennial plants) there is minimal risk of soil erosion caused by either strong wind or high intensity rainfall. Groundcover, or rather soil exposure, is therefore the simplest and most direct indicator of erosion risk. Erosion risk is highest in the summer months when the soil is more

likely to be bare. The introduction of perennial shrubs in the system is expected to decrease the soil erosion risk, although predicting the actual magnitude of this risk reduction is not a straight forward exercise because of variability in soil types, wind speed and direction, livestock activity, role of shrubs as wind breaks, and annual soil loss.

Therefore, a simpler approach was taken for this analysis. Based on the work by Bathgate (1989), we defined a stubble conservation standard for a range of crops and pasture. The conservation standard is the amount of dry matter required to reduce the speed of the wind at ground level to below the threshold level of that on bare ground. At this speed, the risk of erosion is considerably reduced during the summer months, when the soil is more exposed. If the conservation standard is not met, then the assumption is that wind erosion occurs, resulting in soil loss and up to 20% cereal yield reduction.

The onset of wind erosion on the farm led to a significant drop in whole-farm profit due to reduced cereal yields (from loss of top soil). In addition, conservation requirements reduce the availability of stubble for grazing (particularly lupins and pulses), thus (further) affecting profit. In these situations, having a shrub-pasture sward area of up to 50% (optimum 10-20%) of the farm generates a higher profit than a no-shrub situation, probably due to a much lower erosion risk in that area and extra feed on offer to compensate for the retained stubble. These preliminary results are promising and suggest that the full role of shrubs in reducing the risk/impact of soil erosion by wind on the farm requires further investigation.

Cost of establishment

The cost of fodder shrub establishment varied significantly from state to state and even from farmer to farmer within the same regional area. The lowest cost of establishment was an area of tagasaste (in WA) that went into an existing paddock and cost only $100/ha as no additional fencing or water costs were required. The highest cost of establishment, at $820/ha, was for a stand of tagasaste seedlings that were planted in WA. Tagasaste represents the cheapest fodder shrub to establish ($100-150/ha + fencing and stock water supply) due to the highly reliable method of establishment by seed rather than seedling. The establishment of OMSB ranges from $200- $700/ha depending upon whether seed or seedlings were used and the amount of work that the farmer has carried out.

Several farmers commented that the establishment costs for OMSB are similar to the establishment cost for lucerne. However with most lucerne stands only lasting 5-10 years compared to the expected 10-20 years for an OMSB stand there is a clear cost saving available for the establishment of OMSB.

Another important consideration for the establishment of a fodder shrub is the length of time or ‘transition period’ before the shrubs are available for stock grazing. Careful attention must be paid to all aspects of the establishment process to ensure that the shrubs will be able to reach a ‘grazable’ size as quickly as possible. This may mean that only the best quality seedlings should be planted.

The delay in the establishment can also have a significant impact on the cash flow of the property. Many farmers have found that it takes between 3-4 years for OMSB to reach full production. So careful planning is required to ensure to this period of negative cash flow can be financed adequately.

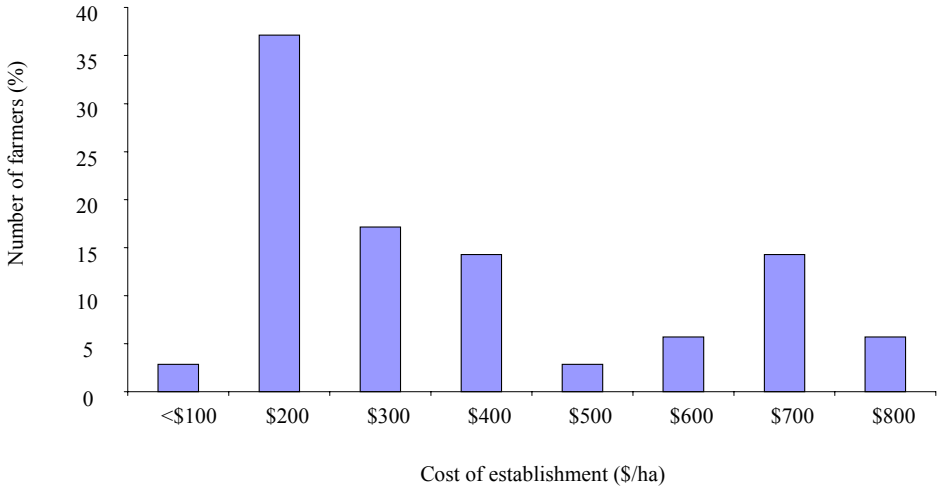


Figure 13. The range of establishment costs reported by producers who were interviewed.

What the economic modeling tells us:

Shrub establishment costs of $0.4 to $1.00/ha were considered. As illustrated in Figure 14, the higher the establishment cost the less economically attractive shrubs become. This impact is relatively small when considering the long life of shrubs, though, with farm profit dropping only 7% across the defined value range and optimal shrub area staying around the optimum 10% of the farm (not shown).

The cost of establishing a shrub sward includes that of establishing inter-row pasture, and relative inter-row pasture production appears to have a positive impact on the profitability of shrubs. In fact, an increase of 20% in inter-row pasture production is responsible for over 10% increase in farm profit, indicating the added value of growing forage shrubs in combination with pasture.

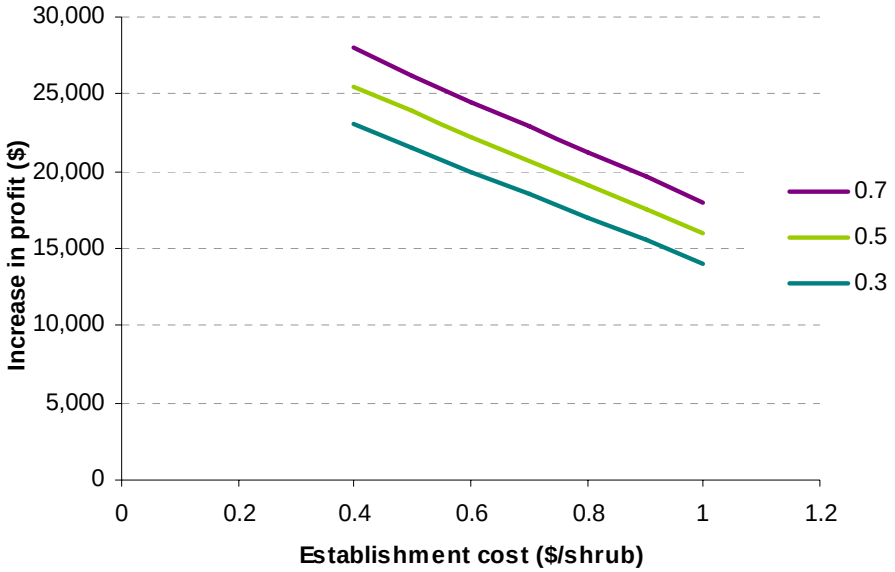


Figure 14. Impact of varying pasture/shrub establishment costs on the farm profit for three levels of inter-row pasture production, expressed as a proportion of pasture growth (productivity) without shrubs.

Impact of ccommodity prices

The impact of a change in wool, prime lamb and wheat prices on whole-farm profit and optimal strategies was also examined in this analysis:

- Wheat price: $160, $200, $240/t of wheat grain (ASW with 10% protein);
- Wool price: 620, 720, 820 c/kg clean wool (WMI);
- Prime lamb price: $1, $2, $3, $4, $5/kg DW.

Generally, an increase in all commodity prices had a positive effect on the overall farm profit, but wheat and prime lamb prices had the largest impact under the current market situation (Figures 15-17).

In addition, the optimal area of shrubs remained unchanged across the range of price scenarios, which strongly indicates that 10% is indeed the shrub area recommended (with the current set of model assumptions). Beyond the optimum shrub area farm profit declines for all prices, confirming the opportunity cost of not growing a more profitable enterprise on the extra land. This is especially the case for wheat prices, where the 'penalty' for having too many shrubs increases as the price of wheat goes up (Fig. 5). At high wheat price, small shrub areas are more valuable as shrubs substitute for more expensive grain feeding. At low wheat price, shrub area remains relatively profitable until 30% as more sheep are carried on the farm when grain prices drop. The lower profitability of a large shrub area at high grain prices (and low sheep numbers) indicates that it could be valuable to develop cropping systems within the shrub alleys, allowing for shrubs to continue to be utilized even when there is a reduced demand for feed.

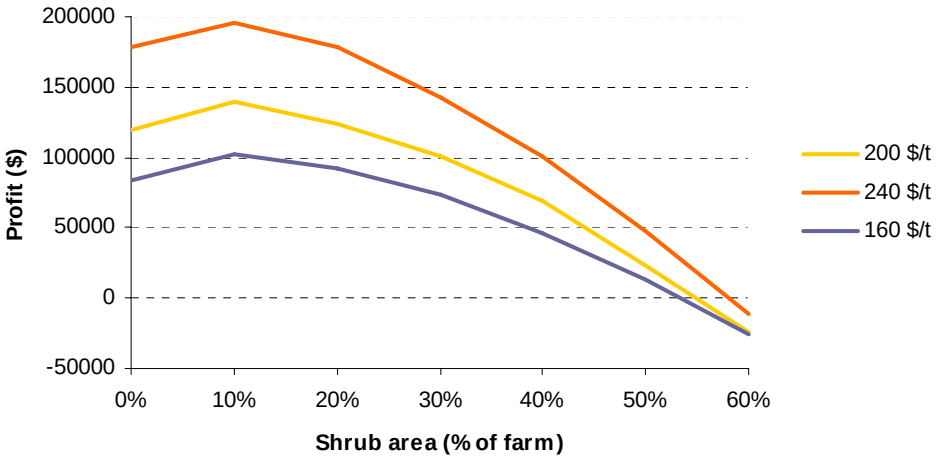


Figure 15. Whole-farm profit against shrub area for a range of wheat prices (ASW 10%).

The 'penalty' for having too many shrubs on the farm does not change much as the commodity prices for wool and prime lamb vary (Figs. 6 and 7). The difference in opportunity cost of having a larger shrub area is reflected in the rate of decline being the same across all wool and lamb prices (parallel lines in Figs. 6 and 7) while varying for wheat prices (converging lines in Fig. 5).

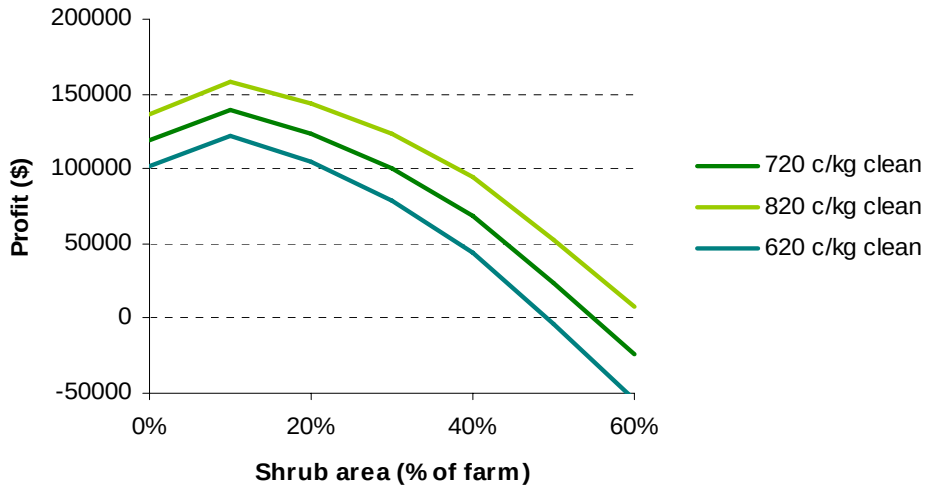


Figure 16. Whole-farm profit against shrub area for a range of wool prices (WMI).

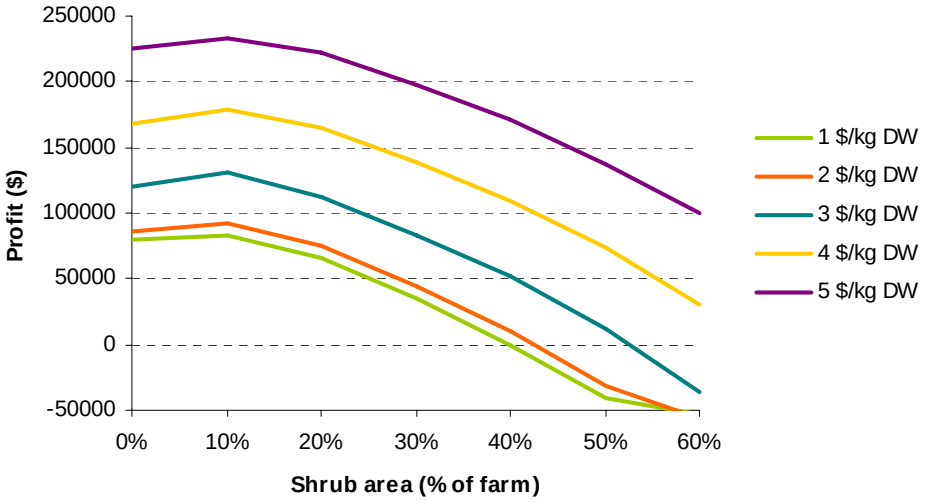


Figure 17. Whole-farm profit against shrub area for a range of prime lamb prices.

General management

Weed and pest control are critically important during the establishment year and are critically important if seeds are being planted instead of seedlings. Beyond the year of establishment very few of the surveyed farmers carry out any annual weed control, or pest control on their fodder shrub plantings.

Some farmers have been applying fertilizer (e.g. 100kg/ha super:potash 5:1, 120kg/ha super and potash 3:1) to their OMSB and tagasaste plants in the years after planting. However the majority of farmers are not applying any fertilizer to their fodder shrubs.

Tagasaste stands can require cutting and several farmers who are grazing their tagasaste with sheep have cut it on an annual or biannual basis. Tagasasate grazed with cattle tends to require less cutting with several farmers cutting their tagasaste approximately every 4th year. One farmer in WA has a 10 year old stand of tagasaste, grazed by cattle that has not yet

required cutting. With appropriate heavy grazing it seems that regular cutting of tagasaste can be avoided and with an approximate cost of $50/ha can represent a significant saving.

A key point raised by several of the farmers interviewed was that they had also began to place a much larger focus of their animal handling techniques and the importance of this in relation to the overall performance of their livestock. Several of the farmers have attended low stress stock handling, or stress free stockmanship, courses and have adopted these principles into their regular farming activities.

Grazing management

One of the key points raised by the survey was that the grazing methods utilised to achieve the maximum grazing potential from forage shrubs are currently evolving and are rarely totally interchangeable from one farmer's situation to the next.
One farmer in NSW (central west region) has established 36ha of OMSB which has been fenced into approximately 5-10ha plots. This enables him graze his sheep on OMSB for a total period of 60 days with a rotation of 5 by 12 day periods within each plot of OMSB. With an additional CMA grant available this year he is currently planning to establish another 90ha of OMSB. With a total of 126ha of OMSB available he will then graze his entire sheep flock through the OMSB paddocks on a rotation of ~10-12 days in each block. This will enable him to carry all of his sheep flock on his OMSB for approximately 90 days. This will enable the pastures on the remainder of his property to 'recover very nicely' and ensure that his lambing ewes will be able to drop their lambs into paddocks with an adequate feed supply.

Small areas of around 10ha have been what many farmers established as individual paddocks of forage shrubs. Some are even moving towards smaller areas of 4-5ha to ensure even grazing pressure can be quickly applied to the shrubs. Some have found that it is much easier to manage the shrubs in this way. Alternative approach, with similar results, has been to increase mob or herd sizes to increase utilisation of the shrubs.

Stock rotation is also an integral part of the successful grazing of fodder shrubs for many producers. High intensity grazing with longer recovery times is being seen as a suitable way to proceed.

How important are shrubs to your whole farm enterprise?

Eighty percent farmers rated forage shrubs as either moderately or highly important to their farm enterprise. Those who did not rate them so highly tended to have a very small proportion of their farm planted to shrubs.

Are you planning to increase or decrease the use of shrubs?

The majority of farmers interviewed indicated that they are intending to increase the use of fodder shrubs on their farm. Comments such as "have suitable soils that are no good for cropping" and "(we) used to think that 10% of our farm in (forage) shrubs would be appropriate but now with more experience/knowledge we feel that 20% may be more appropriate", were common responses to this question.

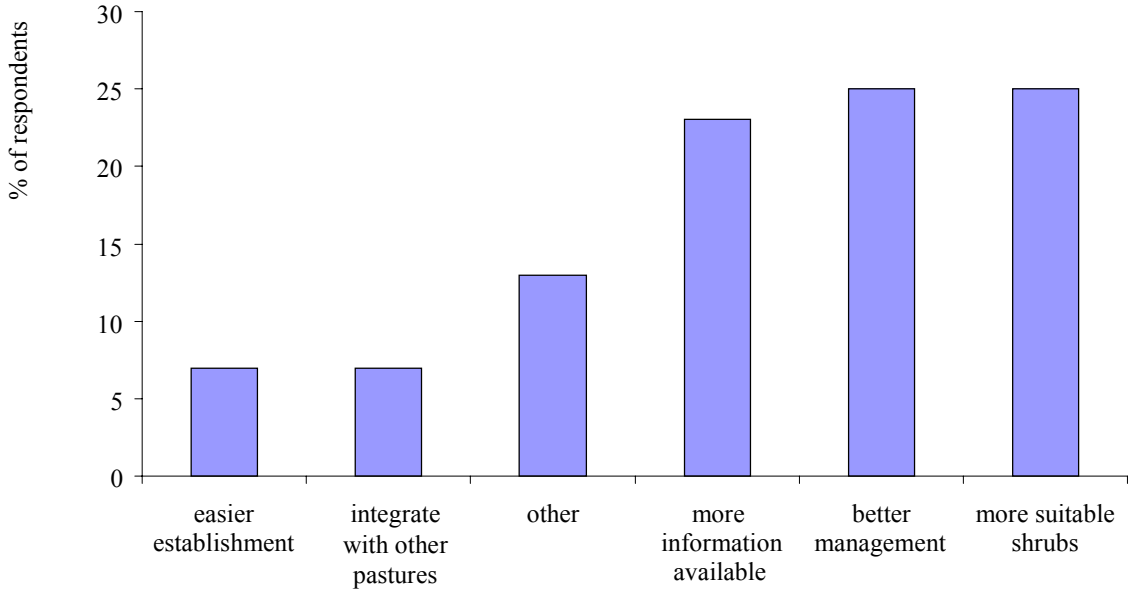
Several farmers who did not intend to increase their area explained that they are "currently trying to fully utilise what we already have" before considering an increase.

Several farmers commented that it often took them a couple of years from the initial establishment of fodder shrubs to fully realise their potential and to understand just what an important asset they can be for their farm particularly during dry or drought periods.

Do you recommend the use of shrubs to friends/ family/ neighbours?

75% of farmers interviewed indicated that they would recommend the use of shrubs with a further 23% indicating that they would 'sometimes' recommend shrubs. Comments such as "especially if they have suitable soils" and "useful alternative coupled with good productivity" were common responses to this question.

How could you improve the usefulness of shrubs on your farm?



Section 2

The identification, biology and ecology of plant species for shrub based grazing systems

This component of Enrich consists of four areas: i) the identification of potential plant species, ii) their initial performance in the field, iii) a more detailed study into a subset's response to grazing and iv) investigations into positive and negative factors that may arise from the growing of polyculture grazing systems.

Overview of the process for selecting native shrub species for evaluation in the Enrich project

The Enrich project was linked to the activities of FloraSearch to capitalise on the knowledge on species performance being generated from that program. FloraSearch established an extensive database on species naturally occurring within and adjacent to the dryland agricultural zone of southern Australia. This resource has been used to develop a systematic approach to identifying species with commercial potential with these species sampled for basic product attribute testing, and to support establishment in trial sites followed by more specific evaluation.

Information on species product areas and plant attributes from other research projects, workshops, literature searches and plant databases has been integrated with the primary species list. For each species, information on taxonomic variations, number of herbarium records in the study area, maximum height and crown width, lifeform, mean annual rainfall, minimum and maximum annual rainfall was collated. Also, where available, information on growth rates, palatability to livestock, coppicing and suckering ability, previous product uses, fodder digestibility, crude protein and drought persistence was tabulated.

Several FloraSearch species also occur in the wheat-sheep zone of WA and have been assessed and have undergone product testing by the WA Search project. These and results of the Acacia Search project have been noted within the FloraSearch database and their results incorporated.

Each species was allocated to one or more potential product areas based on existing knowledge. Some generalisations were made based on known plant Family properties (fodder value of Chenopodiaceae & Fabaceae). For the prioritisation process a minimum height was invoked for each member of the main product areas: multipurpose biomass species only group = 4 metres; fodder species = 0.5 metre; and specialised high-value products = 1.5 metres.

A summary of plant species attributes compiled for the FloraSearch species selection process. To prioritise and rank species for further analysis and collection within the FloraSearch project a series of calculated indices relating to the broad product area have been created:

- Volume Index – Using maximum height and crown width the cylindrical volume (m^3) that each species occupies was calculated. The highly skewed distribution of volumes was normalised using a natural logarithmic transformation. The results were then rescaled into an index ranging from smallest volume to greatest volume. The index is a surrogate for the maximum potential yield at full maturity for each species;
- Rainfall Range Index – To indicate a species' adaptability to rainfall, and in part its spatial distribution, the overlap of each species' minimum and maximum rainfall records with the 200-700mm annual rainfall zone has been expressed as a proportion and rescaled to lowest proportion of the range to across the entire range;

- Growth Rate Index – 3 categories of growth rate, based on expert observations or the literature, have been transformed into an index of growth rate (fast, moderate, slow). Species without reliable information on growth rate were assigned a moderate default value;
- Fodder Palatability Index – 4 categories of fodder palatability to livestock, based on expert observations or the literature, have been transformed into an index of fodder palatability (high, moderate, low, not palatable). Species without reliable information on palatability were assigned a moderate default value;
- Optimal Fodder Height Index – The maximum optimum grazing was nominated at 1.2 metres (fodder height score of 1), to give a selection advantage to species that do not require any mechanical management in a grazing system. Fodder species taller than 1.2 metres had their score reduced by their height above 1.2 metres expressed as a proportion of the height of the tallest fodder species above 1.2 metres. Fodder height scores were scaled from 0.25 (tallest fodder species) to 1 (below 1.2 metres);
- Biomass Priority Index – The average of Volume, Rainfall Range and Growth Rate indices, with double weighting of Growth Rate Index; and
- Fodder Priority Index – The average of Biomass Priority, Fodder Palatability and Fodder Height indices.

Information Type (units or classification)
Species & Infraspecific Variants (subspecies, varieties) **Family** **Number of Records in the Study Area**
Mean Annual Rainfall (mm)
Minimum & Maximum Annual Rainfall (mm)
Maximum Height & Crown Width (metres)
Lifeform (Tree/Mallee/Shrub)
Growth Rate (Fast/Moderate/Slow)
Coppicing & Suckering Ability
Timber Density (kg/m³)
Oil Yield & Constituents (% volume)
Gum Characteristics (compound % volume, optical & physical characters)
Palatability to Livestock (High/Moderate/Low/Not Palatable)
Fodder Digestibility (% dry matter)
Crude Protein (% dry matter) [#]
Drought Fodder Persistence (High/Moderate/Low)
Product Areas – Previous, Current & Potential (Timber/Fodder/Oil/Gum/Tannin)
Prior Product Testing Results
Calculated Indices (indices between 0 = least desirable and 1 = most desirable)
Volume Index – maximum potential space an individual plant occupies **Rainfall Range Index** – rainfall range of a species as a proportion study region **Growth Rate Index*** – growth rate (fast, moderate, slow) **Fodder Palatability Index*** – Palatability to Livestock (high, moderate, low, not palatable) **Optimal Fodder Height Index** – height above optimal grazing height **Biomass Priority Index** – a combination of Volume, Rainfall Range and Growth Rate indices **Fodder Priority Index** – a combination of Biomass Priority, Fodder Palatability and Fodder Height indices

The Biomass Priority Index and Fodder Priority Index were used to rank and prioritise every species in the multi-purpose biomass and fodder product areas.

With the increased focus on forage shrubs arising with the funding of the Enrich project, the geographic range of the region from which to target species has been expanded to include more of the arid region including Central Australia and a re-examination of the WA flora where there has been a lower priority placed on fodder as a product area in the WA Search project (the preceding project to FloraSearch in WA). Some prospective exotic species will also be considered. To facilitate the preparation of a priority species list at short notice, the systemic process outlined above hasn't been undertaken, with additional species selected on a more subjective review of literature and expert opinion. Further collection of information to develop a extensive database of prospective species and to support ongoing systematic selection will be undertaken in the 06/07 financial year.

Selection for the Enrich project has been based on:

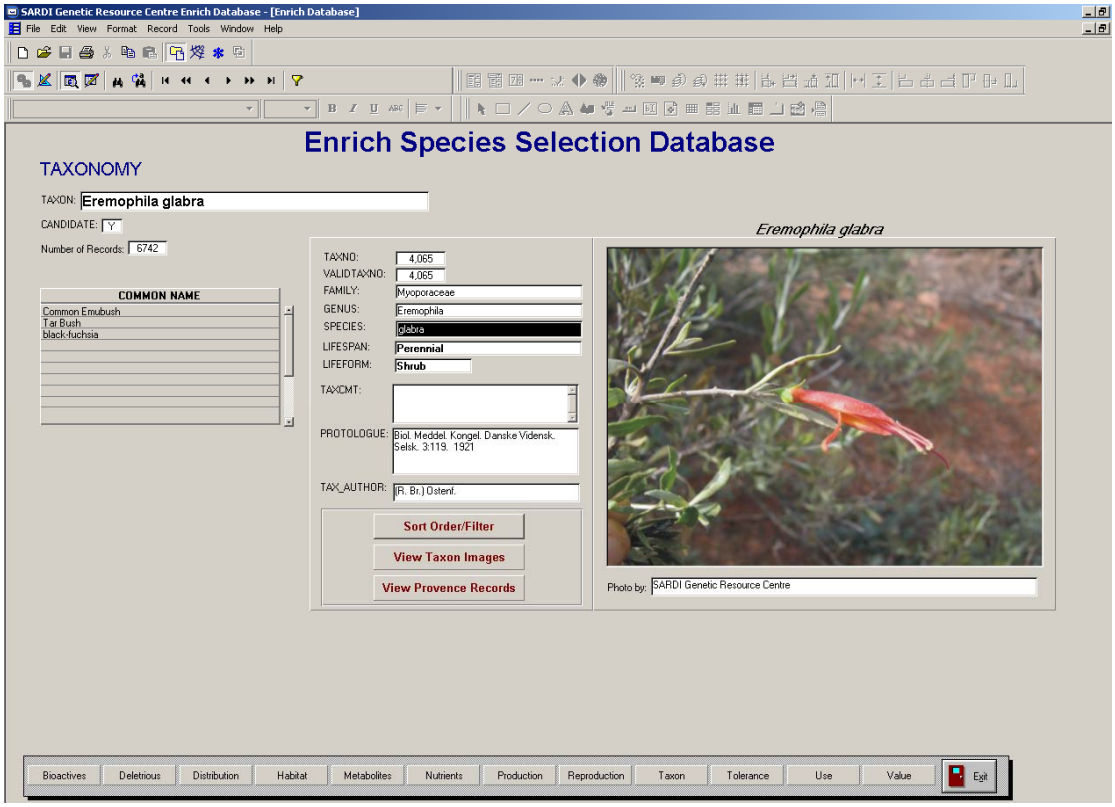
- Palatability – usually rated as being of high, medium, low and no palatability – high to medium level targeted.
- Likelihood to contain toxic secondary compounds – plants with documented animal health risks excluded
- Nutrient value - digestibility and protein content.
- Form of the plant – shrubs with a mature height of .5 to 2.0 metres preferred
- Known weed potential

- The sources for these selections were from expert opinion and published literature including:
- Peter Milthorpe – formerly NSW Department of Primary Industry
- Peter Jessop – NSW Department of Primary Industry
- Tim Wiley – WA Department of Primary Industries.
- Clive Malcom – WA Consultant
- Mitchell AA, Wilcox DG (1994) Arid Shrubland Plants of Western Australia. University of WA Press
- Russell, P. and Fletcher, W. (2003). Relative palatability of selected perennial plants in the southern rangelands of Western Australia – Results of a survey of rangeland practitioners, Range Management Newsletter, No 03/3 Nov 2003, 1-8.
- Cunningham GM, Mulham WE, Milthorpe PL, Leigh JH (1981) 'Plants of Western New South Wales.' (NSW Government Printing Office: Sydney).

Enrich species selection database

An electronic database containing published data of traits from 6,742 potential fodder shrub species has been developed as an output from Enrich 1. This database was developed after the initial species selection process above and has been used as the primary tool to identify the 135 candidate species warranting further investigation. The database was developed using the Paradox for Windows software platform and is fully relational allowing complex querying, filtering and extraction of data.

Detailed information is maintained on 135 candidate species. The database consists of 12 modules of information on each of these species categorised into logical trait based datasets (Bioactives, Deleterious, Distribution, Habitat, Metabolites, Nutrients, Production, Reproduction, Taxon, Tolerance and Uses). As illustrated in the screen capture below of the Taxon module, within each module published trait data is maintained in separate tables specific to each species. In total the database maintains over 80 traits for each species within tables linked by unique taxon identifiers.



In addition to capturing published text based data, predominately anecdotal palatability and production data; the Enrich database builds on information electronically available in other publicly available databases. Datasets incorporated into the Enrich database include the following

- Australian Herbarium. Species distribution.
- Floradata: Propagation and establishment of Australian natives.
- FloraSearch: Lifeform & production data.
- Florabase: Plant images.
- Interim Biogeographic Regionalisation for Australia (IBRA): Unique regions within Australia.
- National Vegetation Information System: Species association and unique vegetation groups.
- USDA GRIN: Plant uses

The Enrich database is unique as it maintains data specific to shrubs species suitable to broad acre farming in the one system. The Enrich database is also unique as it maintains a bibliography to the source of information maintained. Every trait dataset is referenced with a unique citation number linked to an Enrich Endnote database. This system allows for multiple records to be maintained for each trait, captures conflicting published data and allows for the source and integrity of the data to be transparent and confirmed.

It is proposed that further development of the Enrich 'shrub picker' database be supported to fill knowledge gaps and capture trait and performance data generated by the Enrich project team. Capturing Enrich data would greatly add value to the knowledge base and confidence in the data sets. As well as serving as a powerful internal tool to support the development of Enrich species it would provide the backbone for the development of a very useful tool for end users of Enrich outcomes.

For example it would be very useful for end users of Enrich outcomes to have access to a searchable tool which is able to provide information on:

- Plant species.
- What it can be used for (range of purposes) – anything from shelter to recharge to secondary metabolites.
- Its adaptability – what soils, rainfall, regions i.e. where it fits in landscapes.
- How to establish it.
- How to manage it.
- What primary and secondary metabolites it contains – linked to purpose (animal health, other)
- How to utilize the metabolites
- Any information on fit with other species – mix of plants for specific purpose(s) – synergies and risks.

The database is designed to support the development of an interactive, searchable, knowledge base tool which can leave a lasting legacy from Enrich 1 in a form that it can be added to in the future and help producers get ready access to information on adapting their farm business to Enrich principles to suit their particular interests. The system would be flexible enough to also suit particular interests, eg catchment management authorities.

The final system is envisaged to be a web based tool with links to the relevant outputs from Enrich 2 (reports, fact sheets, case studies, papers) and elsewhere (international eg Provenza Behave program). In summary further development and investment in the database has the potential to be a powerful output from the Enrich project team.

Preliminary field evaluation of woody perennial species for inclusion on shrub-based grazing systems

Plant species need to be found which fulfil as many of the benefits (year round feed availability, high water use, nutritious, productive, and healthy) that the Enrich project aims to exploit. The first step in this process is to evaluate a large range of woody perennial germplasm in the field for their suitability for inclusion in low rainfall grazing systems. The aim was to build a profile of these plant species on their agronomic capacity, potential for commercial production and their weed risk.

Methods

From the initial species selection process, 94 species were identified (Table 1). The South Australian Research & Development Institute Genetic Resource Centre (SARDI GRC) undertook the process of sourcing seed of these species. Seed was sourced from commercial suppliers or in a number of cases from the SARDI GRC. This material had been previously collected and had good associated information in terms of collection date, paternal plant details and site details. In an attempt to obtain an accurate representation of the species, three accessions of each species were sourced. However, this was not always possible and in a number of cases no accessions of a species were found (Table 2). Seed of a total of 82 species were sourced.

Table 1. List of species identified in the initial species selection process for investigation in the Enrich project

Taxa	Native/exotic	Successfully sourced
Abutilion otocarpum	Native	Yes
Acacia aneura	Native	Yes
Acacia iteaphylla	Native	Yes
Acacia ligulata	Native	Yes
Acacia loderi	Native	Yes
Acacia minura	Native	No
Acacia mytrifolia	Native	Yes
Acacia neriifolia	Native	Yes
Acacia pycnantha	Native	Yes
Acacia saligna	Native	Yes
Allocasuarina muelleriana	Native	Yes
Allocasuarina verticillata	Native	Yes
Atalaya hemiglauca	Native	Yes
Atriplex amnicola	Native	Yes
Atriplex cinerea	Native	Yes
Atriplex isatidea	Native	Yes
Atriplex leptocarpa	Native	Yes
Atriplex nummularia	Native	Yes
Atriplex paludosa	Native	Yes
Atriplex rhagodioides	Native	Yes
Atriplex semibaccata	Native	Yes
Atriplex vesicaria	Native	Yes
Brachychiton gregorii	Native	Yes
Brachycome ciilaris	Native	Yes
Chameacytisis prolifer	Exotic	Yes
Chenopodium auricomum	Native	No
Chenopodium gaudichaudianum	Native	Yes
Chenopodium nitrariaceum	Native	Yes
Convolvulus remotus	Native	Yes
Cullen australasicum	Native	Yes
Cullen cinereum	Native	Yes
Cullen pallidum	Native	Yes
Chamecytisus proliferus	Exotic	Yes
Dorycnium hirsutum	Exotic	Yes
Enchylaena tomentosa	Native	Yes
Eremophila bignoniiflora	Native	Yes
Eremophila compacta	Native	No
Eremophila debilis	Native	Yes
Eremophila forrestii (stony)	Native	No
Eremophila glabra	Native	Yes
Eremophila latrobei	Native	Yes
Eremophila longifolia	Native	Yes
Eremophila maculata	Native	Yes
Eremophila oppositifolia	Native	Yes
Geijera linearifolia	Native	Yes
Geijera parviflora	Native	Yes
Glycine canescens	Native	Yes
Glycine clandestina	Native	Yes

Taxa	Native/exotic	Successfully sourced
Glycine tabacina	Native	Yes
Grevillea inconspicua	Native	No
Grevillea junncifolia	Native	Yes
Kennedia eximia	Native	Yes
Kennedia macrophylla	Native	Yes
Kennedia nigricens	Native	Yes
Kennedia prorepens	Native	Yes
Kennedia prostrata	Native	Yes
Kennedia rubicunda	Native	Yes
Lavatera plebeia	Native	Yes
Lawrencia squamata	Native	Yes
Lotus australis	Native	Yes
Maireana brevifolia	Native	Yes
Maireana convexa	Native	Yes
Maireana georgei	Native	Yes
Maireana glomerifolia	Native	No
Maireana planifolia	Native	Yes
Maireana platycarpa	Native	Yes
Maireana pyramidata	Native	Yes
Maireana sedifolia	Native	Yes
Maireana tomentosa	Native	Yes
Medicago arborea	Exotic	Yes
Medicago citrina	Exotic	Yes
Medicago strasseri	Exotic	Yes
Medicgao sativa	Exotic	Yes
Myoporum platycarpum	Native	Yes
Owenia acidula	Native	No
Pultanaea largiflorens	Native	Yes
Rhagodia candolleana	Native	Yes
Rhagodia crassifolia	Native	Yes
Rhagodia drummondi	Native	No
Rhagodia eremaea	Native	No
Rhagodia parabolica	Native	Yes
Rhagodia presissii	Native	Yes
Rhagodia spinescens	Native	Yes
Scaevola spinescens	Native	Yes
Sida calyxhymenia	Native	Yes
Sida corrugata	Native	Yes
Sida cunninghamii	Native	Yes
Sida filiformis	Native	Yes
Sida goniocarpa	Native	Yes
Sida intricata	Native	No
Swainsonia greyana	Native	Yes
Swainsona stipularis	Native	Yes
Templetonia retusa	Native	Yes
Ventilago viminalis	Native	No
Viminaria juncea	Native	Yes

Species were germinated using the best available germination stimulating methods described in the literature. After employing the recommended treatment (where available), seeds were pre-germinated in Petri dishes. Germinated seeds were planted when a radicle of over 2cm was present into seedling tubes filled with potting mix. Seedlings were grown for 2-3 months in a sheltered polyhouse before being placed outside for a further 1-2 months in an attempt to acclimatise to outside conditions. Legume species were inoculated with the best available rhizobia after planting where the symbiosis was known.

Table 2. Success of the species selection and germination processes

	Number of species	Number of accessions
Potential species identified	94	n/a
Species sourced (seed)	83	206
Species propagated for field testing in 2006	61	132
Species propagated for field testing in 2007	10	16
Total species for field testing (at June 2008)	71	148

The field experiment employed an unreplicated control plot design to examine as many lines as possible. From the propagation process, adequate tube stock of 132 accessions comprising 61 species were advanced for field testing. *Atriplex nummularia* (cv. Eyre's Green) was used as a frequent control plot throughout the experimental area. Plots consisted of 36 plants, using a 6 x 6 layout with 3m (inter-row) x 1.5m (intra-row) spacing. The experiment took place at Monarto, South Australia. The soil type was a sandy loam over poorly structured clay. Species were established by planting seedlings in September and October 2006. Shrubs were planted into rows which had been deep ripped to a depth of 30-50cm in June 2006. Due to the prevailing conditions, drip irrigation was used to establish the experiment. No further irrigation was used after January 2007. Twelve accessions propagated in 2006 but which were not advanced enough to plant in 2006, were planted in a defined block in August 2007. A further 5 accessions (five species not previously planted) which were sourced in 2007 were planted at the same time. The non-selective herbicide Roundup (glyphosate 450 g L^{-1}) was used for weed control was done with a shielded sprayer to avoid off-target damage.

Canopy dimensions (height and two diameters) were measured in June 2007, December 2007 and May 2008. Only the 24 central shrubs in each plot were measured. Plant architecture, presence of flowers and fruit, physical defences to grazing, volunteer seedlings and pathogenic or environmental damage were also recorded at these times on all individuals (

Table 3). Edible biomass, using the shortcut method of Andrew et al. (1979) was estimated during May 2008 (data being complied at writing). Plots were grazed during June 2008. All lines planted in 2007 began their measuring cycle in May 2008.

Table 3. Variables measured on Enrich candidate species to June 2008

Variable	Date measured
Seed pre treatment required	At germination
Germination time	At germination
Survival	June & November 2007; May 2008
Canopy dimensions	June & November 2007; May 2008
Architecture	June & November 2007; May 2008
Spinesence	June & November 2007; May 2008
Hairiness	June & November 2007; May 2008
Juvenile period	June & November 2007; May 2008
Seed production	June & November 2007; May 2008
Recruitment	June & November 2007; May 2008
Insect/disease/environmental damage	June & November 2007; May 2008
Vegetative reproduction	June & November 2007; May 2008
Edible biomass	May 2008
Sheep preference	June/July 2008

As well as providing species morphological, phenological and production information, this experiment supplied material for the in vitro testing in the nutritive value and bioactive activities (described elsewhere in this report).

Results & Discussion

Seed germination

Overall seed germination was low and variable (Table 4). There were many species where information on a successful seed pre-treatment did not exist. Commonly high germination would be achieved in only one of two or more accessions of one species. This would suggest that seed viability and quality is an issue in native seed stocks. Viability was not examined in this study. Thus for species bought with unknown collection information, no conclusions can be made as to whether poor viability or seed dormancy is the cause of low germination. Unfortunately, such a large number of species did not allow for experimentation to increase germination knowledge for species where a low success rate was achieved.

For successful commercial adoption of these species, effective, economic propagation is essential. The most economical on-farm option would be for direct seeding of the desired species. For this to occur, a much greater knowledge of the germination requirements of the species is needed. This is potentially a highly complex issue as many factors influence germination (e.g. paternal environment, storage conditions, seed dormancy). Therefore this can not occur with such a large number of potential species. Once the most promising species have been identified then further studies can take place.

Table 4. Results of the various seed pre-treatments used in the germination of Enrich species

Species	Pre-treatment	Success (% germination)
Abutilon otocarpum	None	>50%
Acacia anerua	Heat	<50%
Acacia iteaphylla	Heat	>90%
Acacia ligulata	Heat	>90%
Acacia loderi	Heat	>90%
Acacia myrtifolia	Heat	>90%
Acacia neriifolia	Heat	>90%
Acacia pycnantha	Scarification	>90%
Acacia saligna	Scarification	>90%
Allocasuarina muelleriana	None	>50%
Allocasuarina verticillata	None	>50%

Species	Pre-treatment	Success (% germination)
Atalaya hemiglauca	Heat	0
Atriplex amnicola	Soaking with GA3	>90%
Atriplex cinerea	Leaching (water)	<50%
Atriplex isatidea	Leaching (water)	<50%
Atriplex leptocarpa	Leaching (water)	<10%
Atriplex paludosa	Leaching (water)	>50%
Atriplex rhagodioides	Leaching (water)	>50%
Atriplex semibaccata	Leaching (water)	>90%
Atriplex vesicaria	Leaching (water)	>90%
Brachycome ciilaris	None	<50%
Brachychiton gregorii	Heat	>50%
Chameacytisis prolifer	Scarification	>50%
Chenopodium gaudichaudianum	Soak	<50%
Chenopodium nitrariaceum	Soak	<50%
Convolvulus remotus	Scarification	>90%
Cullen australasicum	Scarification	>50%
Cullen cinereum	Scarification	>50%
Cullen pallidum	Scarification	>50%
Dorycnium hirsutum	Scarification	>50%
Enchylaena tomentosa	None	>50%
Eremophila bignoniiflora	Seed removal & leaching	<20%
Eremophila debilis	Seed removal & leaching	0
Eremophila glabra	Seed removal & leaching	<20%
Eremophila latrobei	Seed removal & leaching	<20%
Eremophila longifolia	Seed removal & leaching	<20%
Eremophila maculata	Seed removal & leaching	<20%
Eremophila oppositifolia	Seed removal & leaching	0
Geijera linearifolia	Scarification & Leaching (water)	0
Geijera parviflora	Scarification & Leaching (water)	0
Glycine canescens	Scarification	>50%
Glycine clandestina	Scarification	>50%
Glycine tabacina	Scarification	>50%
Grevillea junncifolia	None	<50%
Kennedia eximia	Scarification	>50%
Kennedia macrophylla	Scarification	>50%
Kennedia nigricans	Scarification	>50%
Kennedia prorepens	Scarification	>50%
Kennedia prostrata	Scarification	>50%
Kennedia rubicunda	Scarification	>50%
Lavatera plebeia	None	>50%
Lawrencia squamata	None	<10%
Lotus australis	Scarification	>50%
Maireana astrotricha	None	>50%
Maireana brevifolia	None	<50%
Maireana convexa	None	<50%
Maireana georgei	None	>50%
Maireana planifolia	None	<10%
Maireana platycarpa	None	0
Maireana pyramidata	None	>50%
Maireana sedifolia	None	>50%
Maireana tomentosa	None	>50%
Medicago arborea	Scarification	>90%

Species	Pre-treatment	Success (% germination)
Medicago citrina	Scarification	>90%
Medicago sativa	Scarification	>90%
Medicago strasseri	Scarification	>90%
Myoporum platycarpum	Soaking	<10%
Pterocaulon sphacelatum	None	<50%
Pultanaea largiflorens	Scarification	<10%
Rhagodia candolleana	Leaching (water)	>50%
Rhagodia crassifolia	Leaching (water)	>50%
Rhagodia parabolica	Leaching (water)	>50%
Rhagodia spinescens	Leaching (water)	>50%
Scaevola spinescens	Heat	0
Sida calyxhymenia	Heat	0
Sida corrugata	Heat	0
Sida cunninghamii	Heat	0
Sida filiformis	Heat	0
Sida goniocarpa	Heat	<10%
Swainsona greyana	Scarification	>50%
Swainsona stipularis	Scarification	<50%
Templetonia retusa	Scarification	>50%
Viminaria juncea	Scarification	>50%

Canopy volume of Enrich species

Species were measured for their maximum canopy dimensions (height, width, breadth) in June and November 2007 as well as May 2008 (data being processed at time of writing) to provide an indication of their early forage production (expressed as canopy volume). From data measured in June (nine months after transplanting) (Table 5) and November (15 months after transplanting) there was large variation between species (Figure 1). The control species, *Atriplex nummularia* had the largest canopy volume. However, a number of other species were still productive when expressed as canopy volume. Over half of the species had less than 10% of the canopy volume of *Atriplex nummularia* (Figure 2). Canopy volume does not always equate to the actual amount of edible biomass as the density of foliage is a particularly critical factor. However, it is still a reasonable predictor of production of young plants. Canopy dimensions are also valuable as they can aid in determining which planting configurations may be the most applicable to each species. The measure of edible biomass in 2008 on plants more applicable to a grazing age will give a more reliable predictor of productivity from a forage perspective.

Table 5. Canopy volume of the largest 10 species measured at June 2007

Species	Canopy volume (% of *Atriplex nummularia*)
Atriplex nummularia	100
Atriplex rhagodioides	67
Chenopodium nitrariaceum	62
Acacia saligna	58
Maireana tomentosa	52
Atriplex amnicola	48
Kennedia nigricans	45
Rhagodia spinescens	40
Cullen australasicum	35
Atriplex cinerea	33

Physical defences to grazing

Physical defences such as spines can be an effective method of protecting plants from being damaged by herbivore grazing. It is also considered a negative trait in many weed risk assessment systems. The Enrich species examined here were largely unaffected by spinesence during their first 18 months of growth. Only one species (*Chenopodium nitrariaceum*) contained spines and by the definition of Cornelissen et al. (Cornelissen et al. 2003) these were only "soft" spines and should not pose a problem for animals or humans.

Reproduction

Over half of the species being examined have entered some form of the reproductive phase. Most have produced some amount of fruit, indicating very short juvenile periods (Table 6). This has implications in the production of commercial seed and is important in the weed risk of species. A short time to reproductive maturity and seed production would seem an advantage for commercial seed production. However, the placement of seed on the plant as well as the synchronicity of seed ripening is critical in determining seed production potential. Short juvenile periods are common in many perennial weeds (Grotkopp et al. 2002) enabling the plant to reproduce early, which results in the rapid formation of self-sustaining populations.

Ten species have been observed to recruit seedlings under or near planted bushes. Some have already shown some degree of spread with seedlings up to 10 m away. Whilst this has been regarded in a positive light in annual pasture systems, it is of less importance in shrub based systems. In fact, it may alter the desired perennial density being targeted by the landholder. Too high a density may create undue competition and reduced productivity (see co-existence section). A large number of recruited seedlings in a relatively short period reflects a colonising plant strategy, typical of many weeds. In a grazed system, many of these recruits may be controlled by grazed but in the absence of grazing or with seed dispersal into ungrazed areas, these species may establish successfully.

Reproduction can also result in changes to the plant's phytochemistry when plants may increase their chemical defences in an attempt to deter herbivory of their propagules. This may alter the nutritive value, bioactivity and palatability of these species which would have important implications in grazing management. Work within Enrich has shown some difference in anethemintic activity with plants at different stages of reproductive maturity (reported elsewhere in this report).

Pathogenic and environmental damage

Whilst many species experienced damage from some form of pathogen during the observation period, none caused any significant damage. However, four frost events occurred at Monarto just prior to data collection in June 2007. There was a significant impact on a number of species and this was recorded. Six species were severely damaged (loss of all green foliage and damage to branch structure) by frost and one species died (Table 6). A further 10 species were moderately affected. There was some genotypic variation in frost tolerance within species. This information is important for identifying a species potential growing region.

Plate 1. The Monarto site showing the initial field evaluation experiment during 2007

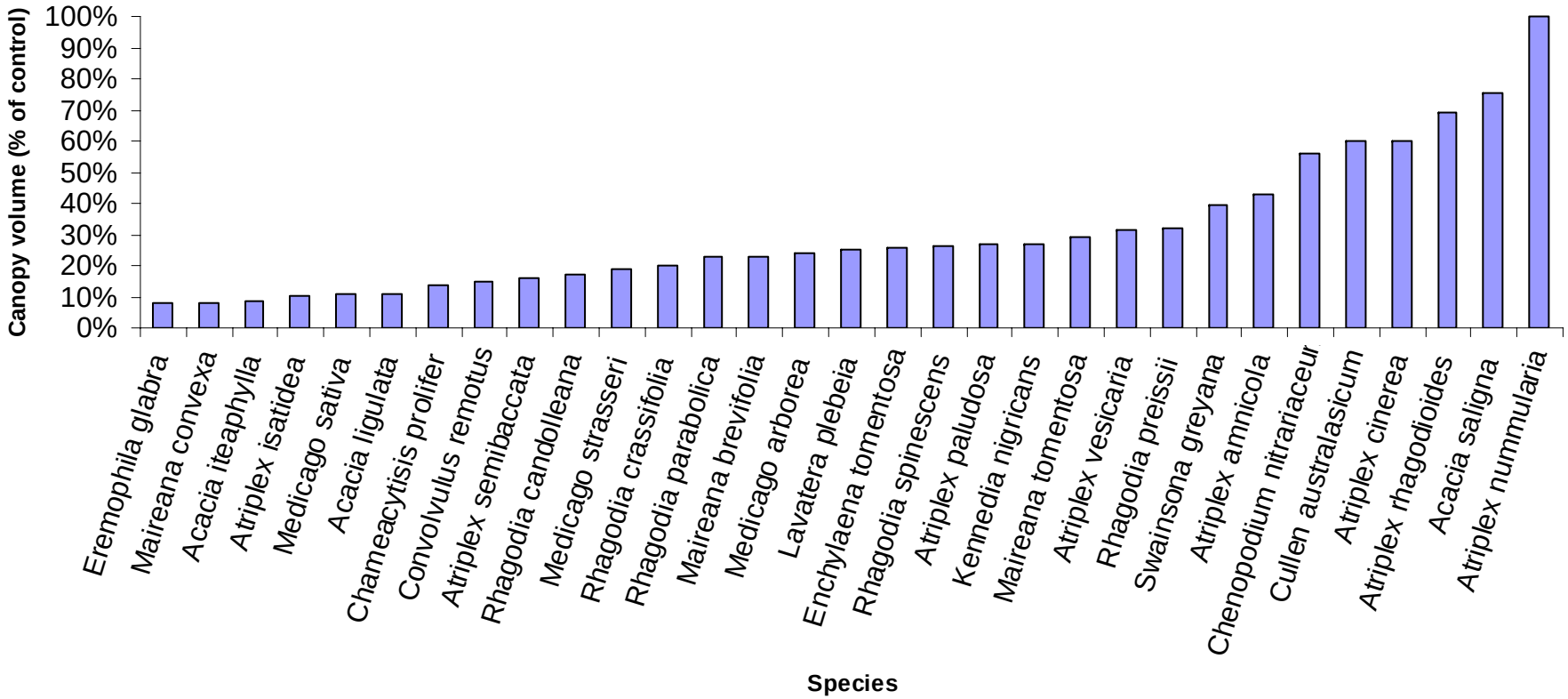


Figure 1. Canopy volume of species in December 2007 (15 months since transplanting tubestock) for the largest 50% of species. Data expressed as a control of *Atriplex nummularia* (100%)

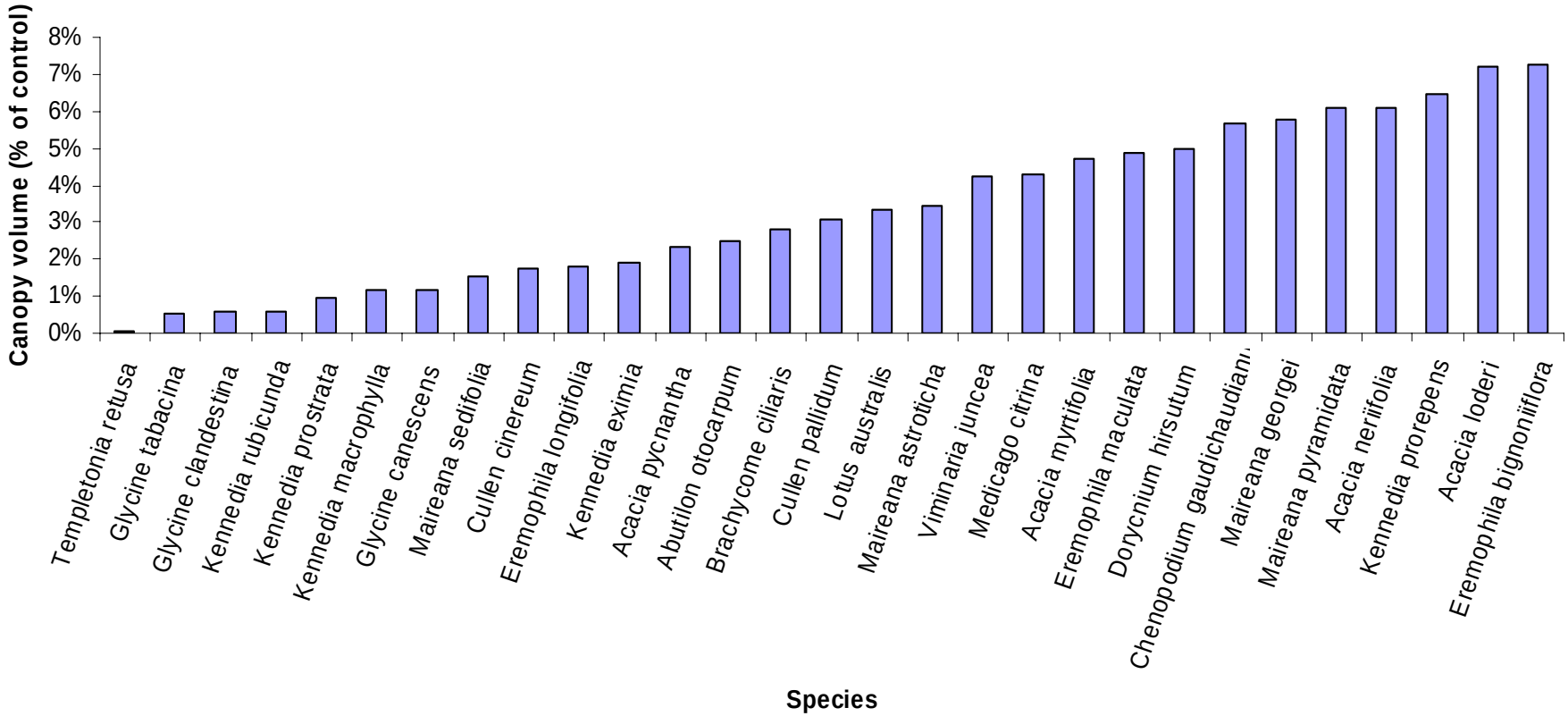


Figure 2. Canopy volume of species in December 2007 (15 months since transplanting tubestock) for the smallest 50% of species. Data expressed as a control of *Atriplex nummularia* (100%)

Table 6. Summary of species measured in 2007 along with the number of accessions per species and the total number of measured plants, reproductive status and frost damage

Species	Number of accessions	Number of measured plants	Growth habit	Flowered	Fruit	Recruitment	Frost damage
Abutilon otocarpum	1	25	Sub-shrub	+	+		Negligible
Acacia iteaphylla	3	71	Shrub				Negligible/Moderate
Acacia ligulata	4	90	Shrub				Negligible
Acacia loderi	1	10	Tree				Negligible
Acacia myrtifolia	2	26	Shrub	+	+		Negligible/Moderate
Acacia neriifolia	3	45	Tree				None/Negligible
Acacia pycnantha	2	32	Tree				Negligible/Moderate
Acacia saligna	1	24	Tree				Negligible
Atriplex amnicola	3	71	Shrub	+	+		None
Atriplex cinerea	4	58	Shrub	+	+		None
Atriplex isatidea	1	16	Shrub				None
Atriplex nummularia	1	658	Shrub	+	+		Negligible
Atriplex paludosa	3	72	Shrub	+	+	+	None
Atriplex rhagodioides	2	47	Shrub	+	+		None
Atriplex semibaccata	4	62	Creeper	+	+	+	Moderate
Atriplex vesicaria	5	118	Shrub	+	+	+	None
Brachycome ciilaris	2	34	Herb	+	+	+	None
Chameacytisis prolifer	1	22	Tree	+	+		None

Species	Number of accessions	Number of measured plants	Growth habit	Flowered	Fruit	Recruitment	Frost damage
Chenopodium gaudichaudianum	2	33	Shrub	+	+		Moderate
Chenopodium nitrariaceum	2	48	Shrub	+	+		None
Convolvulus remotus	1	24	Climber	+	+	+	None
Cullen australasicum	3	46	Sub-shrub	+	+	+	Negligible/Moderate
Cullen cinereum	3	23	Herb	+	+	+	None/Negligible
Cullen pallidum	1	12	Sub-shrub	+			Negligible/Moderate
Dorycnium hirsutum	2	46	Sub-shrub	+	+		None
Enchylaena tomentosa	3	72	Shrub	+	+	+	None
Eremophila bignoniiflora	1	8	Shrub				Negligible
Eremophila glabra	2	7	Shrub	+	+		None
Eremophila latrobei	1	8	Shrub				Severe
Eremophila longifolia	3	26	Tree				Negligible
Eremophila maculata	1	23	Shrub	+			None
Glycine canescens	3	54	Climber	+			Severe
Glycine clandestina	1	24	Climber				Severe
Glycine tabacina	1	23	Creeper	+	+		None
Kennedia eximia	1	18	Creeper	+	+		Negligible
Kennedia macrophylla	1	17	Climber	+			Severe
Kennedia nigricans	2	45	Climber	+			None/Negligible

Species	Number of accessions	Number of measured plants	Growth habit	Flowered	Fruit	Recruitment	Frost damage
Kennedia prorepens	3	30	Creeper	+	+		None/Moderate
Kennedia prostrata	3	39	Creeper	+	+		Negligible
Kennedia rubicunda	2	25	Climber	+	+		Severe
Lavatera plebeia	3	36	Sub-shrub	+	+		None
Lotus australis	3	58	Herb	+	+		None/Negligible
Maireana astrotricha	1	13	Shrub				None
Maireana brevifolia	1	24	Shrub	+	+	+	None
Maireana convexa	3	43	Shrub	+	+		None
Maireana georgei	2	48	Shrub	+	+		None
Maireana pyramidata	4	70	Shrub				None
Maireana sedifolia	1	23	Shrub				None
Maireana tomentosa	2	39	Shrub	+	+		None
Medicago arborea	1	24	Shrub	+	+		None
Medicago citrina	1	13	Shrub				None
Medicago sativa	1	24	Herb	+	+	+	None
Medicago strasseri	1	24	Shrub	+	+		None
Pterocaulon sphacelatum	1	6	Herb	+			Death
Rhagodia candolleana	1	24	Shrub	+	+		None
Rhagodia crassifolia	3	72	Shrub				None

Species	Number of accessions	Number of measured plants	Growth habit	Flowered	Fruit	Recruitment	Frost damage
Rhagodia parabolica	3	87	Shrub				Negligible
Rhagodia preissii	3	71	Shrub	+	+		Negligible
Rhagodia spinescens	4	83	Shrub				None
Swainsona greyana	1	24	Shrub	+	+		None
Templetonia retusa	1	24	Shrub				Negligible/Moderate
Viminaria juncea	3	51	Shrub	+			Severe/Moderate

Identification of promising species

Whilst the species selection activity is not meant to be an open ended process, only 71 out of an identified 135 species have begun evaluation in the field. There is an opportunity to source these outstanding species and subject these to field and in vitro evaluation. With the relatively high success rate that has been found in the range of traits within the initial investigated species there may be value in including these unassessed species.

Despite candidate species not exceeding the canopy volume of the control species (*Atriplex nummularia*), there still appears to be a significant number of promising species that may have potential for inclusion in low rainfall grazing systems. Data shown in this report relate to early growth performance. When the autumn 2008 data is studied, a clearer picture will emerge on species production. This will be more relevant to plants of a grazing age. Additionally, differing results may be obtained on different soil types and climatic regions.

It is known there are various limitations to *Atriplex nummularia* and thus is still scope to continue to investigate alternative or complementary species. For example, the bioeconomic modelling in Enrich has suggested that farm profit will increase more significantly by increasing nutritive value than biomass alone. Therefore species with higher nutritive value and/or which result in improving rumen function may only need moderate biomass production to result in overall net gains. When this is taken into account, more species appear to have potential than when assessed on biomass production alone.

Weed risk of species

The use of Australian native plants does not render weed issues void. The presence of a number of species of Australian origin that are documented as weeds (Groves 2001) indicates that Australian plants can still become weeds when present outside of their original indigenous range. In addition, the use of native plants also creates a different set of weed issues, namely genetic pollution and hybridisation (Byrne and Millar 2004). Particular caution will have to be paid to the weed risk of candidate species if these new systems are going to be environmentally successful. With many Enrich species having a scarce documented history, the information being gathered in this study will contribute to their weed risk being assessed as accurately as possible. A full weed risk assessment has been performed on *Rhagodia preissii* largely from data gathered by Enrich personnel.

The response of 12 woody perennial forage species to grazing

Based on existing knowledge at the start of the Enrich project, 20 species were short listed as having the most potential for further experimentation. As the long-term aim of the Enrich project is for these species to be utilised as grazing/forage plants, the potential for recovery and regrowth after grazing is of paramount importance. The aim of this experiment was to determine the relative grazing preference by sheep and how the tested species respond to grazing.

Methods

A randomised block design was used, comprising 12 species (Table 7) and 3 grazing treatments replicated 4 times. Plots consisted of 36 plants, using a 6 x 6 layout with 3m (inter-row) x 1.5m (intra-row) spacing. The three grazing treatments were 1) no grazing, 2) grazed in autumn only (most common current practice) and 3) grazed twice a year (autumn and late spring). To obtain a better representation of a species, multiple provenances were used where possible. Species were propagated from seed and only 12 species of the proposed 20 germinated in sufficient numbers for experimentation. All species were planted by using established seedlings in August 2006. Shrubs were planted into rows which had been deep

ripped to a depth of 30-50cm in June 2006. Seedlings were supplementary watered to ensure survival. The experiment was conducted at Monarto, South Australia on a gradational calcareous loam.

Lambs were used for short periods for weed control in 2007. Using stock naïve to shrubs and short grazing periods allowed consumption of the inter-row without significant damage to the shrub species. Grazing of the shrub component took place in May 2008.

Edible biomass was estimated during May and November 2007 and April 2008 using the "Adelaide" technique (Andrew et al. 1979). Only the 24 central shrubs in each plot were measured. Plant architecture and the presence of flowering was also recorded at this time.

Table 7. Species used in the experiment

Species	Number of provenances
Acacia saligna	3
Atriplex amnicola	3
Atriplex cinerea	2
Atriplex nummularia	1
Atriplex semibaccata	2
Atriplex vesicaria	3
Cullen australasicum	3
Enchyleana tomentosa	4
Medicago arborea	1
Rhagodia parabolica	5
Rhagodia preissii	3
Rhagodia spinescens	5

Results & Discussion

As only the 2007 biomass data will be shown in this report, all treatments underwent identical management up to this point. Therefore species data are shown as the mean values across grazing treatments.

There was significant variation in edible biomass between the 12 species. *Atriplex nummularia* had the significantly highest biomass at both times of measurement (Figure 3). At the November measurement, this species had over 2000 kg ha^{-1} in biomass with plants less than 18 months of age. Most species increased in biomass with age. However, there appears to be differences in the rate of increase over the cooler winter season. For example *Rhagodia parabolica* appears to grow in more winter than *Atriplex cinerea*. This will be examined once the 2008 data is analysed as both seasons will have a known start point. *Atriplex semibaccata* decreased in biomass over winter. From field observations, it appeared to show some susceptibility to frost.

How the species respond to multiple grazing will be the real test in determining their suitability to grazing systems. The experiment is now set up to deliver these answers in the near future.

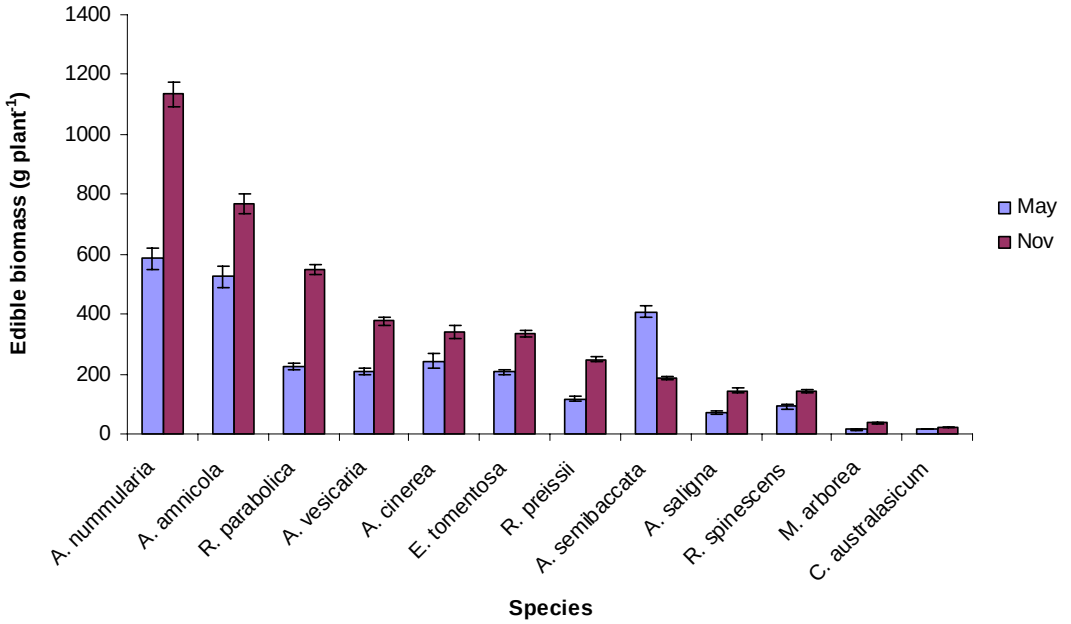


Figure 3. Mean edible biomass at May and November 2007

The co-existence of shrub and understorey pasture species in a shrub based grazing system

It is envisaged that a mixture of plant types will comprise the most beneficial feedbase in a grazing system. A mixture of different plant types creates a substantial change from monocultural production and how complementary these types are needs to be investigated. The aim of this experiment is to determine if growing shrubs with grass and/or herbaceous species results in interference (or facilitation) to one or more components of the feedbase.

Methods

Cultural methods

This experiment used a strip plot design, comprising 4 shrub densities (main plots) and 5 understorey herbaceous/grass components (sub-plots), replicated 5 times. The 4 shrub densities were 0 plants/ha, 1111 plants/ha (3m x 3m spacing), 2066 plants/ha (2.2m x 2.2m spacing) and 3086 plants/ha (1.8m x 1.8m spacing). The shrub species that was used was *Atriplex nummularia* (cv. Eyre's Green). The 5 understorey components consisted of 1) annual legumes (a diverse mix of *Medicago truncatula* cv.'s Jester and Caliph, *M. littoralis* cv. Herald, *M.polymorpha* cv. Scimitar, *M. rugosa* cv. Paraponto and *Trifolium glanduliferum* cv. Prima), 2) lucerne (cv. SARDI 7), 3) perennial grass (red leg grass ;*Bothriochloa macra*), 4) annual legume/perennial grass mixture and 5) bare (kept plant free as much as possible).

Different plot sizes were used for the different shrub density treatments. The area used allowed the same number of measured shrubs per plot (24) across treatments. The 0 plants/ha treatment had the same plot area as the 1111 plants/ha treatment.

The experiment was located at Monarto, South Australia. The soil type was a gradational loam. The understorey components were sown directly into plots in August 2006. The legume species were scarified before sowing but were not inoculated. All seed was tested for germination prior to sowing and the field sowing rate adjusted for viability. The annual legumes were sown at 7 kg ha^{-1}, lucerne at 5 kg ha^{-1} and red leg grass at 8 kg ha^{-1}. The legume

grass mixture was sown together at 3.5 and 4 kg ha^{-1} respectively. The shrub component was planted by using established seedlings in November 2006. Shrubs were planted into rows which had been deep ripped to a depth of 30-50cm in June 2006. Due to dry conditions during 2006, irrigation was used to establish plots. Overhead sprinklers were used during October to aid in understorey growth and to ensure seed set of the annual component. Drip irrigation was used to water the shrub seedlings until March 2007.

Selective herbicides - Broadstike (800 g kg^{-1} Flumetsulam) and Verdict 520 (520 g L^{-1} haloxyfop) - were used to modify the annual legume and lucerne understorey components to reflect their desired composition. Due to insufficient knowledge of the herbicide tolerance of red leg grass to grass selective herbicides, the annual legume/grass treatment was sprayed with Broadstrike only and the grass treatment was treated with a weed wiper containing Gladiator (glyphosate 360 g L^{-1}) at a height above the red leg grass. The bare treatment was kept plant free by the use of the non-selective herbicide Roundup (glyphosate 450 g L^{-1}). The experiment was not grazed before March 2008.

Due to the poor germination of red leg grass over the first year, it was sown again in two replications in October 2007 and irrigated as there was insufficient spring rain for germination. The low success of this procedure resulted in the other replications not being re-seeded.

Measurements

Counts of plant numbers of the understorey component was undertaken in May 2007 after the opening rains of the 2007 season and germination had taken place. Ten counts were taken at random in each plot using a 25 cm square quadrat. Plants were divided into pasture, grass weeds and broadleaf weed components. Shrub numbers were counted in June 2007. Due to a later season in 2008 understorey plant counts were undertaken in June 2008.

Shrub biomass was estimated using the "Adelaide" technique described by Andrew et al. (1979) during June and November 2007 and March 2008. Only the 24 central shrubs in each plot were measured. The biomass of the understorey components was measured in September 2007 and March 2008. This was conducted by placing four 33 cm square quadrats per plot and cutting the above ground green herbage to around a 2 cm height. After cutting, samples were sorted into pasture, grass weeds and broadleaf weed components and dried at 60ºC for 72 hours.

Results & Discussion

Understorey plant numbers

In spite of a short growing season in 2006, regenerating annual legume seedlings numbers were satisfactory to establish a successful pasture in 2007. Whilst having much lower plant numbers than the annual pasture, lucerne plant numbers were high for a perennial pasture sward in autumn 2007. In contrast, red leg grass germinated poorly and very few plants were present at the time of counting (Figure 4). There was no effect of shrub density on understorey pasture numbers.

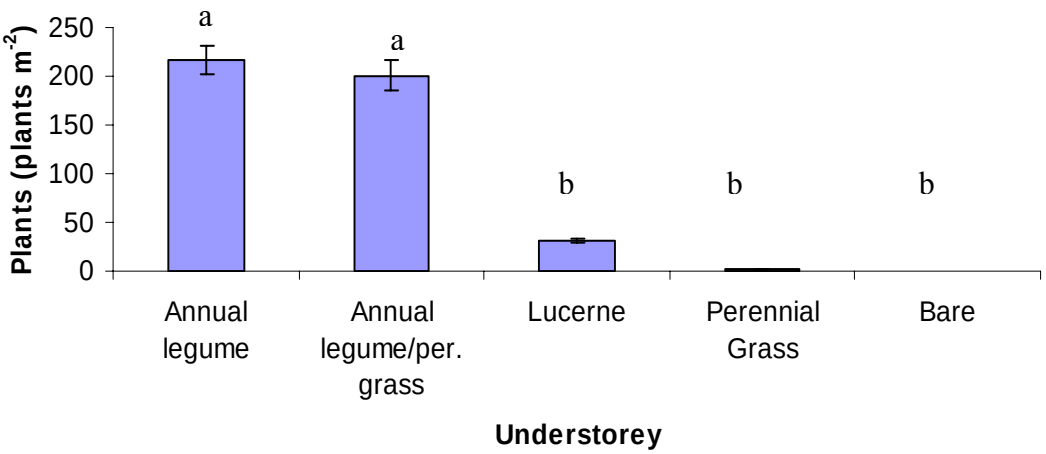


Figure 4. Mean number of pasture plants for the different understorey treatments in May 2007. Different letters represent treatments are different at the 5% level.

Weed numbers were higher than pasture numbers in autumn 2007. There was a significant effect of both shrub spacing ($P<0.05$) and understorey pasture type ($P<0.05$). Weed numbers were significantly higher where the shrub density was the lowest or where shrubs were absent. The lucerne pasture contained the lowest weed numbers (Figure 5). This is most likely due to the lower amount of bare ground available at the time of the opening rains.

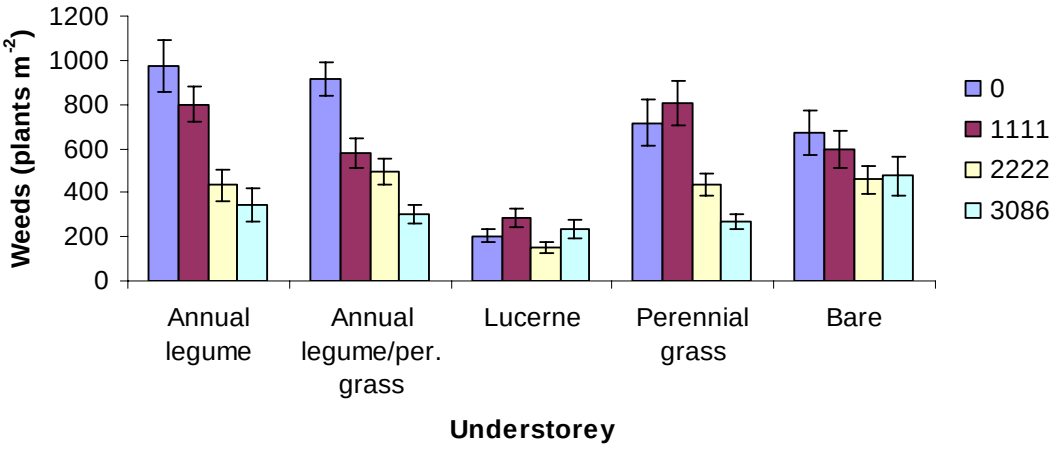


Figure 5. Mean number of weeds for the different understorey treatments and three shrub densities (plants ha^{-1}) in May 2007.

Shrub numbers

The survival of the shrub component of the experiment was over 98%. There was no effect of understorey treatment or shrub density.

Understorey biomass

There was no effect of shrub density on understorey pasture biomass in September 2007. However, the understorey treatments were significantly different ($P<0.001$). The two treatments containing annual legumes had a significantly higher pasture herbage biomass than the other understorey treatments (Figure 6). The poor germination of the red grass pasture

coupled with its high winter dormancy explains the extremely low biomass of this treatment. The annual legume/perennial grass treatment has the highest total weed biomass. This is due to no suitable selective grass herbicide being available to control annual grass weeds in red leg grass. The small weed component of the lucerne treatment (Figure 6) is a function of the lower weed numbers able to establish (Figure 4) with it and demonstrates this species' competitive ability.

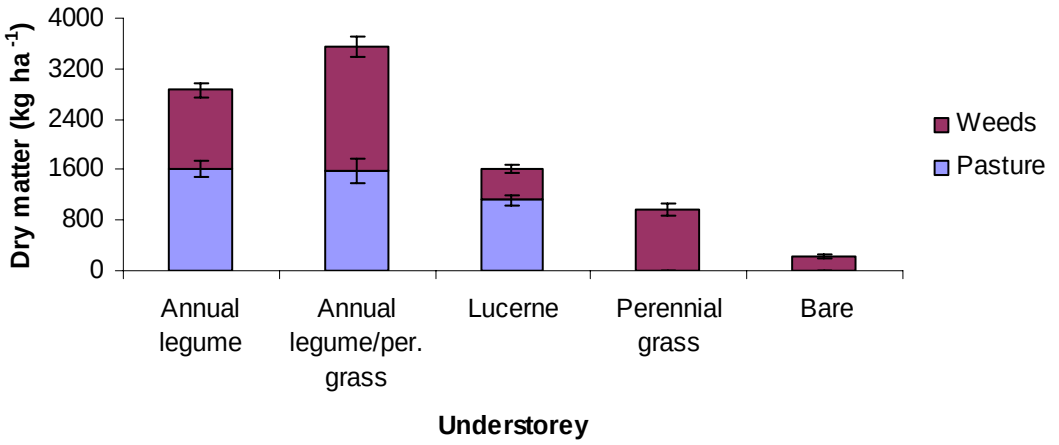


Figure 6. Mean herbage biomass of the different understorey treatments separated into pasture and weed components

When the total biomass of the understorey (pasture plus weeds) is studied there is a significant interaction between shrub density and understorey ($P<0.05$). The effect of shrub density on understorey biomass is dependant on the understorey type where in the annual legume treatments the 0 plants $ha^{-1}$and 3086 plants ha^{-1} have the largest biomass (Figure 7). This is due to the weed component of the understorey. The reasons why the two contrasting densities behaved similarly are unclear.

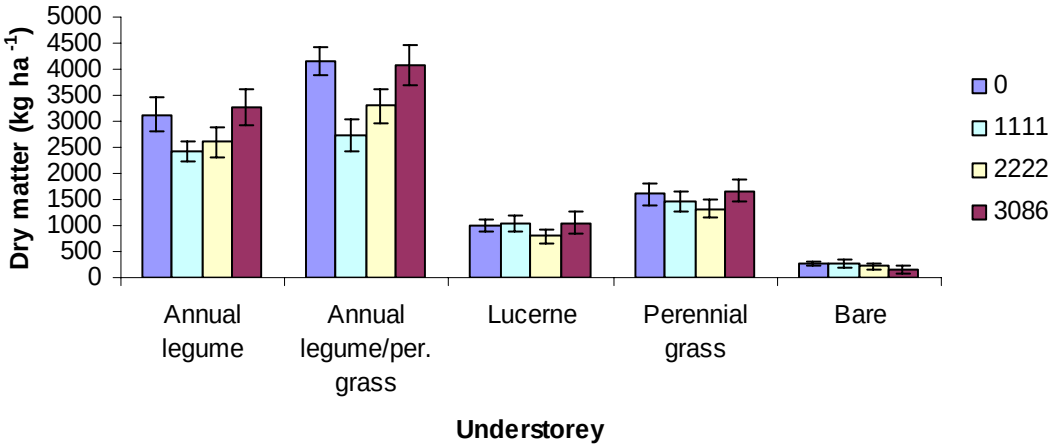


Figure 7. Mean herbage biomass of the total understorey (pasture and weeds) for the different understorey treatments at three shrub densities (plants ha^{-1}) in September 2007.

Shrub biomass

The effect of shrub density ($P<0.05$) and understorey ($P<0.05$) on shrub biomass was significant in June 2007. Shrub biomass was higher at the higher shrub densities. However, at

the later sampling periods shrub density did not significantly effect shrub biomass. The effect of understorey treatment ($P<0.001$) on shrub biomass was significant in November 2007 and March 2008. Shrubs growing with the understorey treatments with the least growth (bare and perennial grass) had significantly higher biomass. Shrubs growing in combination with lucerne had the least biomass (Figure 8 and Figure 9).

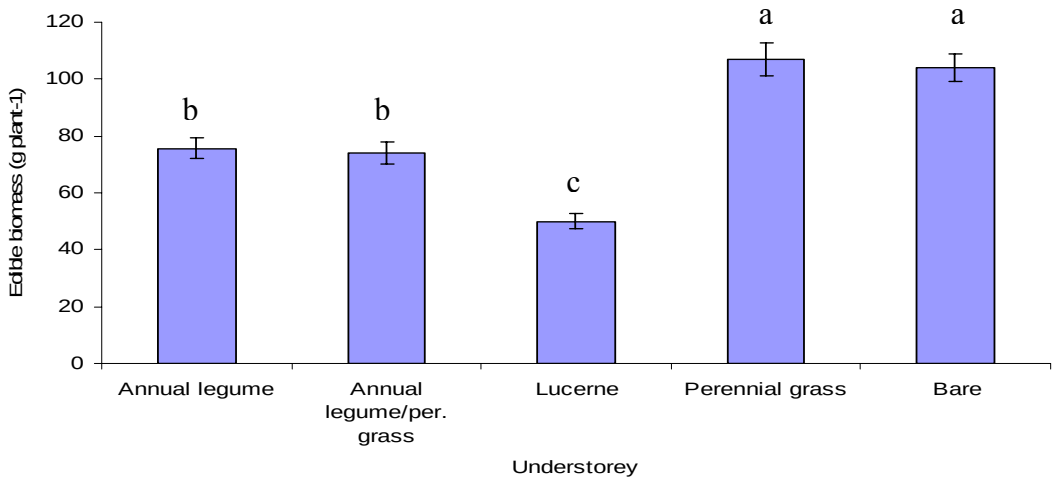


Figure 8. Shrub biomass (g plant^{-1}) with five companion understories at Monarto in November 2007. Different letters represent treatments are different at the 5% level.

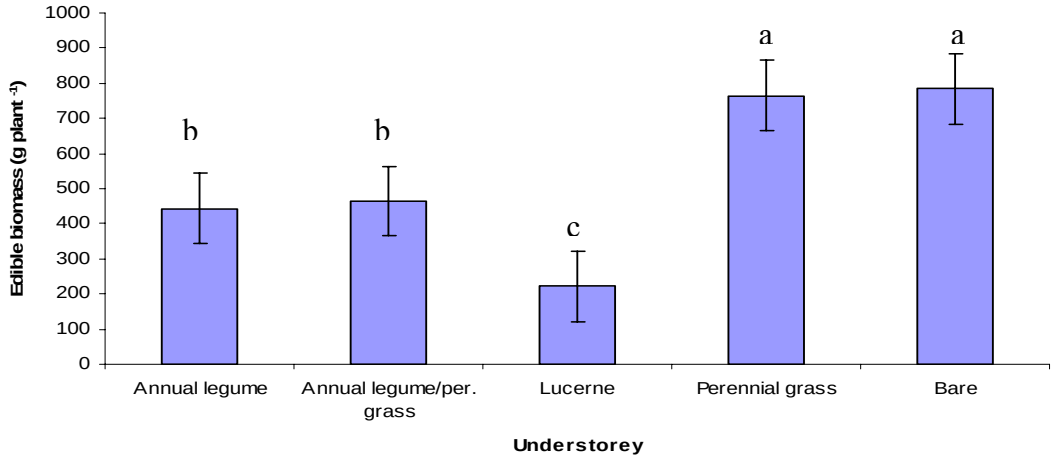


Figure 9. Shrub biomass (g plant^{-1}) with five companion understories at Monarto in March 2008. Different letters represent treatments are different at the 5% level.

The relative growth rate of the shrub component over winter and spring was significantly effected by the understorey type ($P<0.001$) but not shrub density. However, over summer both shrub density ($P<0.05$) and understorey treatments ($P<0.001$) had an effect on shrub relative growth rate. The extra competition of lucerne, particularly over summer resulted in shrub in competition with this plant having a lower relative growth rate than shrubs in combination with other treatments (Figure 10). Shrubs planted at the highest density showed a lower level of biomass increase over summer than the other two densities (Figure 11). This is most likely due to competition for moisture over the dry summer period. It is possible that this trend will continue as the stored soil moisture is exhausted. The continuation of data collection in the future will confirm this.

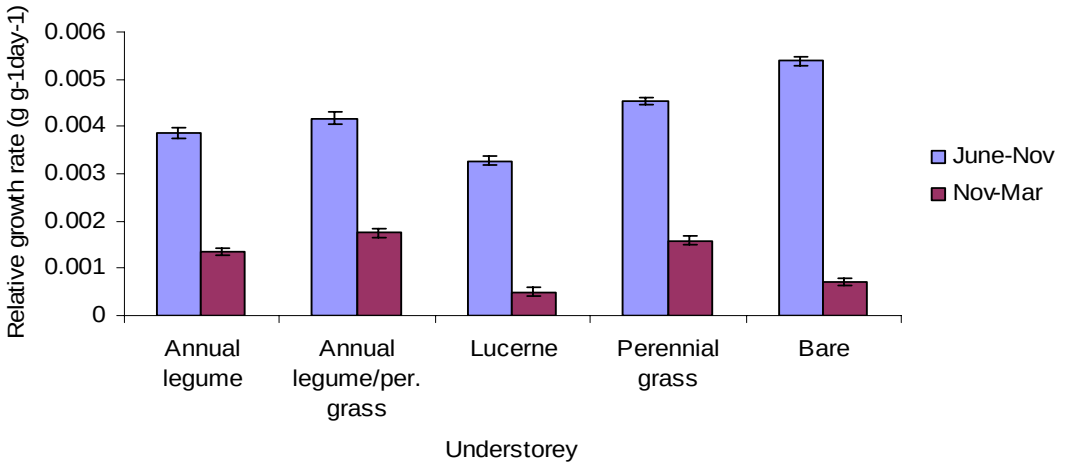


Figure 10. Shrub relative growth rate for plants growing in combination with different understorey treatments for two growing periods, June – November and November - March

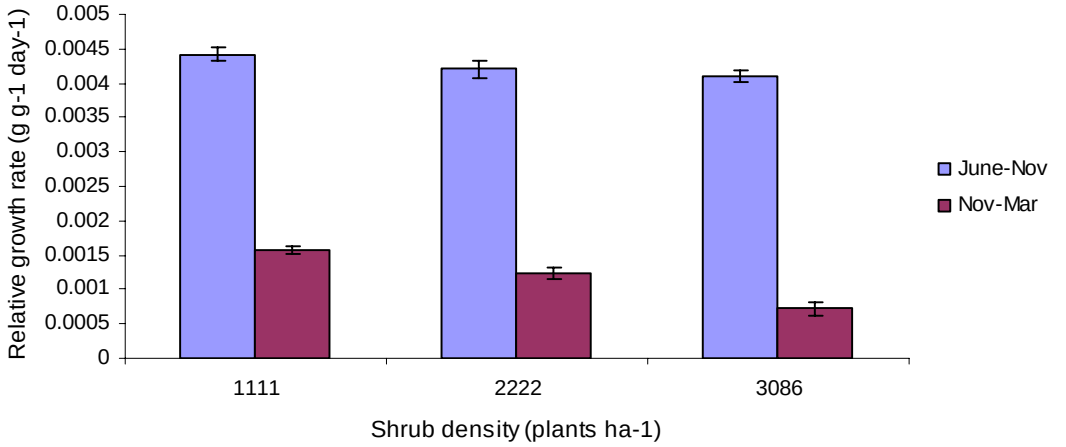


Figure 11. Shrub relative growth rate at the three shrub densities for two growing periods, June – November and November - March

From these early results it appears that to maximise shrub production, competition should be minimised. This particularly applies to year-around competition as occurs from co-existence with other perennial species. Lucerne is known to be a deep rooted species which uses significant amounts of ground water. It is thought that competition for water was the key resource limiting shrub growth in this study. Competition with winter growing annuals also reduced shrub biomass. Even though old man saltbush is deep rooted, there must still be a considerable overlap in the rooting zones of annual species as there was no apparent above ground impendent to the shrub species.

The placement of different plant types (and potentially species) within a system is particularly important. The data gathered here suggests that growing separate blocks of shrubs and herbaceous forages would be the most advantageous. Growing separate areas of species is also advantageous for management. Further data is required to evaluate if the impact of shrubs on the understorey species is negative. However it appears that the density of perennials obtained from any of the combinations of lucerne and saltbush studied was too high for this environment.

The identification, biology and ecology of plant species for shrub based grazing systems – overall conclusions

Enrich has identified over 100 species of woody perennials that have the potential to be used in shrub based grazing systems to deliver a range of benefits. A significant proportion of these have been successfully propagated and planted at Monarto, South Australia for the purpose of evaluating their productivity, morphology, and general suitability to grazing. Productivity as assessed by canopy volume varied widely, as did their ease of germination, reproductive habits, growth architecture and sensitivity to frost.

A replicated experiment designed to study a subset of 12 species' response to grazing has been established and initial edible biomass data shows reasonable production in numerous species. The ability of species to survive and regrow after grazing will be a key factor in their success as forage plants. These factors can be explored now species are advanced enough for grazing.

Grazing systems based purely on shrubs are unlikely to be successful as the production and nutritive value of forage will be limited. The growing of a mixture of plant types therefore creates numerous interactions between the different plant types and the grazing animal. Enrich has attempted to explore if growing shrubs with different understorey species results in interference (or facilitation) to one or more components of the feedbase. It appears that the understorey type grown in companion with shrubs may have a large impact on shrub production. Perennial herbaceous species may result in significant reductions to shrub species biomass and the placement of these species in a forage system needs particular attention. How these components of the systems interact with the introduction of grazing animals also needs to be investigated.

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Section 3

Nutritive value of forage shrubs

The feeding value of plants to grazing animals is determined by voluntary feed intake and nutritive value. Nutritive value is a function of digestibility, protein, minerals, vitamins and secondary compounds, and the efficiency with which they are utilised. Some common indicators of nutritive value are Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), crude protein and in vitro dry matter digestibility (IVD).

Eighty two shrub species have been analysed for nutritive value traits by 'wet chemistry'. The data for 63 growing at the Enrich Monarto site are presented in Table 1 below. Crude protein ranged from 11 to 31%, ADF (representing a relatively indigestible fibre fraction) ranged from 8 to 36%, and hemicellulose content (representing a relatively digestible fibre) ranged from 7 to 32%, although most were in the range of 10-15%.

Pepsin-cellulase in vitro digestibility of dry matter ranged from 28 to 92%. However caution is required in interpreting these data the pepsin-cellulase methodology was developed for oaten hays and there are no calibration equasions to convert this information to meaningful *in vivo* predictions for shrubs. In 2007, the CRC Future Farm Industries funded a project with the aim of developing methods to predict the energy value of shubs. Data analysis from this 'companion' project has recently been completed and we will to apply the equations to the Enrich IVD dataset as one of the first tasks of Enrich phase 2 work.

Table 1. Nutritive value determined by wet chemistry. Values expressed as percentage. CP = crude protein, NDF = neutral detergent fibre; ADF = acid detergent fibre; IVD = pepsin-cellulase in vitro digestibility of dry matter; OM = organic matter.

Taxa	CP	NDF		ADF		hemicellulose		IVD		OM	
	May-07	May-07	Dec-07	May-07	Dec-07	May-07	Dec-07	May-07	Dec-07	May-07	Dec-07
Abutilon otocarpum	27.6	32.1	46.1	23.6	24.4	8.5	21.6	74.2	54.4	89.2	91.0
Acacia iteaphylla	17.6	28.6	35.8	17.6	22.8	11.0	13.0	32.9	28.2	97.3	97.5
Acacia ligulata	11.6	26.4	22.0	20.2	17.4	6.2	4.6	48.6	54.2	84.2	83.8
Acacia loderi	13.4	32.0	34.4	24.1	25.2	7.8	9.2	59.9	63.7	94.6	95.0
Acacia myrtifolia	11.1	32.0	22.7	19.3	16.7	12.7	5.9	68.4	74.8	96.1	96.6
Acacia neriifolia	21.9	24.1	28.7	17.9	21.4	6.2	7.3	55.9	57.2	96.4	97.2
Acacia pycnantha	11.3	31.9	26.7	23.8	18.0	8.1	8.7	51.6	57.4	96.1	95.6
Acacia saligna	15.1	27.0	32.4	22.2	24.7	4.8	7.7	26.4	29.4	93.3	93.7
Atriplex amnicola	27.2	27.2	27.6	12.0	17.0	15.2	10.6	81.6	77.2	79.7	77.7
Atriplex cinerea	13.5	20.3	26.8	12.6	17.3	7.8	9.4	80.3	82.9	76.1	72.0
Atriplex isatidea	10.4	22.2	31.0	14.9	20.3	7.4	10.7	77.4	76.5	72.6	76.1
Atriplex nummularia	21.3	32.0	25.3	15.3	14.6	16.8	10.7	81.6	77.3	71.5	71.7
Atriplex paludosa	21.8	23.8	28.4	14.2	17.7	9.6	10.7	82.4	77.8	73.4	69.8
Atriplex rhagodioides	26.6	28.4	28.2	12.6	15.0	15.8	13.2	79.7	78.5	75.2	74.5
Atriplex semibaccata	18.8	29.1	29.8	17.7	16.4	11.4	13.4	71.7	77.1	77.7	77.1
Atriplex vesicaria	21.6	27.8	33.9	15.5	17.8	12.3	16.0	77.9	75.1	73.2	70.4
Brachycome ciliaris			40.2		27.2		13.0		64.5		94.1
Chameacytisis prolifer	16.1	49.9	32.6	17.6	20.0	32.3	12.7	71.9	69.3	94.6	95.5
Chenopod. gaudichaudianum	22.9	22.3	33.5	8.4	17.8	13.9	15.6	89.2	73.9	70.4	71.1
Chenopodium nitrariaceum	28.0	12.7	19.3	7.7	11.3	4.9	8.0	91.0	88.8	77.7	84.1
Convolvulus remotus	31.3	37.1	36.9	22.2	22.7	14.9	14.2	85.6	70.2	87.6	94.0
Cullen australasicum	28.3	19.7	25.7	10.4	17.5	9.4	8.2	89.5	80.3	90.9	92.6
Cullen cinereum	29.4	16.6	25.3	7.4	14.9	9.2	10.4	93.8	83.9	90.4	93.8

Cullen pallidum	25.5	29.3	46.9	21.1	35.9	8.2	11.0	79.4	65.4	89.4	94.6
Dorycnium hirsutum	18.9	17.1	28.0	11.6	16.8	5.5	11.1	41.0	34.7	92.8	94.7
Enchylaena tomentosa	28.0	32.0	22.8	12.9	13.1	19.1	9.7	74.4	81.3	77.3	79.4
Eremophila bignoniiflora	15.8	25.9	23.7	19.6	12.0	6.2	11.7	83.8	86.6	91.0	93.6
Eremophila glabra	12.9	36.0	28.2	25.1	16.2	10.9	12.0	82.5	81.1	92.3	93.9
Eremophila latrobei											
Eremophila longifolia	16.2	31.0	20.3	25.5	15.9	5.6	4.3	81.5	85.0	91.8	94.4
Eremophila maculata	20.6	17.5	29.1	8.9	12.7	8.7	16.4	92.8	87.4	90.2	91.8
Glycine canescens	25.8	34.0	39.3	17.9	24.3	16.1	15.0	76.7	66.7	97.2	93.4
Glycine clandestina	30.0	30.1	39.8	20.8	25.4	9.3	14.4	82.5	68.5	89.7	94.2
Glycine tabacina		36.0	37.2	19.6	25.1	16.5	12.1	77.7	69.0	88.1	91.5
Kennedia eximia	14.8	26.6	31.8	19.6	23.5	7.0	8.4	46.2	47.9	94.4	95.4
Kennedia macrophylla			30.5		22.0		8.5		39.1		95.0
Kennedia nigricans	19.6	29.5	34.1	22.2	24.9	7.3	9.2	49.4	36.7	93.6	95.2
Kennedia prorepens	12.8	24.9	32.4	20.4	23.6	4.5	8.9	58.3	48.8	85.9	95.1
Kennedia prostrata	13.4	24.6	33.2	15.1	23.0	9.6	10.3	58.7	52.5	92.9	94.2
Kennedia rubicunda	13.6	37.4	34.9	27.0	26.3	10.4	8.6	44.8	41.3	85.1	92.4
Lavatera plebeia	32.4	31.8	51.2	14.2	19.3	17.7	31.9	84.9	68.5	89.2	89.4
Lotus australis	18.9	18.2	20.3	13.5	13.9	4.7	6.4	63.3	47.9	89.6	92.6
Maireana astroticha			41.8		24.1		17.7		68.5		75.6
Maireana brevifolia	25.6	32.2	35.6	13.9	18.0	18.3	17.5	79.4	71.9	73.2	75.8
Maireana convexa	19.8	21.1	35.8	12.1	21.1	9.0	14.7	85.7	70.0	73.2	81.5
Maireana georgei	23.7	31.4	30.3	16.2	17.4	15.2	12.8	78.0	73.9	73.3	80.0
Maireana pyramidata	23.0	35.5	35.5	17.2	20.1	18.2	15.5		73.9		75.6
Maireana sedifolia	22.0	38.6	46.7	21.5	27.3	17.1	19.4	67.6	59.9	77.1	79.3
Maireana tomentosa	23.3	48.1	44.9	32.4	30.3	15.7	14.6	57.3	54.4	83.4	84.8
Medicago arborea	29.3	20.4	28.3	13.6	17.4	6.8	10.9	84.0	78.8	91.2	92.1
Medicago citrina	7.9	32.0	30.7	10.5	15.2	21.5	15.4	89.3	78.4	94.5	94.1
Medicago sativa	43.6	17.5	30.5	13.1	20.0	4.5	10.5	83.0	72.8	90.3	93.5
Medicago strasseri	27.9	23.0	26.2	14.3	15.2	8.7	11.0	80.8	80.3	90.7	91.9
Pterocaulon sphacelatum	16.4	33.7		19.8		13.9		65.5		87.5	
Rhagodia candolleana	20.3	23.9	22.5	12.8	11.4	11.1	11.0	81.9	86.5	73.4	72.5
Rhagodia crassifolia	25.5	13.7	17.1	7.2	9.3	6.5	7.8	88.0	88.7	80.1	81.8
Rhagodia parabolica	27.3	12.4	20.7	8.2	10.9	4.2	9.8	90.4	86.4	78.8	85.9
Rhagodia preissii	22.8	12.7	19.2	6.6	10.6	6.1	8.6	89.5	87.5	80.4	86.9
Rhagodia spinescens	27.1	17.1	28.3	7.7	12.7	9.3	15.6	86.4	79.8	75.5	75.5
Swainsona greyana	27.3	13.9	19.6	8.6	11.7	5.2	7.9	91.8	88.4	90.4	91.8
Swainsona stipularis	31.2	17.3		11.2		6.1					
Templetonia retusa											
Viminaria juncea	9.7	42.2	48.7	26.7	30.9	15.5	17.8	50.3	47.8	94.0	93.7

All samples have been scanned by NIRS (Near Infrared Reflectance Spectroscopy) to allow development of rapid and inexpensive calibration equations to predict nutritive value traits. NIRS requires extensive calibration with a range of plant samples to develop equations that accurately predict nutritive value of pasture samples. For some characteristics we have developed broad-spectrum equations that successfully predict ADF, NDF crude protein and organic matter. In vitro dry matter digestibility (IVD) is proving more difficult and validation of the equations indicate that some 'spectral gaps' were not covered.

Mineral analysis by ICPAES of 68 shrub species is reported in Table 2. The largest range of values was for Na, but all minerals varied considerably between species.

Table 2. Mineral content of shrub species.

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S	Al
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Abutilon otocarpum	280	20	54	6.2	< 1	< 1	1.2	26	19600	6000	910	13300	1390	3000	240
Acacia myrtifolia	104	18	112	3.5	< 1	< 1	< 1	7.4	7000	2100	2800	8400	510	1320	84
Acacia iteaphylla	67	14	41	2.8	< 1	< 1	< 1	10	2600	1360	2400	8500	810	1540	47
Acacia ligulata	54	11	34	1.7	1.3	< 1	< 1	10	38000	5700	7600	12800	1170	23000	29
Acacia loderi	78	8.5	26	2.8	< 1	< 1	< 1	7.9	4800	1850	3300	17100	910	1590	61
Acacia neriifolia	90	7.1	65	3.5	< 1	< 1	< 1	9.9	2100	1840	1410	10900	1060	1360	65
Acacia pycnantha	320	40	27	2.6	1.8	< 1	5.7	7.6	6200	3000	4600	5900	660	1380	230
Acacia pycnantha	50	11	26	2.1	< 1	< 1	< 1	7.9	2400	2200	7100	9700	830	1310	31
Acacia saligna	77	24	99	3.3	< 1	< 1	1.0	14	6500	3500	4100	15400	930	6300	52
Alectryon oleifolius	131	39	35	7.0	< 1	< 1	2.1	39	10300	3100	370	8600	1330	1330	79
Atalaya hemiglauca	230	73	99	3.8	< 1	< 1	4.5	19	14300	2000	420	13300	1280	2300	102
Atriplex amnicola	147	46	58	5.0	< 1	< 1	1.5	20	6800	10500		28000	1870	6100	79
Atriplex cinerea	97	98	58	11	< 1	< 1	2.3	19	12400	7000	64000	33000	1680	4700	72
Atriplex isatidea	130	142	82	19	< 1	< 1	3.9	43	17600	11500	56000	27000	930	3000	110
Atriplex nitrariaceum	149	31	72	3.6	< 1	< 1	1.4	18	7700	7900	67000	12800	1620	3300	106
Atriplex nummularia	124	67	70	8.4	< 1	< 1	1.5	42	14300	7800	73000	25000	1610	7500	87
Atriplex paludosa	115	39	47	5.4	< 1	< 1	1.6	16	9100	5600	77000	31000	1230	5000	82
Atriplex rhagodioides	151	30	48	4.8	< 1	< 1	1.3	22	7100	8200	63000	26000	1920	5900	106
Atriplex vesicaria	128	41	41	3.4	< 1	< 1	1.2	15	10400	3300	80000	31000	1530	4200	91
Brachychiton gregorii	164	18	25	5.3	< 1	< 1	2.2	24	25000	2800	1550	8900	1460	1650	119
Brachychiton populneus	197	20	26	5.9	< 1	< 1	3.6	29	22000	2800	840	12700	1730	1700	79
Brachycome ciliaris	142	17	58	5.1	< 1	< 1	1.3	13	5700	3100	1670	21000	1140	2000	125
Capparis mitchellii	220	125	280	2.5	< 1	< 1	6.8	114	12900	5500	310	11100	870	10500	148
Chameacytisis proliferus	132	63	300	4.0	< 1	< 1	1.1	22	6800	4500	1480	8400	1220	1230	98
Chenopodium gaudichaudianum	147	15	39	3.3	< 1	< 1	1.1	13	4200	4200		68000	1090	3600	124
Chenopodium nitrariaceum	140	24	95	3.3	< 1	< 1	< 1	24	6100	5600	11900	53000	2400	4700	101
Colutea abyssinica	220	290	63	7.3	< 1	< 1	8.3	43	30000	3600	990	18800	3100	10200	76
Convolvulus remotus	240	19	50	5.3	< 1	< 1	5.5	12	5300	3200	1260	16400	1650	1570	220
Cullen australasicum	210	39	134	6.4	1.7	< 1	1.0	18	15100	6000	1060	15900	1650	2400	170

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S	Al
Cullen cinereum	159	23	84	6.6	< 1	< 1	1.2	15	12600	3000	940	16300	1510	2100	111
Cullen pallidum	270	23	85	6.1	4.6	< 1	2.2	13	8200	4400	1720	13000	1120	1530	230
Dorycnium hirsutum	350	57	260	4.1	< 1	< 1	< 1	11	8300	4200	4400	9300	760	1500	350
Enchylaena tomentosa	109	36	34	8.9	< 1	< 1	< 1	15	3400	1840		16700	1110	2100	75
Eremophila bignoniiflora	112	44	34	6.8	< 1	< 1	< 1	14	10400	3600	6300	17100	940	1900	85
Eremophila glabra	192	20	28	9.2	< 1	< 1	2.3	16	7000	2300	620	20000	1450	1760	143
Eremophila longifolia	79	22	17	5.9	< 1	< 1	< 1	12	7900	2900	2000	18200	950	1500	59
Eremophila maculata	118	23	17	4.0	< 1	< 1	1.6	14	4300	2400	18800	13300	1420	1620	100
Glycine canescens	250	41	128	6.0	3.0	< 1	1.8	18	15300	7000	1080	10800	1220	1560	240
Glycine clandestina	200	25	310	6.7	1.2	< 1	2.5	18	11600	4600	440	12800	1590	1500	183
Glycine tabacina	470	62	310	8.6	< 1	< 1	3.0	26	14600	6100	240	13800	1510	1730	450
Kennedia eximia	480	42	230	2.7	< 1	< 1	2.4	11	6200	2500	1610	8400	850	1300	510
Kennedia macrophylla	270	32	134	6.4	< 1	< 1	1.6	11	8800	1880	2800	7400	830	1640	250
Kennedia nigricans	126	53	350	3.2	< 1	< 1	2.4	11	6600	4000	3500	9800	820	1480	105
Kennedia prorepens	490	30	220	4.5	1.1	< 1	2.0	16	8600	3300	1840	8200	1070	1610	480
Kennedia prostrata	440	72	400	4.2	< 1	< 1	2.8	12	9300	5200	3100	6300	900	1410	440
Kennedia rubicunda	490	35	200	7.2	< 1	< 1	7.3	19	12500	4100	1770	11700	1720	1820	480
Lavatera plebeia	420	35	36	6.8	< 1	< 1	1.2	26	21000	7900	6800	10300	2500	10100	350
Lotus australis	167	41	90	2.9	< 1	< 1	1.0	18	8200	6400	3300	19500	1210	4800	143
Maireana sedifolia	210	28	65	4.8	< 1	< 1	1.6	7.5	5700	2500	64000	16500	720	1990	177
Maireana astrotricha	210	60	45	5.1	< 1	< 1	1.7	12	5800	2500	82000	18500	910	1780	171
Maireana brevifolia	210	14	42	6.7	< 1	< 1	< 1	14	2700	1200	78000	21000	1480	2400	144
Maireana convexa	480	14	49	9.9	< 1	< 1	1.0	15	3600	1980	61000	16100	1430	2400	390
Maireana georgei	230	25	45	7.3	< 1	< 1	1.5	12	3200	2300	66000	13800	1070	2500	177
Maireana planifolia	93	18	56	7.8	1.3	< 1	< 1	10	5000	2800	58000	20000	1540	3000	63
Maireana pyramidata	168	39	35	4.8	< 1	< 1	1.3	9.7	4800	2900	69000	23000	980	2200	124
Maireana tomentosa	106	13	60	8.1	< 1	< 1	1.4	15	3100	2500		26000	2300	3400	76
Medicago arborea	149	25	104	6.7	< 1	< 1	1.5	14	18600	2900	6400	13800	1330	2500	121
Medicago citrina	250	29	64	3.1	< 1	< 1	1.5	13	7600	1740	3000	19800	1120	1600	220
Medicago sativa	121	18	70	8.2	< 1	< 1	1.6	31	7800	3000	2200	20000	3100	2400	79
Medicago strasseri	115	32	130	8.0	< 1	< 1	1.1	14	22000	3200	6900	14700	1520	2300	98
Pterocaulon sphacelatum	2100	66	107	19	< 1	< 1	23	28	10100	3200	9100	11900	1820	2100	1680
Rhagodia candolleana	194	43	59	5.8	< 1	< 1	3.2	15	8700	17900	58000	37000	1280	4700	154

	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S	Al
Rhagodia crassifolia	105	19	43	5.3	< 1	< 1	2.3	15	5800	11400	45000	24000	2000	3900	65
Rhagodia parabolica	164	48	53	4.4	< 1	< 1	1.6	16	5300	6000	30000	27000	1370	3000	125
Rhagodia preissii	168	107	74	13	< 1	< 1	2.8	23	7100	8600	31000	24000	1350	3900	121
Rhagodia spinescens	270	64	61	4.6	< 1	< 1	2.2	21	5400	7800	67000	26000	1490	6600	210
Swainsona greyana	198	29	530	6.8	1.4	< 1	1.2	14	13300	8100	5400	14700	1640	3400	152
Viminaria juncea	145	23	154	2.2	< 1	< 1	1.6	9.4	3900	2900	18700	5300	590	2100	126
Minimum value	50	7	17	2	1		1	7	2100	1200	240	5300	510	1230	29
Maximum value	2100	290	530	19	5		23	114	38000	17900	82000	68000	3100	23000	1680
Average	224	42	103	6	2		3	19	9803	4541	21219	17763	1354	3306	180

Section 4

In vitro fermentability and rumen-modulating properties of Australian native shrubs

Introduction

Shrubs

The use of shrubs as fodder for livestock has been restricted due to their low biomass production, poor nutritive value and the anti-nutritive effects of plant secondary compounds (PSC). However, as issues such as Dryland salinity and decreasing rainfall threaten the viability of conventional crop and pasture species, there has been an increase in the adoption of native shrubs for on-farm use. The limitations of these shrubs would be offset by focusing on the positive effects they can have on landscapes, and their bioactive properties.

Native shrubs have developed survival and defence mechanisms and are well adapted to our soils and climate conditions and less prone to variation in rainfall, temperature and salinity. The value of shrubs may be, for example, to complement other pasture species, or supplementary feeding to extend the time of year that green feed is available. It has been observed that inclusion of tree leaves to a straw-based diet had beneficial effects on intake, digestibility and rumen fermentation (Raghuvansi et al., 2007). Shrubs are also important as stabilizers of ecosystem because of their resistance to adverse environmental conditions such as drought and salinity (Aich 1991). Selection programs can help to identify those species with the highest biomass production and nutritive value (Anonn. 2002).

Native shrubs produce (PSC) in response to stressors such as grazing, low soil fertility, drought, high temperatures and attack by microbes and insects (Mueller-Harvey 1999; Wallace 2004). These compounds can have bioactive properties that have the potential to alter intestinal microbial activity (Busquet et al. 2006) and control pathogens in the gut (Si et al. 2006; Voravuthikunchai et al. 2004). This has the potential to lead to production benefits similar to those achieved with other manipulating practices (e.g. in-feed antibiotics), which is important because of the growing pressure to stop the use of in-feed antibiotics and identify 'natural' feed additives. Studying the nutritive value of shrubs and their effect on rumen microbial fermentation could offer a better evaluation of their value in animal production systems and extend their use.

Fermentative properties of shrubs

The nutritive value of conventional feeds can be predicted using methods ranging from chemical analysis (OM, CP, NDF, ADF), simple *in vitro* digestion with pepsin and cellulase enzymes, to more sensitive methods such as near infrared reflectance spectroscopy (NIRS; (Landau et al. 2006). However, these methods cannot predict the interaction between the feed and the rumen microbial population, especially if the feed is complex and contains PSC. Therefore an additional method for forage evaluation should involve investigating their effect on rumen microbial populations and their activity. One such method (*In vitro* Fermentation Technique, IVFT) involves mimicking rumen fermentation: feed is incubated with a mixed rumen population (rumen fluid) in a sealed vessel, and the end-products of microbial fermentation (gas, volatile fatty acids, ammonia) and consequences (e.g. drop in pH) are

measured (Getachew et al. 2004; Theodorou et al. 1994). IVFT is useful for determining how feed value is affected by antinutritive factors, rumen modifiers and feed additives (Getachew et al. 2004). Recently, IVFT has been utilized to assess the potential of plants (Kamra et al. 2006; Soliva et al. 2007) and plant extracts as natural rumen modifiers (Busquet et al. 2006; Kamra et al. 2006; Newbold et al. 2004; Wallace 2004).

Manipulating fermentation

Nutrients consumed by ruminants are fermented by rumen microbes, producing gas, volatile fatty acids (VFA), and NH_3, providing animals with energy and (microbial) protein. However, fermentation also results in energy and dietary protein losses that can limit animal production and contaminate the environment.

Large amounts of gas are normally produced in the rumen during fermentation of feedstuff and eliminated by belching. The gas is mainly composed of carbon dioxide (45%) and methane (CH_4, 30%) (Clarke and Reid 1974). Methane represents a loss of 2–12% of the gross energy consumed by ruminants, and is a potent greenhouse gas (Beijer 1952; Johnson and Johnson 1995). Agriculture is the main contributor to methane emissions, with 2/3 coming from enteric fermentation (Moss et al. 2000). Fermentation of feeds in the rumen is the largest source of CH_4 from enteric fermentation (Johnson and Johnson 1995) and research efforts are therefore being directed towards manipulating rumen fermentation to inhibit CH_4 production and emission.

Volatile fatty acids are end products of ruminal fermentation that provide 70% of the ruminants energy requirements (Annison and Armstrong 1970). While acetate levels reflect cell wall fermentation, levels of propionate indicate fermentation of soluble carbohydrates (Soest 1982). In sheep, propionate produced in the rumen is used for glucose synthesis (Leng et al. 1967) and is associated with greater efficiency of energy utilisation (Armstrong and Blaxter 1957). Further, acetate and butyrate promote CH_4 production while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen (Moss et al. 2000). When the acetate:propionate ratio decreases, CH_4 production also declines and energy retention increases.

A portion of dietary protein provided to ruminants is lost in the rumen due to its microbial degradation to ammonia (NH_3). A portion of NH_3 can be reassimilated and utilized by the microbes for microbial protein synthesis, but it is of lower quality than the dietary protein, and the process is considered wasteful for the animal. Ruminal concentration of NH_3 is often used as an indicator of proteolysis by rumen microbes. Reduced NH_3 concentration in the rumen is therefore indicative of reduced proteolysis or increased microbial protein synthesis, both being marked as favourable processes. Apart from dietary loss of protein, release of NH_3 contributes to N excretion to the environment and pollution from animals.

Furthermore, there are ruminal disorders that occur as a consequence of microbial imbalances. A key microbial-related disorder in ruminants is lactic acidosis, which occurs after the ingestion of rapidly fermentable carbohydrates (typically grains), resulting in a microbial imbalance and accumulation of lactic acid in the rumen, and in cessation of rumen function (Telle and Preston 1971).

There are many management practices that are used to try to manipulate rumen fermentation in order to improve growth, feed efficiency and animal health and welfare. Antimicrobials are added to ruminant diets in intensive production because of their positive effects on feed efficiency, VFA production, reduction in proteolysis and CH_4, as well as control of ruminal disorders such as lactic acidosis and bloat, and inhibition of gut pathogens (Walton, 1977; Dennis *et al.*, 1981; Godfrey *et al.*, 1993; Fellner, *et al.*, 1997). While some have broad-spectrum activities (e.g. monensin), others, like virginiamycin, have more specific targets

(Nagaraja and Taylor 1987; Odongo et al. 2007). However, routine feeding of additives has its downfalls – apart from the cost and withholding periods - there is also the possibility of microbial adaptation and transmission of bacterial resistance to humans.

Synthetic additives are not commonly used in extensive production, mostly because of the cost of implementation, but also because of the somewhat limited effect in forage diets (Calsamiglia et al. 2007). However, extensive production systems still face some of the negative consequences of ruminal fermentation. For example, CH_4 production is greater when animals are fed poor diets, high in fibre (DeRamus et al. 2003; Johnson and Johnson 1995), and acetete:propionate ratios are greater in grazing than in concentrate-fed animals. Finally, animals introduced to lush pastures can be at risk of developing frothy bloat (Clarke and Reid 1974). Strategies to minimize CH_4 emissions from grazing ruminants are a priority in CH_4 mitigation protocols, while improving other factors (nutrient utilization) may offer the potential for more efficient use of marginally productive land.

Shrubs as potential rumen modulators

Managing microbial activity in the rumen to enhance productivity and animal welfare while meeting consumer demands still remains a task. Since the 1950s, nutritionists have sought chemical additives to decrease fermentation loses and increase useful end-products of ruminal fermentation. However, there is a need to find safe and natural modifiers, and plants containing PSC may be promising candidates for satisfying these needs. For example, feeding tannin- or saponin- containing plants results in decreased ruminal protein degradation, gas formation (especially CH_4), occurance of bloat and gastrointestinal numbers, and increased microbial protein synthesis (Carulla et al. 2005; Getachew et al. 2000; Waghorn 2003; Wallace et al. 1994). Flavonoid-rich plants (Broudiscou *et al.*, 2000), some essential oils (Mohammed *et al.*, 2004), plant extracts and common culinary spices (Bodas et al. 2007; Busquet et al. 2006; Garcia-Gonzalez et al. 2004; Soliva et al. 2007) can inhibit CH_4 production by the rumen microbes. *In vitro,* an increase in ruminal propionate was also observed with some plant extracts and common culinary spices (Busquet et al. 2005b; Busquet et al. 2006; Patra et al. 2006).

All these features may be valuable in addressing the needs of both the producer (cheap, robust and resilient fodder) and the increasing demand of consumers for high quality animal products obtained in production systems that are chemical free and have less impact on the environment.

Experimental outline

A preliminary investigation (**Experiment - Preliminary**) was conducted with 29 opportunistic samples from the Florasearch collection to obtain some initial data on *in vitro* fermentability (pH, gas, and VFA production), indication of rumen modulating potential (i.e. reduction of CH_4 production, and increased propionate production) of the plants that will be further examined and the suitability of the methodology.

Following this, another 127 plant samples were collected and fully examined for their *in vitro* fermentability (pH, gas, and VFA production) and rumen-modulating properties (reduction of CH_4 production, increased propionate production, and reduction of ammonia nitrogen release) (**Experiment - Fermentability**). To investigate rumen-modulating properties more specifically, selected plants were included as ethanolic extracts (**Experiment - Compounds**) and another set of plants was examined for the potential to reduce acidosis *in vitro* (**Experiment - Acidosis *In vitro* (Plants)**).

Experiment - Acidosis *In vitro* (*E. glabra* dose) was conduced to investigate the optimal dose for inclusion of the plant in an *in vivo* experiment: **Experiment - Acidosis *In vivo.***

Finally, two experiments were designed to help translate the effects observed *in vitro* to *in vivo*. **Experiment - Dose** was conducted with the five most prominent plants to examine the level of inclusion of each plant that would be needed to achieve an effect. **Experiment - Shrub Mixes *In vitro*** was undertaken to examine the fermentability of shrub mixes that were grazed *in situ* at Monarto in the summer of 2008.

Experiment - Preliminary

A total of 29 plants were examined in this part of the Enrich project. Plants were opportunistic samples collected in Florasearch from Waite and Murray Bridge, SA. The objective of this experiment was to get some preliminary data on the fermentability (gas production) and rumen-modulating potential (reduction of CH4 production) of the plants that will be used in Enrich. It was also conducted to examine and validate the suitability of the IVFT for this purpose.

Plant material

Plant material was collected in summer 2005/2006 from Waite and Murray Bridge, South Australia. Edible parts of the plants (leaf, stems 5 cm long) were collected and material was freeze-dried and ground to pass through a 1 mm screen. Material was stored at room temperature in sealed containers until analysis (Table 1).

Table 1. Plant names, sources, sampling conditions for plants used in the Experiment Preliminary

Botanical name	Source	Location	Sampling time	Plant age (years)	Growth Stage
Acacia pycnantha	Florasearch	Murray Bridge	winter 2006	2	r
Acacia saligna	Florasearch	Murray Bridge	winter 2006	2	v
Atriplex amnicola	Florasearch	Murray Bridge	winter 2006	1 to 2	r
Atriplex cinerea	Florasearch	Murray Bridge	winter 2006	1 to 2	r
Atriplex nummularia	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Atriplex rhagodioides	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Atriplex semibaccata	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Atriplex vesicaria	Florasearch	Murray Bridge	winter 2006	1 to 2	r
Chenopodium auricomum	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Chenopodium nitrariaceum	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Cullen australasicum	Florasearch	Waite	summer 2005	<1	f,p
Cullen pallidum	Florasearch	Waite	summer 2005	<1	f,p
Enchylaena tomentosa	Florasearch	Murray Bridge	winter 2006	1 to 2	r
Eremophila bignoniiflora	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Eremophila glabra	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Eremophila longifolia	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Eremophila maculata	Florasearch	Murray Bridge	winter 2006	1 to 2	r
Maireana convexa	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Maireana pyramidata	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Maireana sedifolia	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Medicago sativa	Florasearch	Waite	summer 2005	<1	f,p
Myoporum platycarpum	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Plantago lanceolata	Florasearch	Waite	summer 2005	<1	f,p
Rhagodia candolleana	Florasearch	Murray Bridge	winter 2006	1 to 2	f
Rhagodia crassifolia	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Rhagodia parabolica	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Rhagodia spinescens	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Templetonia retusa	Florasearch	Murray Bridge	winter 2006	1 to 2	v
Viminaria juncea	Florasearch	Murray Bridge	winter 2006	2	v

f – flower, p – pod, v – vegetative, r – reproductive (fruit)

In vitro *Fermentability Test (IVFT)*

The fermentability of plants was examined in an *in vitro* batch fermentation system designed to mimic the rumen environment (Busquet et al. 2006). One day prior to the experiment, 0.1 g of plant material was weighed into Belco tubes and transferred to anaerobic chamber (Coy anaerobic chamber; 80% N_2 : 10% CO_2 : 10% H_2) to expel the oxygen. On the day of the experiment, rumen fluid was collected 2 h after feeding from two fistulated sheep fed a lupin and oaten chaff-based diet. Rumen fluid was removed through the rumen cannula with a suction device via a plastic pipe fitted with a 1 mm metal screen to eliminate large feed particles. After collection, rumen fluid was pooled, transferred into an anaerobic chamber and buffered to pH 7.2 (McDougall 1948). Ten ml of this mix was dispensed into prepared Belco tubes. A negative control (buffered rumen fluid, NC), a positive control (buffered rumen fluid and 0.1 g of oaten chaff, PC), and an antibiotic control containing 20 μg of Rumensin 100 (10% monensin, Elanco, Advanced Feeds, Midvale, Australia) were included in the assay. The antibiotic treatment was used as a model for a rumen-modulating effect in the rumen. Each treatment and the controls were done in triplicate. Tubes were sealed with a rubber stopper, crimped and incubated for 24 h at 39°C with constant shaking at 50 rpm. At the end of the incubation period, tubes were placed in a water bath at 39°C and gas pressure was measured using a pressure transducer (Greisinger Electronic GmbH, Regenstauf, Germany).

Chemical Analyses

CH_4 concentrations in the headspace were then estimated using an infrared gas analyser (GA 2000, Geotechnical Instruments, UK) (Makkar and Vercoe 2007). Methanogenic potential of the plant was calculated and expressed as total CH_4 produced/g DM, taking into account methane-to-total gas produced (Soliva et al. 2007), Tubes were then opened, and 2 mL of the liquid phase was mixed with 1 mL of NaOH for VFA analysis, and the pH was measured in the remainder. VFA were analysed by GC, using Agilent 6890 Series GC with HP 6890 injector, FID detector and HP Chemstation software, Capillary column HP-FFAP, 30 m x 0.53 mm x 1.0 micron, Hydrogen was used as carrier gas at 6.6 mL per min, oven 240°C, injector 260°C, detector 265°C.

Statistical Analysis

Data were analysed by one-way ANOVA using JMP® software (SAS Institute Inc., USA). Treatment means were compared to the positive control, and the differences between means were considered significant at $P<0.05$.

Results and Discussion

As indicated by gas and VFA production during the 24h incubation period, the majority of plants had low fermentability, with only four plants having gas and VFA production similar to oaten chaff (Table 2). The gas and VFA production were generally in good correlation ($r^2 = 0.86$), but there were five plants where VFA were still high despite low gas production.

CH_4 was detected in the headspace of all treatments, with amounts ranging from 0.9 – 4.5% of total gases in the headspace (data not shown), or 4 – 43 mL/g DM (Table 2). When compared to the positive control, 16 produced significantly ($P<0.05$) less CH_4.

Based on the results of total gas and CH_4 production, plants were divided into four groups; 1) gas not reduced, CH_4 not reduced, 2) gas not reduced, CH_4 reduced, 3) gas reduced, CH_4 reduced, and 4) gas reduced, CH_4 not reduced (Table 2). There were four plants that did not affect gas or CH_4 production. Although these plants can be grazed, a production loss might be expected due to high CH_4 production. There were no plants that maintained gas production without reducing CH_4 production. However, there were plants where reduction in gas production was not more than 20% of the PC, but that had significant reductions in CH_4. In two of these plants (*Eremophila maculate* and *Rhagodia spinescens*) VFA concentrations also remained comparable to PC. Further investigation of these plants may indicate their suitability as fodder. Some other plants also reduced CH_4 production (*Acacia pycnantha, Acacia saligna, Eremophila glabra, Enchylaena tomentosa,* and *Maireana sedifolia*) but they also inhibited rumen fermentation severely. It is possible that these could be fed as a supplement or additive to control CH_4 emissions. Finally, seven plants that reduced gas production but failed to reduce CH_4 production would not be recommended for grazing.

Propionate produced at the expense of acetate was observed in two plants - *Eremophila maculata* and *Eremophila longifolia* (Table 2). In addition, they both had reduced CH_4 production, while gas was not inhibited by more than 15%, and VFA concentrations were comparable to the antibiotic treatment. These plants are already known to contain PSC with antimicrobial properties (Ndi et al. 2007), and it is possible that this effect was related to specific moderating effects of these antimicrobial compounds.

Table 2. Fermentation characteristics and rumen-modulating properties of plants examined *in vitro*. Species are divided into four groups based on their effects on total gas and methane production.

Treatment	Fermentability		Rumen-modulating properties	
	Gas (kPa)	VFA (mmol/L)	CH_4 (mL/g DM)	A : P
Rumen fluid only (NC)	14[b]	63[b]	0[b]	3.4[a]
Oaten chaff (PC)	83	114	31	2.9
Oaten chaff + Antibiotic (AB)	85	120		2.1[b]
Gas not reduced, CH4 not reduced				
Cullen australasicum	98[a]	120	43[a]	3.4[a]
Cullen pallidum	98[a]	122	41[a]	3.2[a]
Medicago sativa	101[a]	121	42[a]	3.4[a]
Plantago lanceolata	91[a]	105	33	3.6[a]
Gas reduced, CH4 reduced				
Acacia pycnantha	28[b]	69[b]	6[b]	3.2[a]
Acacia saligna	43[b]	72[b]	13[b]	3.5[a]
Atriplex rhagodioides	51[b]	87[b]	24[b]	3.8[a]
Enchylaena tomentosa	35[b]	86[b]	13[b]	4.3[a]
Eremophila glabra	31[b]	57[b]	4[b]	4.8[a]
Eremophila longifolia	74[b]	102[b]	23[b]	2.7[b]
Eremophila maculata	73[b]	115	19[b]	2.4[b]
Maireana sedifolia	23[b]	67[b]	7[b]	4.9[a]
Maireana convexa	54[b]	85[b]	26[b]	3.6[a]
Maireana pyramidata	44[b]	91[b]	17[b]	4.0[a]
Rhagodia candolleana	64[b]	101[b]	26[b]	3.8[a]
Rhagodia crassifolia	63[b]	103[b]	23[b]	3.8[a]
Rhagodia parabolica	50[b]	88[b]	19[b]	3.6[a]
Rhagodia spinescens	56[b]	108	23[b]	3.7[a]
Templetonia retusa	67[b]	93[b]	27[b]	3.1[a]
Viminaria juncea	46[b]	78[b]	16[b]	3.6[a]
Gas reduced, CH4 not reduced				
Atriplex amnicola	67[b]	109	30[a]	3.5[a]
Atriplex cinerea	75[b]	104[b]	40[a]	3.6[a]
Atriplex nummularia	73[b]	93[b]	43[a]	3.8[a]
Atriplex semibaccata	68[b]	102[b]	32	3.6[a]
Atriplex vesicaria	68[b]	99	36	4.0[a]
Chenopodium auricomum	73[b]	106	40[a]	3.7[a]
Chenopodium nitrariaceum	62[b]	92[b]	34	4.2[a]
Eremophila bignoniiflora	83[b]	111	33	3.3[a]
Myoporum platycarpum	75[b]	97[b]	31	3.1[a]

a - significantly ($P<0.05$) higher than PC; b - significantly lower ($P<0.05$) than PC

Experiment - Fermentability

Material for these experiments were collected in during the period winter 2007 – summer 2008 from various sites in SA and WA (Table 3), in a similar manner as described previously. Material that could not be obtained from Enrich sites was again sourced from Florasearch.

Table 3. Plant names, sources, sampling conditions, and any known bioactive compounds/properties for plants used in the Experiment Fermentability

Botanical name	Source	Location	Sampling time	Plant age (years)	Growth Stage	Bioactive compound or activity (if known)
Abutilon otocarpum	Enrich	Monarto	autumn 2007	<1	v	
Acacia iteaphylla	Enrich	Monarto	autumn 2007	<1	v	saponin
Acacia iteaphylla	Enrich	Monarto	summer 2007	1-2	v	saponin
Acacia kempeana	Enrich	Mt Finke	autumn 2007		v	antibacterial
Acacia ligulata	Enrich	Monarto	autumn 2007	<1	v	medicinal
Acacia ligulata	Enrich	Monarto	summer 2007	1-2	v	medicinal
Acacia loderi	Enrich	Monarto	autumn 2007	<1	v	
Acacia loderi	Enrich	Monarto	summer 2007	1-2	v	
Acacia minyura	Enrich	Yellabinna	autumn 2007		v	
Acacia myrtifolia	Enrich	Monarto	autumn 2007	<1	v	triterpene
Acacia neriifolia	Enrich	Monarto	autumn 2007	<1	v	
Acacia neriifolia	Enrich	Monarto	summer 2007	1-2	v	
Acacia pycnantha	Enrich	Monarto	autumn 2007	<1	v	tannins
Acacia pycnantha	Enrich	Monarto	winter 2007	<1	v	tannins
Acacia pycnantha	Enrich	Monarto	summer 2007	1-2	v	tannins
Acacia saligna	Enrich	Monarto	winter 2007	<1	v	tannins, myricetin
Alectryon oleifolius	Enrich	Arboretum	autumn 2007	31	r	cyanide
Atalaya hemiglauca	Enrich	Arboretum	autumn 2007	48	v	
Atriplex amnicola	Enrich	Monarto	winter 2007	<1	r,f	oxalates
Atriplex cinerea	Enrich	Monarto	winter 2007	<1	v	oxalates
Atriplex isatidea	Enrich	Monarto	autumn 2007	<1	v	oxalates
Atriplex nummularia	Enrich	Monarto	autumn 2007	<1	v	oxalates, antifungal
Atriplex paludosa	Enrich	Monarto	autumn 2007	<1	v	oxalates, antifungal
Atriplex rhagodioides	Enrich	Monarto	autumn 2007	<1	v	oxalates
Atriplex rhagodioides	Enrich	Monarto	summer 2007	1-2	v	oxalates
Atriplex semibaccata	Enrich	Monarto	autumn 2007	<1	r	oxalates, antifungal
Atriplex vesicaria	Enrich	Monarto	autumn 2007	<1	r	oxalates
Brachychiton gregorii	Enrich	Arboretum	autumn 2007	60	v	
Brachychiton populneus	Enrich	Arboretum	autumn 2007	77	r	medicinal
Brachycome ciliaris	Enrich	Monarto	summer 2007	1-2	r,f	
Calotis scapigera	Florasearch	Waite	summer 2005	1-2	f,p	
Capparis mitchellii	Enrich	Arboretum	autumn 2007	63	v	
Chameacytisis proliferus	Florasearch	Waite	summer 2005	>3	f,p	
Chameacytisis proliferus	Enrich	Monarto	winter 2007	<1	v	tannins, alkaloids
Chenopodium auricomum	Enrich	Millers Creek	autumn 2007		r	
Chenopodium gaudichaudianum	Enrich	Monarto	winter 2007	<1	r,f	
Chenopodium nitrariaceum	Enrich	Monarto	autumn 2007	<1	r	
Cichorium intybus	Florasearch	Turretfield	summer 2005	<1	f,p	antimicrobial, antinematodal
Colutea abyssinica	Enrich	Waite	winter 2007	<1	v	
Convolvulus remotus	Enrich	Monarto	autumn 2007	<1	v	
Cratystylis conocephala	Enrich	Mt Finke	autumn 2007		r	
Cullen australasicum	Enrich	Monarto	autumn 2007	<1	v	
Cullen australasicum (young)	Florasearch	Waite	summer 2005	<1	f	
Cullen australasicum (mature)	Florasearch	Waite	summer 2005	1-2	f,p	
Cullen cinereum	Florasearch	Waite	summer 2005	<1	f,p	
Cullen cinereum	Enrich	Monarto	winter 2007	<1	v	
Cullen discolor	Florasearch	Waite	summer 2005	<1	f,p	
Cullen pallidum	Enrich	Monarto	winter 2007	<1	v	
Cullen parvum	Florasearch	Waite	summer 2005	<1	p	
Cullen patens	Florasearch	Waite	summer 2005	<1	p	

Cullen tenax (a)	Florasearch	Waite	summer 2005	<1	f,p	tannins
Cullen tenax (b)	Florasearch	Waite	summer 2005	<1	f,p	tannins
Dorycnium hirsutum	Enrich	Monarto	winter 2007	<1	v	
Enchylaena tomentosa	Enrich	Monarto	winter 2007	<1	r r	
Enchylaena tomentosa	Enrich	Monarto	summer 2007	1-2	r,f	
Eremophila alternifolia	Enrich	Yellabinna	autumn 2007		v	antibacterial
Eremophila bignoniiflora	Enrich	Monarto	winter 2007	<1	v	medicinal
Eremophila glabra	Enrich	Monarto	autumn 2007	<1	v	antibacterial
Eremophila glabra	Enrich	Monarto	summer 2007	1-2	r	antibacterial
Eremophila longifolia	Enrich	Monarto	autumn 2007	<1	v	antibacterial
Eremophila longifolia	Enrich	Monarto	summer 2007	1-2	v	antibacterial
Eremophila maculata	Enrich	Monarto	winter 2007	<1	v	antibacterial
Geijera parviflora	Enrich	Arboretum	summer 2007	66	r	medicinal
Glycine canescens	Florasearch	Waite	summer 2005	1-2	f,p	
Glycine canescens	Enrich	Monarto	winter 2007	<1	r,f	
Glycine clandestina	Florasearch	Waite	autumn 2006	1-2	p	
Glycine clandestina	Enrich	Monarto	winter 2007	<1	v	
Glycine tabacina	Florasearch	Waite	autumn 2006	1-2	p	tannins
Glycine tabacina	Enrich	Monarto	winter 2007	<1	v	tannins
Indigofera australis	Florasearch	Waite	summer 2005	1-2	r	indospicine
Jasminium didymium	Enrich	Telowie Gorge	autumn 2007		r	
Jasminium didymium	Enrich	Telowie Gorge	summer 2007		r	
Kennedia eximia	Enrich	Monarto	autumn 2007	<1	v	antibacterial
Kennedia macrophylla	Florasearch	Waite	autumn 2006	1-2	v	
Kennedia nigricans	Florasearch	Waite	autumn 2006	1-2	v	
Kennedia nigricans	Enrich	Monarto	winter 2007	<1	v	
Kennedia nigricans	Enrich	Monarto	summer 2007	1-2	v	
Kennedia prorepens	Enrich	Monarto	summer 2007	1-2	r,f	tannins, antibacterial
Kennedia prorepens	Enrich	Monarto	autumn 2007	<1	v	tannins, antibacterial
Kennedia prorepens	Florasearch	Waite	autumn 2006	1-2	r	tannins, antibacterial
Kennedia prostrata	Florasearch	Waite	summer 2005	1-2	r	
Kennedia prostrata	Enrich	Monarto	winter 2007	<1	r,f	
Kennedia rubicunda	Enrich	Monarto	autumn 2007	<1	v	
Kennedia rubicunda	Florasearch	Waite	autumn 2006	1-2	v	
Lavatera plebeia	Enrich	Monarto	winter 2007	<1	v	medicinal
Lavatera plebeia	Florasearch	Waite	summer 2005	1-2	f,p	medicinal
Lomandra longifolia	Florasearch	Waite	summer 2005	1-2	v	
Lotus australis	Enrich	Monarto	autumn 2007	<1	v	tannins
Lotus australis	Enrich	Monarto	summer 2007	1-2	r,f	tannins
Maireana convexa	Enrich	Monarto	autumn 2007	<1	v	
Maireana sedifolia	Enrich	Monarto	winter 2007	<1	v	
Maireana astrotricha	Enrich	Monarto	summer 2007	1-2	v	
Maireana brevifolia	Enrich	Monarto	autumn 2007	<1	v	
Maireana georgei	Enrich	Monarto	autumn 2007	<1	v	
Maireana georgei	Enrich	Monarto	summer 2007	1-2	v	
Maireana planifolia	Enrich	Monarto	summer 2007	1-2	v	
Maireana pyramidata	Enrich	Monarto	winter 2007	<1	v	
Maireana tomentosa	Enrich	Monarto	winter 2007	<1	v	
Medicago arborea	Enrich	Monarto	winter 2007	<1	v	
Medicago citrina	Enrich	Monarto	winter 2007	<1	v	
Medicago citrina	Florasearch	Turretfield	summer 2005	<1	v	
Medicago sativa	Enrich	Monarto	winter 2007	<1	r	tannins
Medicago sativa	Florasearch	Waite	autumn 2006	<1		tannins
Medicago strasseri	Enrich	Monarto	winter 2007	<1	v	
Medicago strasseri	Florasearch	Turretfield	summer 2005	<1	v	
Nitraria billardieri	Florasearch	Waite	summer 2005	1-2	v	antifungal
Pterocaulon sphacelatum	Enrich	Monarto	autumn 2007	<1	r	antiviral
Pterocaulon sphacelatum	Florasearch	Waite	autumn 2006	<1	v	antiviral

Rhagodia candolleana	Enrich	Monarto	autumn 2007	<1	v	
Rhagodia candolleana	Enrich	Monarto	summer 2007	1-2	v	
Rhagodia crassifolia	Enrich	Monarto	winter 2007	<1	v	
Rhagodia parabolica	Enrich	Monarto	winter 2007	<1	v	
Rhagodia preissii	Enrich	Monarto	winter 2007	<1	v	
Rhagodia spinescens	Enrich	Monarto	winter 2007	<1	v	
Senna barclayana	Florasearch	Waite	summer 2005	1-2	f,p	medicinal
Senna planitiicola	Florasearch	Waite	summer 2005	1-2	f	
Sida corrugata	Enrich	Tarcoola	autumn 2007		r	toxin?
Sida intricata	Enrich	Morgan	summer 2007		r,f	
Swainsona galegifolia	Florasearch	Waite	summer 2005	1-2	f,p	PSC
Swainsona greyana Lindl.	Enrich	Monarto	autumn 2007	<1	r	toxic?
Swainsona greyana Lindl.	Florasearch	Waite	summer 2005	<1	f	toxic?
Swainsona stipularis	Enrich	Waite	winter 2007	<1	v	
Templetonia retusa	Florasearch	Waite	summer 2005	1-2	v	medicinal
Teucrium racemosum	Enrich	Ingomar	autumn 2007		r	acaricidal
Viminaria juncea	Enrich	Monarto	winter 2007	<1	v	medicinal
Viminaria juncea	Florasearch	Waite	summer 2005	1-2	f,p	medicinal

f – flower, p – pod, v – vegetative, r – reproductive (fruit)

Materials and Methods

The testing was conducted in the same manner as described for Experiment -Preliminary, with the following modifications: In addition to oaten chaff (OC) as PC, another diet (MD) based on oaten chaff supplemented with lupins and vitamins (1 kg oaten chaff + 250 g lupins + 25 vitamin mix) was also included in the assay to represent a more complex sheep diet. At the end of the incubation period, and after measuring gas pressure, 5 mL of headspace gas was transferred to an exetainer tube (Labco, UK) for subsequent CH_4 analysis by GC (instead of by IR). Methane concentration in the gas sample was determined using a Varian 3600 fitted with a 60 m HP-1 capillary column using helium as carrier gas. The injector temperature was at 190°C, detector temperature was 200°C. The column was held at a constant temperature (isothermal) at 37°C during analysis. In addition to measuring VFA, a 2 mL sample was collected for NH_3 analysis and acidified with 0.5 mL of 2M HCl. The NH_3 in the fermentation fluid was determined using UV Method and Boehringer Mannheim Test kit 1112732 on Roche Cobas Mira S autoanalyser. The concentrations of NH_3 were corrected for protein content of each individual plant, and expressed as mg of NH_3 produced per g of protein supplied, and this was used as an indicator of proteolysis in the system.

Results

Plants were fermented at a different rate, producing variable amounts of gas, VFA, CH_4 and NH_3 (Table 4). More than half the plants had fermentability (gas or VFA) that was similar to the positive controls (OC and MD). Addition of monensin did not affect the fermentation. There were 10 plants that produced significantly more gas than PC, with five of these (two Cullen, two Medicago and one Swansonia species) also having significantly higher VFA concentrations.

Plants were further divided into four groups, based on their combined effect on fermentation and CH_4. There were 21 plants that did not reduce fermentation (as judged by gas or VFA), but had lower CH_4; 53 that reduced neither; 29 that reduced both; and 16 that reduced fermentation and not CH_4. There were 22 plants that had CH_4 values more than 50% lower

than the OC treatment. The plants that were the least methanogenic were *Cullen australasicum* (mature), *Jasminium didymium* and *Acacia loderi.*

All plants had lower NH_3 values than PC and AB. Plants that had the most prominent effect were also those that reduced overall fermentation. However, there were several plants that had very low NH_3, without affecting ferementation, ie *Eremophila longifolia*, *Brachychiton populneus, Eremophila glabra and Chenopodium auricomum*.

There were 12 plants that promoted propionate production (as judged by lower A:P ratio), with the most prominent effect (below 3.0) observed with *Eremophila maculata, Eremophila glabra, Cullen discolor, Jasminium didymium, Lomandra longifolia Swainsonia greyana Acacia myrtifolia,* with the first four also significantly producing less CH_4, but there was no overall strong correlation ($r^2 = 0.10$) between CH_4 and A:P ratio in this system.

Table 4. Fermentation characteristics and rumen-modulating properties of plants examined *in vitro*

Treatment	Site & season	Fermentability			Rumen-modulating		
		pH	Gas (kPa)	VFA (mmol/L)	CH_4 (mL/g DM)	NH_3 (mg/g CP)	A:P
Rumen fluid only (NC)		7.0 [a]	26 [b]	62 [b]	18 [b]	0 [b]	3.8 [a]
Oaten chaff (PC)		6.1	84	92	45	384	3.3
Mixed diet (MD)		6.2	92	90	44	379	3.6 [a]
Oaten chaff + monensin (AB)		6.1	86	97	tba	411	2.1 [b]
Fermentation not reduced, CH_4 reduced							
Templetonia retusa	Waite summer '05	6.3 [a]	73	101 [a]	34 [b]	227 [b]	3.1
Eremophila longifolia	Monarto autumn '07	6.4 [a]	84	96	21 [b]	50 [b]	3.1
Cullen australasicum (mature)	Waite summer '05	6.3 [a]	92	115 [a]	4 [b]	184 [b]	3.3
Enchylaena tomentosa	Monarto summer '07	6.6 [a]	79	68 [b]	24 [b]	95 [b]	3.5
Maireana astrotricha	Monarto summer '07	6.7 [a]	83	68 [b]	33 [b]	tba	4.1 [a]
Kennedia rubicunda	Monarto autumn '07	6.5 [a]	48 [b]	90	28 [b]	112 [b]	3.6 [a]
Capparis mitchellii	Arboretum autumn '07	6.5 [a]	53 [b]	92	26 [b]	120 [b]	4.3 [a]
Brachychiton populneus	Arboretum autumn '07	6.5 [a]	53 [b]	89	30 [b]	44 [b]	4.4 [a]
Rhagodia candolleana	Monarto autumn '07	6.8 [a]	53 [b]	84	18 [b]	84 [b]	5.3 [a]
Cullen discolor	Waite summer '05	6.5 [a]	55 [b]	102 [a]	26 [b]	170 [b]	2.7 [b]
Kennedia prostrata	Monarto autumn '07	6.6 [a]	56 [b]	90	30 [b]	102 [b]	3.3
Eremophila glabra	Monarto autumn '07	6.6 [a]	57 [b]	85	13 [b]	43 [b]	2.4 [b]
Kennedia nigricans	Monarto autumn '07	6.4 [a]	58 [b]	110 [a]	27 [b]	117 [b]	3.6 [a]
Nitraria billardieri	Waite summer '05	6.4 [a]	62 [b]	89	27 [b]	113 [b]	3.2
Atriplex vesicaria	Monarto autumn '07	6.8 [a]	63 [b]	86	28 [b]	124 [b]	4.8 [a]
Abutilon otocarpum	Monarto autumn '07	6.6 [a]	64 [b]	91	28 [b]	96 [b]	4.2 [a]
Chenopodium auricomum	Millers Creek autumn '07	6.5 [a]	64 [b]	94	27 [b]	54 [b]	4.4 [a]
Chenopodium nitrariaceum	Monarto autumn '07	6.8 [a]	68 [b]	89	28 [b]	96 [b]	4.1 [a]
Teucrium racemosum	Ingomar autumn '07	6.3 [a]	69 [b]	104 [a]	30 [b]	tba	3.8 [a]
Eremophila alternifolia	Yellabinna autumn '07	6.1	70 [b]	102 [a]	28 [b]	tba	4.3 [a]
Pterocaulon sphacelatum	Waite summer '05	6.5 [a]	70 [b]	86	17 [b]	83 [b]	4.9 [a]
Fermentation not reduced, CH_4 not reduced							
Pterocaulon sphacelatum	Monarto autumn '07	6.4 [a]	74	104 [a]	56 [a]	132 [b]	3.9 [a]
Calotis scapigera	Waite summer '05	6.1	76	104 [a]	47	155 [b]	3.3
Geijera parviflora	Arboretum summer '07	6.2	80	87	78 [a]	tba	4.1 [a]
Medicago sativa	Monarto autumn '07	6.4 [a]	82	86	53	166 [b]	3.2
Lavatera plebeia	Monarto autumn '07	6.3 [a]	83	89	49	177 [b]	3.5
Cullen parvum	Waite summer '05	6.1	83	126 [a]	39	219 [b]	3.3
Cullen patens	Waite summer '05	6.2	83	121 [a]	53	208 [b]	3.3
Swainsona stipularis	Monarto autumn '07	5.9	84	88	49	153 [b]	3.2
Chameacytisis proliferus	Monarto autumn '07	5.8 [b]	84	95	39	137 [b]	3.4
Senna planitiicola	Waite summer '05	6.3 [a]	85	113 [a]	38	166 [b]	3.0 [b]
Convolvulus remotus	Monarto autumn '07	6.5 [a]	85	98	46	102 [b]	3.8 [a]
Cullen pallidum	Monarto autumn '07	6.1	85	94	42	165 [b]	3.1
Cullen tenax (B)	Waite summer '05	6.2	86	118 [a]	52	170 [b]	3.2
Swainsona greyana	Monarto autumn '07	6.4 [a]	86	106 [a]	44	102 [b]	3.2
Maireana georgei	Monarto summer '07	6.8 [a]	86	85	36	128 [b]	3.4 [a]
Glycine canescens	Waite summer '05	6.1	87	86	59 [a]	172 [b]	3.1
Cullen australasicum (young)	Waite summer '05	6.3 [a]	88	113 [a]	48	172 [b]	3.3

Medicago sativa	Waite summer '05	6.3 [a]	88	87	56 [a]	138 [b]	3.3
Glycine canescens	Monarto autumn '07	6.3 [a]	88	106 [a]	48	193 [b]	3.0 [b]
Glycine clandestina	Monarto autumn '07	6.1	89	102 [a]	47	76 [b]	3.2
Glycine tabacina	Waite summer '05	6.1	90	85	53	241 [b]	3.4
Chameacytisis proliferus	Waite summer '05	6.1	90	115 [a]	46	155 [b]	3.4
Senna barclayana	Waite summer '05	6.3 [a]	91	111 [a]	47	160 [b]	3.0 [b]
Colutea abyssinica	Monarto autumn '07	5.9	92	88	35	159 [b]	3.3
Glycine clandestina	Waite summer '05	6.0	92	86	67 [a]	152 [b]	3.2
Medicago strasseri	Turretfield summer '05	5.7 [b]	93	89	68 [a]	120 [b]	3.3
Swainsona galegifolia	Waite summer '05	6.0	93	117 [a]	48	228 [b]	3.0 [b]
Medicago arborea	Monarto autumn '07	5.9	94	86	48	127 [b]	3.2
Sida intricata	Morgan summer '07	6.3 [a]	94	87	36	tba	3.8 [a]
Cullen cinereum	Waite summer '05	5.9	95 [a]	90	59 [a]	149 [b]	3.3
Medicago strasseri	Monarto autumn '07	6.2	96 [a]	116 [a]	54	158 [b]	3.2
Cullen cinereum	Monarto autumn '07	6.2	96 [a]	120 [a]	50	203 [b]	3.1
Swainsonia greyana	Waite summer '05	6.0	97 [a]	121 [a]	70 [a]	163 [b]	2.9 [b]
Medicago citrina	Turretfield summer '05	5.4 [b]	97 [a]	93	72 [a]	91 [b]	3.1
Cullen australasicum (young)	Monarto autumn '07	6.4 [a]	98 [a]	106 [a]	37	116 [b]	3.5
Atriplex rhagodioides	Monarto summer '07	6.5 [a]	99 [a]	90	47	162 [b]	3.5
Rhagodia candolleana	Monarto summer '07	6.4 [a]	101 [a]	84	35	179 [b]	3.6 [a]
Medicago citrina	Monarto autumn '07	6.0	102 [a]	117 [a]	56 [a]	185 [b]	3.0
Acacia ligulata	Monarto summer '07	6.3 [a]	73	66 [b]	40	72 [b]	3.4
Chenopodium gaudichaudianum	Monarto autumn '07	6.5 [a]	80	77 [b]	63 [a]	159 [b]	3.2
Atriplex amnicola	Monarto autumn '07	6.6 [a]	81	84 [b]	84 [a]	172 [b]	3.5
Maireana planifolia	Monarto summer '07	6.7 [a]	85	69 [b]	44	tba	3.9 [a]
Glycine tabacina	Monarto autumn '07	6.1	85	81 [b]	45	169 [b]	3.4
Eremophila bignoniiflora	Monarto autumn '07	5.9 [b]	86	80 [b]	48	149 [b]	3.2
Brachycome ciliaris	Monarto summer '07	6.0	92	80 [b]	43	tba	3.5 [a]
Convolvulus remotus	Monarto summer '07	6.0	102 [a]	81 [b]	49	78 [b]	3.7 [a]
Acacia myrtifolia	Monarto autumn '07	6.6 [a]	55 [b]	84	45	28 [b]	2.9 [b]
Atriplex rhagodioides	Monarto autumn '07	6.7 [a]	57 [b]	86	39	119 [b]	5.0 [a]
Indigofera australis	Waite summer '05	6.3 [a]	64 [b]	110 [a]	50	82 [b]	3.5
Sida corrugata	Tarcoola autumn '07	6.3 [a]	64 [b]	98	36	tba	4.4 [a]
Cullen tenax (A)	Waite summer '05	6.2	71 [b]	99	45	141 [b]	3.0
Atriplex semibaccata	Monarto autumn '07	6.7 [a]	71 [b]	86	40	102 [b]	4.3 [a]
Kennedia prostrata	Waite summer '05	5.7 [b]	73 [b]	85	35	41 [b]	3.2
Fermentation reduced, CH_4 reduced							
Alectryon oleifolius	Arboretum autumn '07	6.8 [a]	20 [b]	67 [b]	18 [b]	48 [b]	4.3 [a]
Acacia loderi	Monarto autumn '07	6.9 [a]	21 [b]	60 [b]	5 [b]	36 [b]	3.6 [a]
Acacia pycnantha	Monarto autumn '07	7.0 [a]	25 [b]	65 [b]	7 [b]	45 [b]	3.9 [a]
Jasminium didymum	Telowie Gorge autumn '07	6.3 [a]	27 [b]	81 [b]	4 [b]	tba	2.3 [b]
Acacia neriifolia	Monarto autumn '07	6.8 [a]	28 [b]	63 [b]	12 [b]	30 [b]	3.9 [a]
Brachychiton gregorii	Arboretum autumn '07	6.7 [a]	32 [b]	75 [b]	16 [b]	58 [b]	4.3 [a]
Acacia iteaphylla	Monarto autumn '07	6.9 [a]	34 [b]	66 [b]	12 [b]	43 [b]	4.6 [a]
Acacia pycnantha	Monarto autumn '07	6.4 [a]	34 [b]	58 [b]	19 [b]	84 [b]	3.2
Kennedia eximia	Monarto autumn '07	6.6 [a]	36 [b]	76 [b]	25 [b]	20 [b]	3.9 [a]
Atalaya hemiglauca	Arboretum autumn '07	6.9 [a]	37 [b]	74 [b]	17 [b]	53 [b]	4.2 [a]
Enchylaena tomentosa	Monarto autumn '07	6.7 [a]	39 [b]	59 [b]	25 [b]	97 [b]	3.6 [a]
Acacia minyura	Yellabinna autumn '07	6.6 [a]	39 [b]	83 [b]	13 [b]	tba	4.1 [a]

Cratystylis conocephala	Mt Finke autumn '07	6.6 [a]	40 [b]	83 [b]	24 [b]	tba	4.8 [a]
Lotus australis	Monarto autumn '07	6.8 [a]	40 [b]	76 [b]	8 [b]	26 [b]	4.0 [a]
Acacia kempeana	Mt Finke autumn '07	6.5 [a]	41 [b]	83 [b]	26 [b]	tba	4.2 [a]
Maireana brevifolia	Monarto autumn '07	6.9 [a]	42 [b]	75 [b]	20 [b]	72 [b]	4.3 [a]
Maireana convexa	Monarto autumn '07	6.8 [a]	45 [b]	72 [b]	21 [b]	67 [b]	4.4 [a]
Kennedia prorepens	Monarto autumn '07	6.5 [a]	45 [b]	75 [b]	26 [b]	106 [b]	3.2
Acacia saligna	Monarto autumn '07	6.2	47 [b]	70 [b]	25 [b]	106 [b]	3.7 [a]
Acacia ligulata	Monarto autumn '07	6.8 [a]	47 [b]	73 [b]	16 [b]	67 [b]	4.3 [a]
Rhagodia preissii	Monarto autumn '07	6.5 [a]	50 [b]	78 [b]	19 [b]	151 [b]	3.7 [a]
Kennedia rubicunda	Waite summer '05	6.7 [a]	53 [b]	77 [b]	30 [b]	41 [b]	4.4 [a]
Lomandra longifolia	Waite summer '05	6.4 [a]	53 [b]	74 [b]	33 [b]	271 [b]	2.7 [b]
Kennedia prorepens	Waite summer '05	6.6 [a]	56 [b]	79 [b]	18 [b]	26 [b]	3.9 [a]
Cichorium intybus	Turretfield summer '05	6.5 [a]	58 [b]	79 [b]	27 [b]	207 [b]	3.5
Lavatera plebeia (A)	Waite summer '05	6.6 [a]	64 [b]	80 [b]	30 [b]	117 [b]	3.6 [a]
Maireana georgei	Monarto autumn '07	6.8 [a]	67 [b]	83 [b]	32 [b]	109 [b]	4.1 [a]
Rhagodia parabolica	Monarto autumn '07	6.2	68 [b]	76 [b]	33 [b]	142 [b]	3.3
Eremophila maculata	Monarto autumn '07	6.0	72 [b]	77 [b]	31 [b]	119 [b]	2.3 [b]
Fermentation reduced, CH_4 not reduced							
Viminaria juncea	Monarto autumn '07	6.5 [a]	45 [b]	61 [b]	35	137 [b]	3.3
Dorycnium hirsutum	Monarto autumn '07	6.2	51 [b]	67 [b]	35	57 [b]	3.6 [a]
Kennedia macrophylla	Waite summer '05	6.4 [a]	51 [b]	71 [b]	42	103 [b]	3.3
Atriplex paludosa	Monarto autumn '07	6.8 [a]	53 [b]	83 [b]	46	84 [b]	4.4 [a]
Maireana sedifolia	Monarto autumn '07	6.7 [a]	55 [b]	77 [b]	34	144 [b]	3.7 [a]
Kennedia nigricans	Waite summer '05	6	59 [b]	72 [b]	38	49 [b]	3.8 [a]
Jasminium didymum	Telowie Gorge summer '07	6.5 [a]	60 [b]	65 [b]	41	tba	2.7 [b]
Atriplex isatidea	Monarto autumn '07	6.8 [a]	62 [b]	84 [b]	38	93 [b]	4.2 [a]
Rhagodia spinescens	Monarto autumn '07	6.6 [a]	63 [b]	77 [b]	45	149 [b]	3.7 [a]
Atriplex nummularia	Monarto autumn '07	6.9 [a]	66 [b]	80 [b]	36	79 [b]	4.5 [a]
Viminaria juncea	Waite summer '05	6	66 [b]	74 [b]	37	166 [b]	3.6 [a]
Lavatera plebeia (B)	Waite summer '05	6.5 [a]	68 [b]	82 [b]	51	136 [b]	3.5
Maireana pyramidata	Monarto autumn '07	6.8 [a]	68 [b]	79 [b]	43	147 [b]	3.3
Maireana tomentosa	Monarto autumn '07	6.7 [a]	68 [b]	72 [b]	48	168 [b]	3.7 [a]
Rhagodia crassifolia	Monarto autumn '07	6.4 [a]	71 [b]	80 [b]	45	147 [b]	4.0 [a]
Atriplex cinerea	Monarto autumn '07	6.7 [a]	72 [b]	74 [b]	62 [a]	222 [b]	3.6 [a]

a - significantly ($P<0.05$) higher than PC; b - significantly lower ($P<0.05$) than PC

There was some variability observed between the plants depending on location, the sampling site (at the same location), season, and age of the plant (Table 5). The most prominent difference was observed with mature *Cullen australasicum,* which was more potent in reducing CH_4 than young plant. A similar difference was observed with seasonal variation in *Jasminium didymium*: plant material collected in autumn was more potent in reducing CH_4 than material from the same plant collected in summer. While *Pterocaulon sphacelatum* and *Cullen australasicum* from Monarto in autumn were more methanogenic than the same species collected from Waite in summer, the opposite was observed with *Kennedia nigricans*, however these were not as prominent effects as those of season and maturity.

Table 5. Examples of the effect of plant age, sampling site, season and plant location on variability in rumen modulating properties (data extracted from Table 4).

Botanical name	Site & season	CH_4 (ml/g DM)	NH_3 (mg/g CP)	A:P
Different age				
Cullen australasicum (young)	Waite summer '05	48	172	3.3
Cullen australasicum (mature)	Waite summer '05	4	184	3.3
Different season				
Jasminium didymum	Telowie Gorge summer'07	41	tba	2.7
Jasminium didymum	Telowie Gorge autumn '07	4	tba	2.3
Different location and season				
Kennedia nigricans	Waite summer '05	38	49	3.8
Kennedia nigricans	Monarto autumn '07	27	117	3.6
Pterocaulon sphacelatum	Waite summer '05	17	83	4.9
Pterocaulon sphacelatum	Monarto autumn '07	56	132	3.9
Cullen australasicum (young)	Waite summer '05	48	172	3.3
Cullen australasicum (young)	Monarto autumn '07	37	116	3.5
Different sampling site (same location)				
Cullen tenax (A)	Waite summer '05	45	141	3.0
Cullen tenax (B)	Waite summer '05	52	170	3.2
Lavatera plebeia (A)	Waite summer '05	30	117	3.6
Lavatera plebeia (B)	Waite summer '05	51	136	3.5

Discussion

Using IVFT, we demonstrated that there is a wide range in the effects of shrubs on rumen fermentation, and that some shrubs can have fermentability comparable to common feeds (i.e. oaten chaff). We also demonstrated that some shrubs could have advantageous rumen fermentation profiles, indicative of rumen-modulating properties. While some of the plants with these properties are the ones with well-established bioactivity (i.e. *Eremophilas*), for others this is the first report on their bioactivity. Of special interest for further investigation are the plants that did not inhibit rumen fermentation (gas, VFA), while exhibiting multiple rumen-modulating properties (reduced methane, reduced proteolysis, increased propionate) such as *Cullen discolor* and *Eremophila glabra*, or those having the most significant effect on methane reduction, such as *Cullen australasicum* (mature). There were also shrubs such as *Jasminium didymum* and *Eremophila maculata* that reduced rumen fermentation, but had profound effects on methane and A:P, and could be considered as dietary supplements. It is also possible to select for plant accessions with less detrimental effects –i.e. *Eremophila maculata* from Murray Bridge (Experiment Preliminary) did not affect VFA, but maintained low CH_4 and A:P.

Grazing saltbush is associated with higher methane production (Mayberry et al. 2007), and not surprisingly, the most methanogenic plant in our study was *Atriplex amnicola*. However, in our study only two out of nine saltbush species tested had significantly higher values of CH_4 than oaten chaff, and one (*Atriplex vesicaria*) even had lower CH_4 values than oaten chaff. Other groups that were also highly methanogenic were *Cullen*, *Medicago* and *Glycine*.

These leguminous plants contain high levels of tannins, which should reduce CH_4 production, but only a few species demonstrated this effect. This underlines the need for careful selection of a particular species and accessions.

The values obtained in our study align with other data in the literature using similar systems for *in vitro* testing (Busquet et al. 2006; Kamra et al. 2006; Patra et al. 2006; Soliva et al. 2007). In relation to particular plants, Soliva et al (2007) found that *Acacia* species produced between 5 and 18 mL of CH_4 per gram of DM in an *in vitro* incubation, while Acacia species in the current study produced between 5 and 45 mL of CH_4 per gram of DM, although we examined a different range of species. Furthermore, the VFA concentrations and A:P also had a similar range of values for these species, but NH_3 concentrations were lower than those reported by Soliva et al (2007), and it is possible that the particular range of *Acacia* species tested in our study had a more protective effect against proteolysis.

Tannins are very potent protectors of proteolysis in the rumen (Min et al. 2002; Mueller-Harvey 1999), and as expected in our study, plants that had the most prominent effect on preventing proteolysis were those from tannin-containing plant groups such as *Acacia* and *Kennedia*. We also found several non-tannin containing plants with similar effects. *Eremophilas* are already well-known for their specific antimicrobial properties (Ndi et al. 2007; Palombo and Semple 2001; Pennacchio 2005), while *Brachychiton populneus* and *Chenopodium* species are also reported to have some bioactivity (Alanis et al. 2005; Nash 2004). These plants must have some proteolytic-protective mechanisms in the rumen other than tannins, which is exciting and has been suggested elsewhere in the literature (Wallace et al. 1994).

Propionate and CH_4 compete for hydrogen in the rumen and reduction in CH_4 is often associated with an increase in propionate (Moss et al. 2000; Patra et al. 2006), However, in the current study, while there were plants that followed this rule, there was no general correlation between the CH_4 and propionate production. It is possible that plants switched to other H_2 sinks, or it is an anomaly of the in vitro system. It is exciting if an alternative H sink is being used and this is something we should investigate further. Based on information presented here it is possible to select plants with both favourable pathways (i.e. reduction in methane resulting increased propionate).

It has been observed that same plant species can differ in their bioactivity, depending on season, maturity etc. For example, methanogenic potential varied between the accessions of *Acacia angustisima* collected by (Soliva et al. 2007). In the current study, we had similar observations, as there was variability between the same species collected at different times, locations and stages of growth. However, this needs to be investigated further with a greater set of plants and over longer periods of time, applying more rigor when selecting plants.

Experiment - Compounds

This experiment was done to examine whether the rumen-modulating effects observed by the plants *in vitro* were related to the secondary compounds contained in these plants. A total of 14 plants were selected, based on known bioactivity (Table 3) and/or their fermentation and rumen modulating properties (Table 4), to be examined as ethanol extracts. The extracts were obtained according to the procedure of (Semple et al. 1998) from an amount of the plant material that was equivalent to that included in the Experiment - Preliminary (i.e. 0.1 g). This was then dried and resuspended in 100 μL of 70% ethanol to be included in the *in vitro* (IVFT) assay as described previously. However, this time each tube was provided with 0.1 g of oaten chaff as substrate. The negative control had no addition (rumen fluid only), positive control was oaten chaff + 100 μL ethanol, and the antibiotic control had 0.1 g oaten chaff + ethanol + Rumensin (as described in the preliminary experiment). Treatments were incubated

for 24 h, and at the end of the incubation period gas and CH_4 production were measured for all treatments, while NH_3 concentrations were measured only in those that had a significant effect on rumen NH_3 in the earlier experiments.

Table 6. Effect of selected plant extracts on gas production, CH_4 and NH_3

	Established PSC or bioactivity	Possible rumen-modulating effect on	Gas (kPa)	CH_4 (mL/g DM)	NH_3 (mg/g CP)
Rumen fluid only (NC)			15.9b	0.2b	0a
Oaten chaff + EtOH (PC)			84.7	35.2	384
Oaten chaff + EtOH + monensin (AB)	Yes	CH_4 & NH_3	68.8b	36.3	501a
Cullen australasicum (mature)	-	CH_4	79.7	35	-
Cullen australasicum (young)	-	CH_4	86.6	50a	-
Cullen discolor	-	CH_4	73.1b	26b	-
Nitraria billardieri	Yes	CH_4	80.5	42a	-
Senna barclayana	Yes	CH_4	89.1	44a	-
Templetonia retusa	Yes	CH_4	87.4	49a	-
Indigofera australis	Yes	CH_4 & NH_3	92.4a	59a	354
Kennedia macrophylla	-	CH_4 & NH_3	79.1b	42	264b
Kennedia rubicunda	-	CH_4 & NH_3	76.7b	41	258b
Kennedia prorepens	Yes	CH_4 & NH_3	81.2	30	320b
Lomandra longifolia	-	CH_4 & NH_3	85.5	42a	367
Viminaria juncea	Yes	CH_4 & NH_3	91.9a	63a	431
Kennedia nigricans	-	NH_3	88.9	-	243b
Kennedia prostrata	-	NH_3	83.8	-	221b

a - significantly ($P<0.05$) higher than PC; b - significantly lower ($P<0.05$) than PC

Results and discussion

When selected plants were included as ethanolic extracts, only one plant - *Cullen discolor* maintained rumen modulating effects on CH_4. Extracts from Kennedia species (*Kennedia macrophylla, Kennedia nigricans, Kennedia prorepens, and Kennedia rubicunda*) also had inhibiting effects on NH_3 concentrations similar to those observed with the whole plants (Table 6). Three plant extracts also inhibited fermentation (gas production) even when provided with external substrate, indicating the presence of secondary compounds with general antimicrobial activity. Interestingly, when included along with ethanol, monensin also exhibited an inhibitory effect on gas production suggesting an interaction between monensin and ethanol that broadens the effect of monensin.

The lack of effect with extracts compared to what we observed with the whole plants could be assigned to many factors. First, the dose of the compound might have been too low. We opted to extract the amount corresponding to what was used in IVFT, however other authors have used much higher doses when testing plant extracts (Broudiscou et al. 2000; Busquet et al. 2005a). Second, ethanol was used both as extractant and solvent, and it is possible that the right bioactive compound(s) and/or amounts of bioactives were not extracted with this procedure. Finally, and probably more importantly, the "bioactivity" is a complex action, requiring consortium of bacteria, substrates and compounds. Providing oaten chaff as a substrate in our system might have failed to provide the type of substrate as the plant itself (in the first part of the study) and therefore did not stimulate the right consortium of bacteria for the effect to become apparent.

Experiment - Acidosis In vitro

This experiment was performed to examine if the plants had another important rumen - modulating effect - on the bacteria and events involved in lactic acidosis (i.e. prevent rapid starch fermentation when animals are exposed to grain). Plants were selected based on the results of gas production and pH from the first part of the study (Table 4, i.e. only plants that maintained pH comparable or higher to the positive control, without affecting gas production were tested).

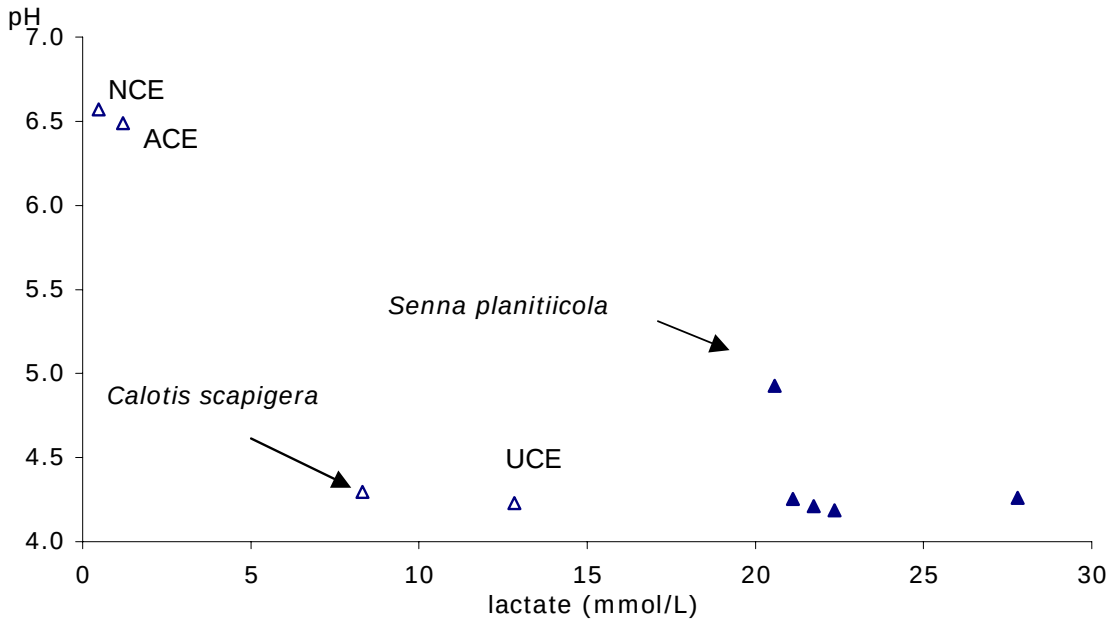
Materials and Methods

Six plants, namely *Calotis scapigera*, *Cullen australasicum* (young and mature), *Senna barclayana, Senna planitiicola* and *Templetonia retusa* were randomly selected from the first part of the study based on their potential to maintain pH (6.3 and above) without significantly affecting gas production *in vitro*. These plants were included in a modified *in vitro* system - "carbohydrate challenged *in vitro* system" (CCIV) that was designed to mimic conditions of lactic acidosis *in vitro* (Hutton et al. 2006). In this system 0.1 g glucose was added as a substrate, and the indicators of rapid glucose fermentation (drop in pH and accumulation of lactate) were measured after 24 h. In this system, RF was not buffered to magnify the effect of the carbohydrate challenge and observe differences between plant treatments. The other treatments included in the experiment were: Non-challenged environment (NCE, oaten chaff only), uncontrolled challenged environment (UCE, oaten chaff + 0.1g glucose), antibiotic-controlled environment (ACE, oaten chaff + 0.1g glucose + 0.0012 g/mL virginiamycin, VM - Eskalin 500, Phibro Animal Health Pty. Ltd., Wentworthville NSW, Australia; dissolved in methanol prior to testing). At the end of the incubation period, gas production and pH were measured, and a 2 mL sample of rumen fluid was taken and mixed with 1 mL 2M NaOH to analyse for D-lactate. D (-)-lactate was determined using the Boehringer Mannheim Lactic acid kit (Product No 1112821) using a Roche Cobas Mira S autoanalyser for readings.

Results and Discussion

Senna planitiicola demonstrated significantly higher pH value ($P<0.05$) to that of the uncontrolled environment (UCE), while *Calotis scapigera* produced significantly less lactate than any other plant treatment ($P<0.05$), although it did not differ significantly to lactate production in the UCE (Figure 1). None of the selected plants were capable of maintaining pH above 5.0 or have lactate values comparable to the ACE. However, in this experiment, the lack of drop in pH in ACE treatment was also accompanied by a significant inhibition of gas production (data not shown), indicating that there was severe inhibition of normal rumen fermentation by the antibiotic. The dose of 12 μg VM per 1 g of substrate was not too high, as the recommended inclusion rate *in vivo* is 40 mg VM/kg feed (Godfrey, *et al.*, 1995), so it should equal 40 μg VM per 1 g of the substrate. It remains unclear why AB had this effect, but it is possible that addition of methanol (used as solvent) released or activated compounds from the antibiotic that are not normally released when fed to the animal.

Figure 2. pH and lactate production in the presence of plant extracts in the CCIV. NCE – non-challenged environment, ACE – antibiotic-controlled environment, UCE – uncontrolled environment



This work was done in parallel to work that was being done by Dr Peter Hutton, who was undertaking his PhD under the supervision of Dr Vercoe. Dr Hutton has now completed his PhD and some of his results are published (see list of publications section). We used the data Peter had obtained from a much broader collection of species to identify plants with exciting anti-acidosis properties. E. glabra was one of those plants and is the subject of the next sections.

Experiment Acidosis *In vitro* – *E. glabra* dose

This experiment was conducted in preparation for the *in vivo* experiment. Based on the previous experiment, none of the candidates we tested demonstrated complete protective effect in the IVCC. However based on Dr Hutton's studies, *E. glabra* was shown to be capable of preventing a significant drop in pH and production of lactate in an IVCC system, with some dose-response effect observed *in vitro* (levels tested 0-10%) (Hutton et al. 2006). However, when the plant was included *in vivo* at a level of 10% of the amount of grain supplied to the animal, there was a lack of complete protection against acidosis. It was necessary to establish whether higher levels could be effective without impeding rumen function.

Materials and methods

In this experiment, several levels of inclusion of *E. glabra* (EG) were tested. The IVCC system was similar to that described above, with the exception that antibiotic was not dissolved in methanol but in rumen fluid. *E. glabra* was included at 0.1 – 1.0 g (10% EG - 100% EG) per gram of glucose. In addition, oaten chaff treatments were included as a control for the effect of fibre on acidosis, at levels of 0.1 g, 0.3 g and 0.6 g (10%, 30% and 60% OC) per gram of glucose. Higher levels of inclusion of oaten chaff could not be achieved because

of a lack of physical space to make it practical. Measurements were taken after 5 h (gas pressure and pH only) and 24 h (gas pressure, pH, lactate and VFA). Samples were analysed as described previously.

Results

After 5h of incubation, there was a drop in pH in UCE and all OC treatments, but not in EG treatments. However, this was also accompanied with inhibited gas production in all EG treatments. After 24 h, pH declined further in all treatments, but in all EG treatments it remained above 5.0 and was significantly higher than UCE and comparable to ACE. Lactate was also lower in all EG treatments than UCE, with levels of 60% EG and above having significant effects. In all EG treatments below 80% inclusion, gas was significantly higher than UCE, while those of 80% and above had significantly lower gas production. VFA in all treatments remained comparable to UCE, but lower than ACE.

Discussion

All 10 levels of inclusion of *E. glabra* in IVCC demonstrated protective effects against a drop in pH and accumulation of lactic acid. There was a dose-response effect for gas and pH, but only at 5 h. After 24 h, this linearity was lost, as all treatments had a narrower range of pH (5.1 – 5.6), while still differing in gas production. In this closed system, when the substrate, buffer and outflow are limited, it is necessary to find optimal "stopping time", i.e. when the effect will be the most prominent, allowing enough time for the release of compounds, without loosing the linearity and correlation of normal fermentation parameters. Batch systems are commonly incubated over a period of 24 h, but when the system is challenged with more specific substrates it was discovered that the optimal time for observation is 6 h of incubation (Durmic et al. 2008; Hutton 2008). It is worth noting that that apart from the dose itself, incubation time plays another significant role (for example i.e. time of release of compounds vs time of their degradation).

It has been established previously that the protective effect of *E. glabra* is due to specific inhibitory effects against lactate-producing bacteria (Hutton 2008), rather than non-specific inhibition or poor digestibility. However, at very high doses *E. glabra* appears to effect rumen fermentation more generally. In support of this, in our experiment addition of OC did not prevent the decline in pH or accumulation of lactate, and the gas production was similar to UCE. This indicates that addition of fibre as such did not prevent acidosis, and that the protective effect is more likely be due to bioactive compounds present in the plant.

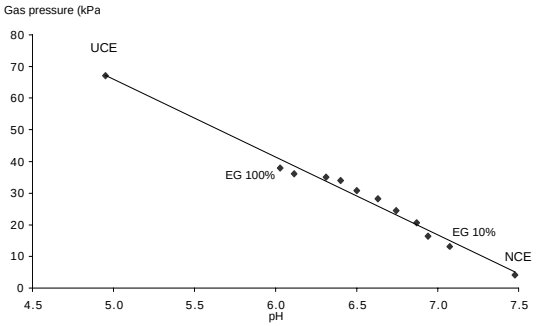
Table 7. *In vitro* fermentation parameters in IVCC system incubated with different levels of *E. glabra* after 5 h and 24 h incubation periods

Treatment	Incubation time					
	5 h		24 h			
	pH	Gas	pH	Gas	Lactate	VFA
NCE	7.5a	4b	7.4a	12b	1b	55b
UCE	5.0	67	4.2	87	27	82
ACE			5.2a	171a	0.3b	153a
10% OC	4.6b	71a	4.2	89	40a	92
30% OC	4.6b	67	4.1	86	54a	95
60% OC	4.7b	69	3.9b	93	67a	106a
10% EG	7.1a	13b	5.3a	135a	20	84

20% EG	6.9a	16b	5.1a	140a	25	78
30% EG	6.9a	21b	5.4a	125a	22	76
40% EG	6.7a	25b	5.5a	134a	22	84
50% EG	6.6a	28b	5.2a	135a	22	85
60% EG	6.5a	31b	5.3a	116a	17b	92
70% EG	6.4a	34b	5.3a	96a	11b	93
80% EG	6.3a	35b	5.4a	75b	6b	93
90% EG	6.1a	36b	5.5a	57b	3b	101a
100% EG	6.0a	38b	5.6a	59b	2b	98

a - significantly (P<0.05) higher than PC; b - significantly lower (P<0.05) than PC

5 h



24 h

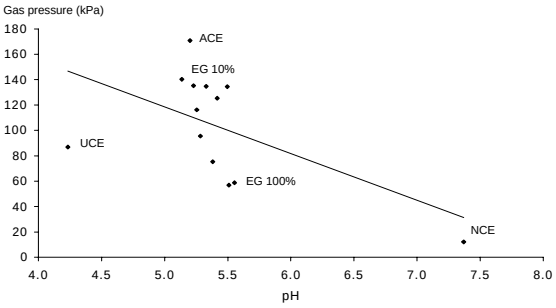


Figure 3. pH and gas pressure after 5 h and 24 h incubation (plotted from Table 6)

Experiment - Acidosis In vivo

This experiment was conducted as "proof of concept" that the effect observed *in vitro* corresponds to that *in vivo*. Testing plants in sheep where lactic acidosis is induced provides a good model for this. We chose this system because the experiment is conducted over a 24 h period, requiring a relatively small number of animals and a relatively small amount of plant material that can be applied as a one-off dose, as opposed to feeding over several weeks to observe the effect on rumen fermentation, for example, methane production. In addition, the correlation between *in vivo* CH_4 production and our *in vitro* method was validated by Mayberry et al (2008), so it was not necessary to measure CH_4 production *in vivo.* The experiment can also be conducted easily in the animal house, as opposed to grazing, which reduces variability. The effect (i.e. rapid drop in pH and accumulation of lactate) is immediate and prominent and can be monitored and arrested easily, so that the health and welfare of the animals are not jeopardised.

The purpose of this study was to determine if *E. glabra* could be as efficient at preventing acidosis *in vivo* as observed *in vitro* when included at levels established in the dose response experiment.

Materials and Methods

Forty sheep (n=10 per treatment) were dosed via the ruminal cannula with ground wheat (UCE), ground wheat + virginiamycin (ACE), or ground wheat + one of three levels of *E. glabra*: 200 g/kg wheat (corresponding to EG 20%); 400 g/kg wheat (EG 40%) and 800 g/kg wheat (EG 80%) of dried and ground plant. These levels of plant inclusion were chosen because i) previous *in vivo* studies with lower level of inclusion (10%) did not offer significant protection (Hutton 2008), and ii) because 40% and 80% showed the most promising effect *in vitro* (Table 7). After the inoculation, rumen fluid samples were collected over a 24 h-period and analyzed for pH, D-lactate and VFA concentrations. Sheep that had a ruminal pH $\leq$ 5.0 were removed from the trial and their rumen flushed with warm water, then inoculated with rumen fluid from donor sheep.

Results

Ruminal pH varied between the treatments and sampling times. Potentially acidotic values (below 5.5) were recorded only in sheep in UCE treatment, with values below 5.0 (indicative of acidosis) observed in only three UCE sheep at 8 h and 10 h after dosing. Except for one sheep in EG 20%, animals in other treatment groups maintained pH above 5.5 throughout the experiment. The highest levels of lactate were detected in EG 20 % at all sampling times and UCE at 12 h after dosing. While lactate levels in UCE became similar to ACE at 16 h, in EG 20% they were still significantly higher than ACE throughout the experiment. Reduced VFA concentrations compared to UCE (indicative of inhibited fermentation) were detected in EG 40% (8 h) and EG 80% (all post-dosing times), but these were not significantly different to ACE except for EG 80% at 16 h. In addition to this, there was one sheep in EG 80% treatment that maintained an unusually high pH, reaching above 7.0 at 8 h post-dosing (indicative of inhibition of fermentation), which was accompanied with an accumulation of gas (bloating) and rumen stasis. This animal was removed from the experiment.

Table 8. pH, D-lactate and VFA concentrations in the rumen fluid collected before dosing (0 h) and at 8 h, 12 h and 16 h after dosing in sheep receiving wheat and different additives. UCE – wheat only; ACE – wheat + antibiotic; EG 20% – wheat + 200 g plant/ kg wheat; EG 40% – wheat + 400 g plant/ kg wheat; EG 80% - wheat + 800 g plant/ kg wheat

	Time after dosing											
	0h			8h			12h			16h		
	pH	Lactate (mmol/L)	VFA (mmol/L)	pH	Lactate (mmol/L)	VFA (mmol/L)	pH	Lactate (mmol/L)	VFA (mmol/L)	pH	Lactate (mmol/L)	VFA (mmol/L)
UCE	6.9	0	21	5.8^{b}	$4^{a,b}$	51^{a}	5.7^{c}	7^{a}	64^{a}	5.4^{b}	$4^{a,b}$	70^{a}
ACE	6.9	0	22	6.7^{a}	0^{c}	$40^{a,b,c}$	6.7^{a}	0^{b}	$57^{a,b}$	5.9^{a}	0^{b}	67^{a}
EG 20%	6.9	0	24	6.0^{b}	8^{a}	$48^{a,b}$	$6.1^{b,c}$	7^{a}	71^{a}	5.8^{a}	8^{a}	68^{a}
EG 40%	6.9	0	22	6.1^{b}	$5^{a,b}$	$37^{b,c}$	$6.4^{a,b}$	$5^{a,b}$	$52^{a,b}$	6.2^{a}	$2^{a,b}$	59^{a}
EG 80%	6.9	0	23	6.1^{b}	$3^{b,c}$	36^{c}	$6.0^{b,c}$	9^{a}	40^{b}	6.0^{a}	$5^{a,b}$	38^{b}

Note- within the same column, values not sharing the same superscript are significantly different ($P<0.05$)

The results obtained in this study correlate to some extent to what was observed *in vitro*. In all EG treatments pH remained above 5.0, and significantly higher than UCE at the later sampling (24 h *in vitro* and 16 h *in vivo*). The medium level of inclusion (EG 40%) seem to offer a level of protection comparable to antibiotic (after 8 h) and, amongst the EG treatments, maintained the highest pH and lowest lactate (both *in vitro* and *in vivo*) without inhibiting fermentation (non-inhibited gas *in vitro*, non-inhibited VFA *in vivo*). Inhibition of normal rumen fermentation occurred with high levels of plant - EG 80% (inhibition of gas in *in vitro*; high pH, bloat and stasis *in vivo*). Dose-response effect was observed in earlier stages of inoculation (i.e. 5 h *in vitro*, 8 h *in vivo*) but this linearity of dose-response is lost at later stage *in vivo*.

Intraruminal inclusion of EG at 400 g/kg of wheat (EG 40%) produced pH and lactate levels comparable to the virginiamycin treatment achieved at 12 hours after dosing (Table 8), but higher levels of EG (80%) did not offer significant protection against acidosis and also inhibited rumen fermentation, as judged by a significant reduction in VFA concentrations and observations of animal behaviour. Some dose-response effects were observed but only at earlier stages, and it is possible that repeated inoculations would prove beneficial. Further experiments are needed to confirm the appropriate levels, frequency of inclusion, and finding more practical ways of administering the plant.

It is not clear why in this experiment, only three sheep in UCE had pH below 5.0, and lactate levels were much lower than previously observed (Hutton 2008). The lack of a "proper" positive (acidosis) control (that is a dose of grain that made all animals in the control group become acidotic) may have obscured further the full potential of the protective effect of *E. glabra*. However, variation in the response of animals to acidosis is common and the fact that the only animals to suffer acidosis were in the control (UCE) group, suggest that E. glabra does have some potential to offset the effects of a rapid introduction to grain.

Experiment - Dose

The experiments conducted *in vitro* presented above assumed that the shrubs were the sole substrate for fermentation. However, observations on consumption of shrubs *in situ* indicates that they would be eaten only at a limited level, along with pasture. In this experiment we used the five most prominent plants from Experiment - Fermentability to examine the level of inclusion of each plant that would be needed in a plant mix to achieve an effect. Several shrubs from have exhibited inhibitory effect on CH_4, either with or without inhibiting gas. To investigate the amounts that were required to have an effect, five plants were tested at 0, 25, 50, 75 and 100% inclusion in combination with OC (Table 8). The plants used were CA- *Cullen australasicum (mature, oven dried), KP - Kennedia prorepens (oven dried), EG- Eremophila glabra, EL- Eremophila longifolia and MB- Maireana brevifolia*.

Table 8. Treatments used in the experiment

Treatment	Plant	OC
OC	0.000g	0.100g
25	0.025g	0.075g
50	0.050g	0.050g
75	0.075g	0.025g
100	0.100g	0.000g

Results

In some plants, like *E. glabra* and *K. prorepens*, the fermentability (gas and VFA) decreased as the dose of the plant increased, while in the others it was not affected (Table 9). We are currently waiting to receive the CH_4 results and once they become available, and NH_3 concentrations are corrected for the protein content, we will be able to discuss further the effect on rumen-modulating properties.

Table 9. Rumen fermentation parameters in IVFT system with selected plants included at different levels

	Gas	pH	VFA (mmol/L)	CH4 (mL/g DM)	NH3 (mg/L)
NC	25 b	7.16 a	53 b	tba	256 a
OC	78	6.05	87	tba	215
Rumensin	80	6.07	92	tba	225
				tba	
CA 25	77	6.06	108 a	tba	264 a
CA 50	92 a	6.10	98 a	tba	322 a
CA 75	85	6.16 a	102 a	tba	307 a
CA 100	90	6.20 a	95	tba	336 a
				tba	
EG 25	76	6.17 a	91	tba	196
EG 50	69	6.30 a	77 b	tba	198
EG 75	58 b	6.38 a	79	tba	216
EG 100	52 b	6.46 a	76 b	tba	220
				tba	
EL 25	86	6.05	84	tba	240
EL 50	80	6.15 a	89	tba	254 a
EL 75	89	6.10	92	tba	281 a
EL 100	93 a	6.09	92	tba	296 a
				tba	
KP 25	87	6.06	92	tba	276 a
KP 50	81	6.14 a	84	tba	263 a
KP 75	78	6.31 a	80	tba	296 a
KP 100	72	6.38 a	80	tba	279 a
				tba	
MB 25	93 a	6.30 a	92	tba	330 a
MB 50	116 a	6.52 a	85	tba	368 a
MB 75	98 a	6.86 a	84	tba	392 a
MB 100	94 a	7.01 a	84	tba	413 a

a - significantly ($P<0.05$) higher than PC; b - significantly lower ($P<0.05$) than PC

Experiment - Shrub Mixes

Sheep were grazing a mix of 5 shrubs (+2 planted but refused to eat, Acacia and Rhagodia) along with pasture at Badgi. It was estimated that shrubs were representing 10% of their diet. We tried to replicate this combination *in vitro* to see if this marginal level of inclusion has any effect on *in vitro* fermentability and rumen-modulating properties. Two mixes were tested: "Badgi Mix 1" composed of 5 shrubs (oven dried and ground) that were readily eaten, mixed with pasture (oven dried and ground) at ratio 10% : 90%, and "Badgi Mix 2" contained an additional two shrubs (Acacia and Rhagodia) that were not grazed, and mixed at the same ratio.

Results

There were no differences in fermentability of pasture on its own, or pasture supplemented with 10% shrub mixes. Rumen-modulating effects (if any) will be presented once CH_4 data become available and NH_3 values are recalculated on the basis of CP%.

Table 9. *In vitro* fermentability of shrub mixes

Level	Gas (kPa)		pH	VFA (mmol/L)	CH_4 (ml/g DM)	NH_3 (mg/mL)
Pasture	110		5.94	95	tba	296
Badgi Mix 1	112	a	5.98	94	tba	311
Badgi Mix 2	111		5.99	96	tba	323

General discussion

A series of experiments were conducted to examine fermentability and rumen-modulating properties of Australian native shrubs that are being investigated as new fodder for ruminants. Plants were initially selected based on their agronomic properties and nutritive value. There was limited knowledge on the bioactive properties of these plants, especially their effect on rumen fermentation.

Fermentability of the plants varied, ranging from those that were readily digestible and comparable to conventional fodder (i.e. oaten chaff) to those that were poorly digestible, as judged by low gas and VFA production *in vitro*. There were plants that exhibited rumen-modulating properties, by reducing CH_4, NH_3 and/or A : P ratios. While some of these plants did not affect normal fermentation, others also inhibited fermentation but had more pronounced rumen-modulating effect. Limited attempts were made to distinguish if the effect of these plants was due to poor fermentability and/or bioactive compounds, and further work is required to investigate this.

One of the most attractive areas in rumen manipulation, especially in grazing animals consuming large quantities of fibrous food, is reducing methane production through the development of new products with anti-methanogenic activity (Moss et al. 2000). Plant species with antimethanogenic properties have already been identified in Europe (Bodas et al. 2007) and the results from our study suggest that plants with similar properties exist in Australia. Using shrubs that produce lower levels of methane production in sheep systems may offer a viable strategy for reducing CH_4 emissions and could have additional environmental benefits in reducing the contribution to greenhouse gas emissions from livestock. However, potential candidates may need to be tested more rigorously and in more challenging environments, for example combining with plants promoting high methnogenic activity (i.e. fibrous foods).

In the past, rumen-modulating effects usually have been tested for plants with already established and often well-known bioactivity. The plants in this study were selected in the first instance on their agronomic potential, rather than their bioactivity. In addition, we only tested major rumen-modulating activities that might be of major interest when grazing shrubs. However, we have already discovered plants with multiple bioactive properties, and further investigation might reveal some other beneficial effect of these plants, for example on the animals physiology and digestion. In addition, the control of diseases in livestock is an important part of the animal production, and part of aim of the programme was to promote the welfare of farm animals along with the economic and marketing benefits associated with improved animal welfare. Plants containing antimicrobial compounds may prove helpful in controlling other harmful microbes in animals (other than acidosis), and help producers to raise animals with less chemical inputs and comply with the National Antibacterial Residue Minimisation Program (NARM 2000).

Compared to the laborious nature and expense of *in vivo* testing, the IVFT method provided a quick and easy way to evaluate nutritive value of feeds, relating well to *in vivo* observations (Rymer and Givens 2002). In the current study the IVFT method allowed the evaluation of a large number of plant samples in a relatively short time and the ranking of them based on their potential nutritive value (fermentability) while monitoring more specific changes in microbial fermentation. However, some caution should be taken when interpreting results from the IVFT used here.

In the current study, we only used 100 mg of plant as substrate in 10 mL of rumen fluid. This proportion is often used in *in vitro* systems (Bodas et al. 2007; Soliva et al. 2007), but it is not realistic and it does not translate to the level of feed that an animal consumes *in vivo*. Further, we monitored changes after 24 h, without adding additional substrate or buffer, so it is

possible that the system goes out-of balance. As we discovered in the Experiment - E. glabra-dose, shorter periods of incubation (i.e. 5-6 hours) would be better representative than 24 h. The IR measurement of CH_4 (used in Experiment - Preliminary) was less reliable than the GC method for estimating methane production in this system; it seems to underestimate methane production compared to GC. Although it provided fast, inexpensive and direct measurement, it would require more optimisation and calibrations if it were to be considered for future use. It would be worthwhile investing some money and time to optimise it because of the ease and speed with which it can be used.

Another impediment in assessing potential rumen modulating effect of plants in this system was that the effect was not estimated using high-quality diets. It has been observed that some other plant-derived compounds have an effect (increase in dry matter intake and total VFA, and reduction in the acetate : propionate ratio and ammonia N concentration) when used with high-concentrate diets, but appear to have small effects in high-forage diets (Calsamiglia et al. 2007). The effects of some of these plants in our study could be diet-dependent and their use may be more beneficial under more defined conditions and a variety of production systems.

Further, we monitored levels of ammonia as the end product in proteolysis, but it would be valuable to measure the full suite of intermediates and enzyme activities involved in proteolysis (McIntosh et al. 2003). Also, we used strained rumen fluid to minimize the influence of the donor sheep diet on fermentation, but this could have an impact on other proteolytic members (i.e. fungi) that could possibly be under-represented due to this procedure.

In our system, we observed only some rumen-modulating effects of monensin (Busquet et al. 2005a; Castillejos et al. 2006). It is a common practise in these tests to include antibiotic dissolved in ethanol or other solvents to enhance their solubility, but instead, in most of our experiments we included antibiotics in the way that they are presented to the animal, i.e. dissolved in rumen fluid. However, we observed more pronounced effects of antibiotics once they were dissolved in ethanol (monensin, testing ethanolic compounds of plants), or methanol (virginamycin, Experiment - Acidosis *In vitro*).

Conclusions

A range of Australian native shrubs have been demonstrated to have fermentation characterisitcs (gas production, and/or VFA) comparable to a common supplementary fodder, oaten chaff. Within these, there were plants that had rumen-modulating properties. There were also plants that had more pronounced effect on these properties, but also affected rumen fermentation. Some of the effects observed *in vitro* translated well *in vivo*, but further studies are needed to confirm this.

- A total of 156 (29 in preliminary examination and 127 in full examination) plant samples were tested in an *in vitro* rumen culture system. Plants were tested for overall fermentability (pH, gas, VFA) and specific metabolic pathways indicative of rumen-modulating properties (methane production, proteolysis, propionate production).

- Plants having good fermentability as well as rumen-modulating properties were identified. The most promising plants seem to be *Cullen australasicum (mature), Cullen discolor*, *Eremophila glabra* and *Eremophila longifolia.*

- Some variability in responses was observed in relation to plant location, age and season

- Several plants were tested to investigate rumen-modulating more specifically (i.e. as ethanolic extracts or *in vitro* method designed to simulate acidosis), but these tests provided limited additional information.

- An *in vivo* experiment was conducted as a proof-of-concept. The *in vivo* experiment confirmed some of the results observed *in vitro*.

Future work

Moving towards on-farm application

- Investigate rumen fermentation profiles and rumen-modulating effects in *in vivo* grazing studies
- Investigate aspects of possible applications - when, how much, in what way, in what combinations?
- Design of innovative grazing systems built on this knowledge

"Science behind"

- Identify the specific compounds that are involved, what their mode of action is, is there a persistency of effect and possible acclimatization. Expand the work to include continuous flow system (Rusitec) for deeper understanding of bioactive plants
- Examine plant variation further - site, species, season
- Work on pure cultures of rumen bacteria

Extension

- Other applications in animals (i.e. to improve public health through reduced carcass contamination and improved fatty acid profiles in product; improve animal health through control of gut)
- Application in other animal species
- Plant selection and breeding for specific compound/effect

Commercialisation

- "Designer shrub mixes" to improve both health and productivity (i.e. small plots of active plants along with highly-grazable plants)
- Designing "new and safe additives for better production, health and the environment" as cut-and-carry additives
- Isolating compounds and developing a commercial product

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Section 5

Can native shrubs play an anthelmintic role for livestock?

Methods

1) Parasite egg recovery

The *Haemonchus contortus* used for this study were from the anthelmintic susceptible Kirby 1982 strain isolated from the field at the University of New England Kirby Research Farm in 1986 (as described by Albers and Burgess, 1988). Infected animals were housed at McMaster Laboratory, CSIRO Livestock Industries, Armidale, NSW. Faeces was sent regularly by courier to the Queensland Biosciences Precinct laboratory at St. Lucia, Brisbane. Nematode eggs were recovered from the faeces by passage through a series of fine sieves (250 μm and 75 μm) followed by centrifugation in a stepwise sucrose gradient (10, 25 and 40% sucrose). The eggs were recovered from the interface between the 10 and 25% sucrose layers, and washed over a 25 μm sieve with water to remove residual sucrose. They were then agitated gently in a solution of 8.4 mg/L sodium hypochlorite for 12 minutes, and then washed again with copious amounts of water. The eggs were prepared in distilled water at a concentration of 50-60 eggs per 30 μl after the addition of amphotericin B (final concentration 37.5 μg/ml), and used immediately for larval development assays.

2) Plant material

a. Collection
Plant samples were received from the following sources:

1) Monarto field site, SA
2) Badgingarra field site, WA
3) Roadside collections made by Jason Emms in SA in April 2007
4) Collections made by Peter Jessop in south west NSW in September 2007.

Plants were received either as ground material which had been either oven- or -freeze dried, or as fresh specimens. These later samples were cut into small pieces and ground using a mortar and pestle on dry ice.

b. Extraction

Plant material was extracted in either water or 70 % ethanol. For water extraction, 1 ml of water was added to 100 mg of plant material, and the tube placed onto a roller wheel for 48 hours at room temperature. The mixture was then centrifuged at 10,000 rpm for 30 minutes and the supernatant recovered, and stored at – 20^{o}C for use in bioassays. For ethanol extraction, 1 ml of 70 % ethanol was added to 100 mg of plant material and placed onto a roller for 48 hrs at room temperature. The mixture was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was recovered and dried overnight in a Savant SpeedVac. The pellet was resuspended in 80 μl DMSO with shaking for 4 hours, and stored at -20^{o}C for use in bioassays.

In some cases, the weight of dry matter in extracts was determined. For water extracts, an aliquot of the final water extract was dried overnight in a pre-weighed tube, the tube was then reweighed, and the weight of dried material calculated by difference. For ethanol extracts, an aliquot of the initial extract (prior to transfer into DMSO) was dried, and tube weights used to calculate dry matter weight as for the water extracts.

A number of plant extracts were treated with polyvinylpolypyrrolidine (PVPP) prior to use in bioassays in order to remove tannins. Extracts (300 µl) in water or ethanol (prior to transfer into DMSO) were added to 30 mg amounts of PVPP (that is, final concentration of PVPP of 10 % w/v) and shaken for 4 hours. The mixture was centrifuged at 5000 rpm for 5 minutes, and the supernatant removed and either used directly in assays for water extracts, or dried and resuspended in 30 µl of DMSO for ethanol extracts.

3) Nematode larval development assay

Assay plates were established in two ways according to the type of extract being examined:

a. ethanol extracts: 0.5 ul aliquots of plant extract (in DMSO) were added to individual wells of 96-well assay plates (DMSO alone was added to control wells). Agar (200 ul of 2% (w/v)) was added to each well, and allowed to solidify. Egg solution (30 µl) was added to each well.
b. water extracts: plain agar was added to wells of a 96-well plate, and allowed to solidify. Egg solution (30 µl) was added to each well. Aliquots of plant extract (10 µl) were added to each well (control wells received water only).

The plates were placed into bags to prevent drying, and incubated overnight at 26^{o}C. The next day, 2 µl of a growth medium (consisting of Earle's salt solution (10% v/v), yeast extract (1% w/v), sodium bicarbonate (1 mM) and saline solution (0.9% sodium chloride w/v) was added, along with 2 µl of a solution of live *E. coli* cells. The plates were incubated for a further 5 days, and then killed using Lugol's iodine solution (10 µl per well). The number of fully developed infective stage larvae (L3) present in each well was counted. Percent larval development was calculated by expressing the numbers of larvae in treated wells as a percentage of the mean number in control wells (at least 8 control wells were present on each plate).

4) Adult worm motility assays.

The effects of several plant extracts on adult H. contortus worms were assessed by observing the degree of motility shown by worms over a period of exposure to the extracts in vitro. Adult worms were recovered from sheep housed at the QDPI animal facility at Yeerongpilly, Brisbane The adult worm recovery and culture methods were as described by Kotze and McClure (2001). Briefly, worms were recovered from sheep abomasa (approximately 6-10 weeks post infection) by manual picking from the gut contents. They were placed in culture medium (RPMI-1640, HEPES buffer, glucose, bovine serum, antibiotics and fungicide) for several hours, and then groups of ten were placed in separate tubes in 0.9 ml of culture medium. The only significant change from the previously described method was the inclusion of 20% newborn bovine serum in the culture medium, and the subsequent maintenance of the worms in an atmosphere of 20% CO2, 5% O2 and 75% N2 (compared to 5% CO2 in air for the earlier study). Aliquots of plant extracts (100 ul) were added to tubes, which were then kept at 37□C. At 24 hr intervals, the worms were observed and their degree of motility was scored. The assay tubes were placed onto a warm tray and tubes were held individually near a light for assessment of motility. Each tube was swirled to thoroughly disturb the nematodes and was scored according to the degree of motility shown by the worms using the scoring system described by O'Grady and Kotze (2004). Briefly, the worms were scored as: 3- most individuals showing significant smooth sinusoidal motion, similar to motion at the start of the culture period; 2- significant movement shown by a small number of individuals, at least one individual able to move in a normal sinusoidal fashion; 1- only very limited movement in a small number of individuals, no sinusoidal motion; 0- no movement.

Results

Samples from 85 plant species were examined using nematode larval development assays through this study. Some plants were tested only as oven – or freeze- dried samples, while

others were tested after both preparation methods. All plants were tested after extraction with water, while most were also tested after ethanol-extraction. A small number (n = 6) were tested as freshly ground samples, without being dried prior to the water or ethanol extraction procedure. Figure 1 shows frequency distributions of anthelmintic activity across the water extracts of both freeze- and oven-dried samples. It is apparent that a large number of samples had no anthelmintic activity, while others showed a spread of activities ranging up to complete inhibition of larval development at the screening concentration of approximately 1.4 mg extracted material / ml for water extracts. For both freeze- and oven-dried samples, there was a significant number which reduced larval development to less than 40 %. This was subsequently used as a cut off point to identify plants with 'significant' anthelmintic activity. A list of all plants examined in the study, subdivided into those which reduced larval development to less than 40 %, and those with less or no effect on development, is shown in Table 1. In cases where multiple plant species from single genera were examined, the prevalence of activity within the genera differed. For example, all seven *Cullen spp*, and 7 out of 8 *Atriplex spp*. were not active, while 6 out of 7 *Acacia spp* showed significant activity. *Rhogodia spp*., *Maireana spp*. and *Kennedia spp*. were more evenly spread between those showing a presence or absence of activity.

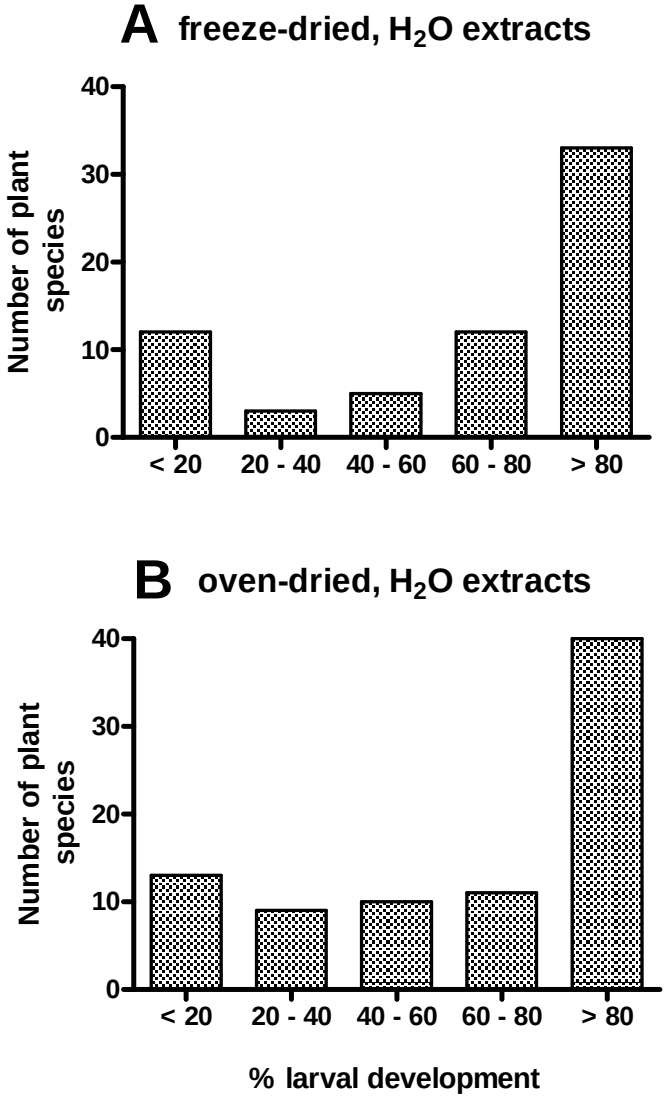

Figure 1 Frequency distribution of anthelmintic activity in water extracts of freeze-dried (**A**) and oven-dried (**B**) samples of various plant species. Total numbers of samples: **A** = 65; **B** = 83.

Table 1. Anthelmintic activity in extracts from 85 plant species. Extracts were considered to have significant anthelmintic activity if they reduced larval development to less than 40 % compared to control assays.

Genus	Species – Inhibition of larval development	
	yes	no
Eremophila	*glabra, maculate, longifolia*	*bignoniiflora*
Rhagodia	*parabolica, crassifolia, preissii*	*candolleana, spinescens*
Acacia	*pycnantha, neriifolia, myrtifolia loderi, iteaphylla, saligna*	*ligulata*
Lomandra	*longifolia*	
Myoporum	*platycarpum*	
Medicago	*citrina*	*sativa, arborea, strasseri*
Cullen		*palladium, tenax, parvum cinereum, patens, discolor australasicum*
Plantago		*lanceolata*
Atriplex	*isatidea*	*cinerea, semibaccata, vesicaria nummularia, amnicola, paludosa rhagodioides*
Maireana	*brevifolia, planifolia, tomentosa astrotricha*	*convexa, sedifolia, georgei pyramidata*
Templetonia		*retusa*
Chenopodium		*auricomum, gaudichaudianum nitrariaceum*
Viminaria		*juncea*
Swainsona	*stipularis*	*galegifolia, greyana*
Nitraria		*billardieri*
Calotis		*scapigera*
Lavatera		*plebia*
Indigofera	*australis*	
Glycine		*canescens, clandestine, tabacina*
Senna		*planitiicola, barclayana*
Chameacytisis		*prolifer*
Kennedia	*nigricans, prorepens, eximia rubicunda*	*prostrate, macrophylla*
Flindersia		*maculosa*
Cichorium		*intybus*
Enchylaena	*tomentosa*	

Pterocaulon	*sphacelatum*	
Exocarpus		*aphyllus*
Alectryon		*oleifolium*
Convolvulus	*remotus*	
Lotus		*australis*
Brachyome	*ciliaris*	
Dorycnium	*hirsutum*	
Abutilon		*otocarpum*
Colutea	*abyssinica*	
Dodonea		*viscosa*
Geijera		*parviflora*
Santalum		*acuminatum*

The sample preparation methods of freeze- or oven-drying, as well as the sample extraction methods using water or 70% ethanol, were compared directly in order to assess the impact of the different methods on subsequent measurement of anthelmintic activity (Figure 2). Figure 2A shows a comparison between the different extraction methods (note that the data represents a subset of the total data set as ethanol extractions were not performed on all plant samples). It is clear that for both sample preparation methods, there was a deal of overlap in samples identified as anthelmintic by the two extraction procedures, but there was also a significant number of samples that were only identified as toxic after one of the extraction methods was used. This was particularly the case for water extractions of freeze-dried material. This indicates that neither extraction method can be relied on solely to identify all anthelmintic plants, and also suggests the presence of anthelmintic agents with markedly different solubility characteristics in the different plants. Similarly, Figure 2B shows that while many samples were identified as anthelmintic after both freeze-and oven-drying, a significant number were only active if prepared by freeze drying. Only one plant sample was anthelmintic only after being over-dried. This indicates a range of susceptibilities of the plant bioactive agents to the extremes of oven-drying.

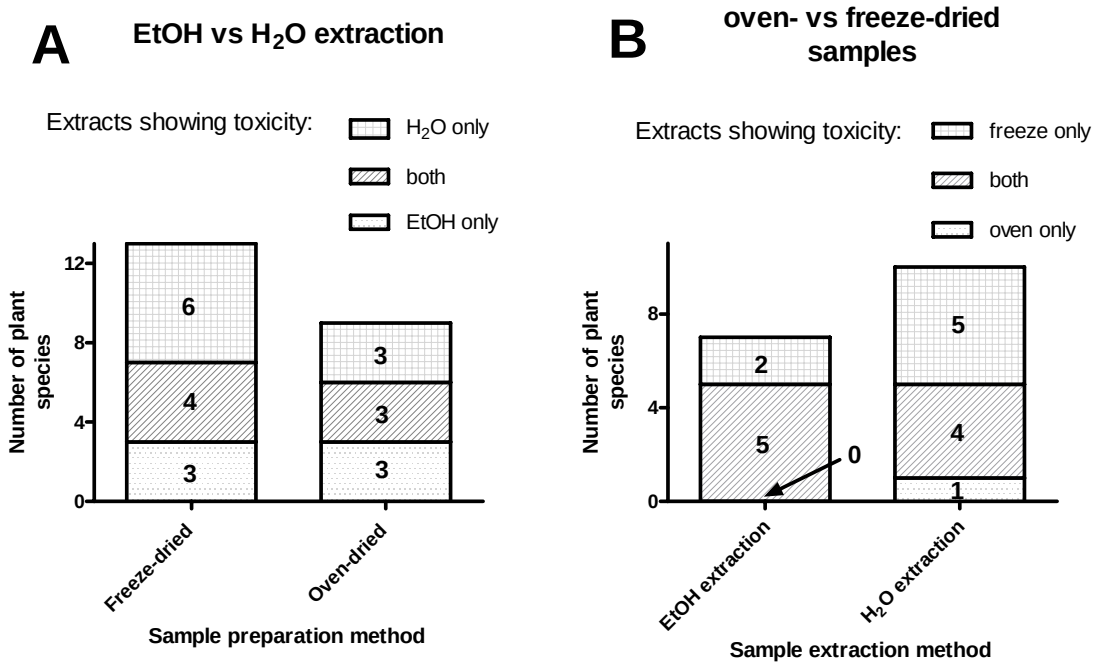

Figure 2. Relationship between the different sample preparation methods (freeze- or oven-dried) and different extraction methods (water- or ethanol-extraction).
A: comparison of water and ethanol extractions within each sample preparation method;
B: comparison of oven and freeze-dried extractions within each sample extraction method.

Several of the most active plant extracts (inhibiting development completely at the screening concentration from Figure 1) were examined further in dose response assays, alongside anthelmintic drugs (Figure 3). The extracts showed activity at least 400-fold less than levamisole, 7,600-fold less than thiabendazole, and 175,000-fold less than ivermectin. This assay is not ideal for demonstrating activity of levamisole, so it is the latter two comparisons that are more informative. The comparisons indicate that the plant extracts are much less active than the commercial anthelmintics. However, it should be noted that while the anthelmintic drugs are pure compounds, the anthelmintic compounds in the plant extracts may represent only a very small proportion of the total material in the extract, and hence in terms of toxicity on the basis of weight of the bioactive compound(s) alone, the activity against worms could be much greater than indicated by the IC_{50} of the whole extract. Direct comparison with anthelmintic drugs will need to wait until the bioactives are identified and purified from plant extracts.

The role of tannins in the observed anthelmintic activity was examined by comparing toxicity of some abstracts with or without pretreatment with the tannin-removing polymer PVPP. Figure 4 shows the results for some water extracted samples, while Figure 5 presents data from ethanol extracted samples. It is apparent that the activity of some extracts was unaffected by the PVPP-treatment, indicating no role for tannins in the observed toxicity (eg. *Eremophila maculata*, *E. glabra*, *Rhagodia parabolica*), while in other cases the toxicity was significantly reduced following PVPP treatment (eg, *Myoporum platycarpum*, *Acacia pycnantha*, *Rhagodia crassifolia*), indicating that tannins contribute at least partly to the observed activity. In general the impact of PVPP treatment was greater for ethanol extracts than water extracts. All cases in which PVPP was assessed with ethanol extracted samples in Figure 5 showed a significant reduction in toxicity, compared to only selected plants for the water extractions (from Figure 4).

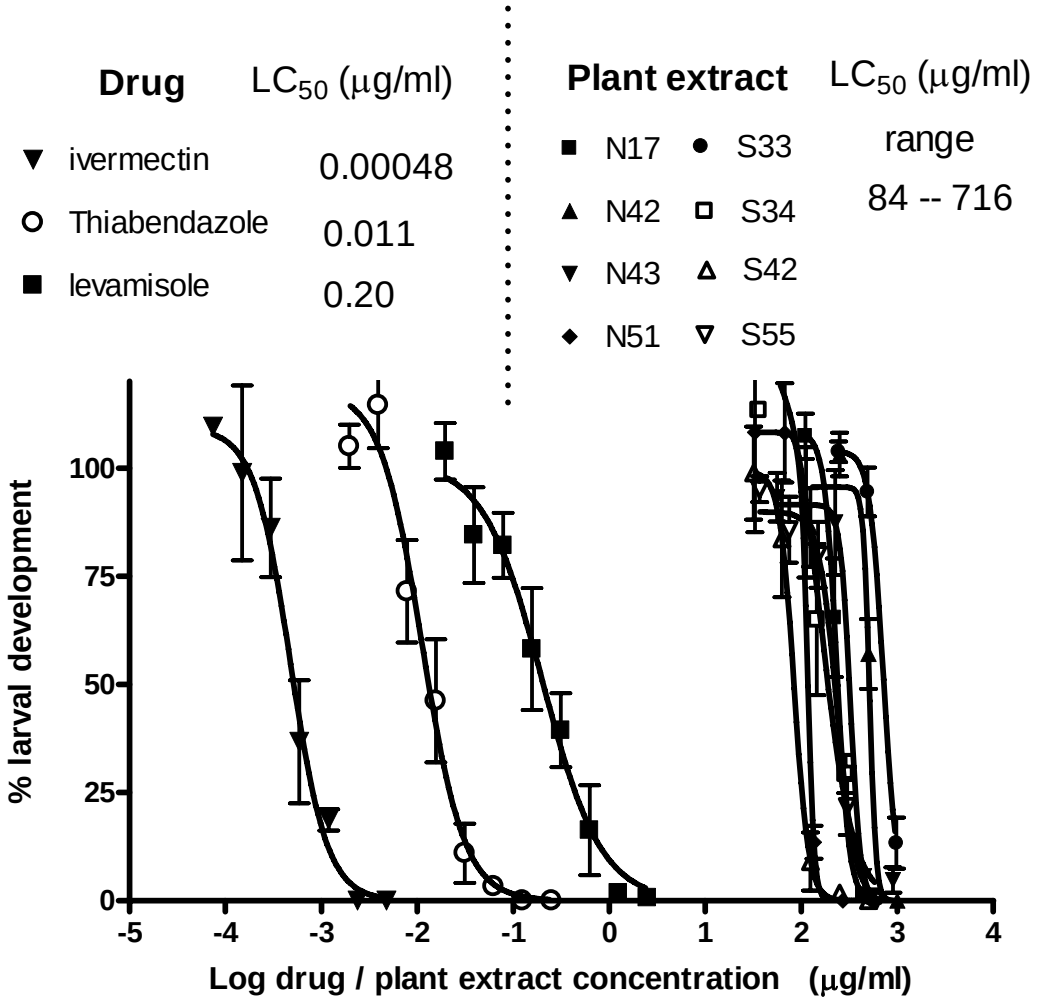

Figure 3. Anthelmintic activity of ethanol extracts of plant samples and commercial anthelmintic drugs. LC_{50} values represent the concentration of material required to reduce the larval development to 50 %.

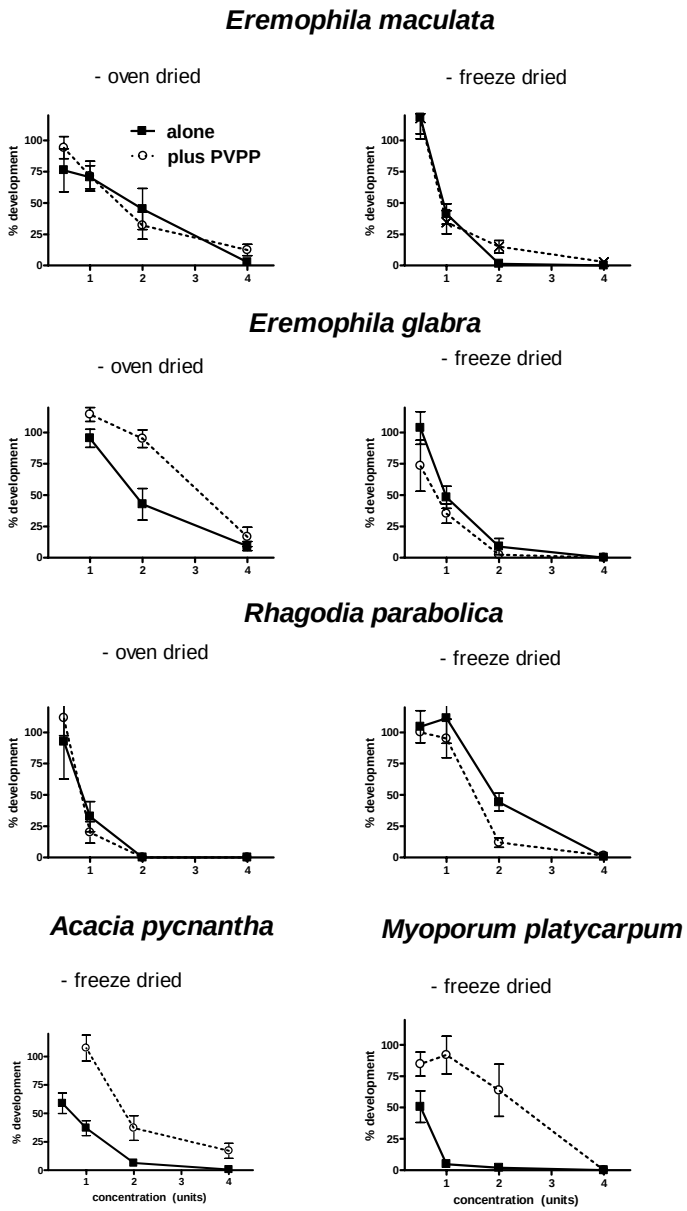

Figure 4. Effect of pretreatment with PVPP on the anthelmintic activity in water extracts of oven- or freeze-dried material from various plant species. Extracts not treated with PVPP are shown as solid lines, extracts pretreated with PVPP are shown as dotted lines. The X-axis represents arbitrary concentration units, with the highest concentration for assays in which 10 µl of plant extract was added to the assay, and lower concentrations representing 2- and 4-fold dilutions of extract before addition to the assay.

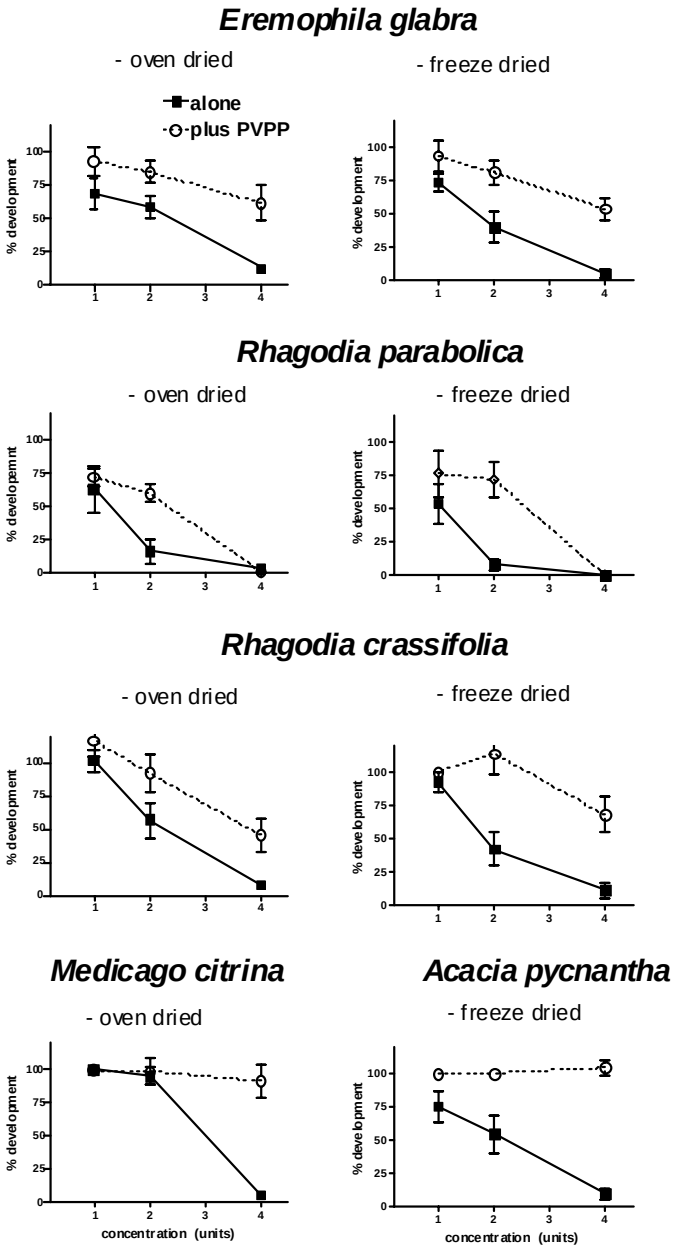

Figure 5. Effect of pretreatment with PVPP on the anthelmintic activity in ethanol extracts of oven- or freeze-dried material from various plant species. Extracts not treated with PVPP are shown as solid lines, extracts pretreated with PVPP are shown as dotted lines. The X-axis represents arbitrary concentration units, with the highest concentration for assays in which 10 µl of plant extract was added to the assay, and lower concentrations representing 2- and 4-fold dilutions of extract before addition to the assay.

Having identified some plant species with anthelmintic activity, we were interested in examining whether this activity varied among individual plants over time and space. That is, whether individual plants showed variation through the season, as well as whether activities differed between plants from the same species across different geographical zones, ranging from different parts of a single field to different places within a wider region (hundreds of kilometres wide). Figure 6 shows activity in water extracts from five *R. preissii* plants collected at the Badgingara field site in April 2007 and August 2007. The two plants in Figure 6A showed markedly different activities at the time of the first sampling, with plant 5 being not active against the worm larvae. These two plants both showed little change in activity between the two sampling times. On the other hand, plants 2,3 and 4 showed a significant increase in activity at the second sampling time compared to the first (Figure 6B). This was particularly marked for plants 3 and 4 which were designated as not active in April (larval development > 40 %) but both inhibited larval development significantly by August 2007. The difference in plant samples between the two time points may be associated with the increasing maturity of the plants as they approach flowering in October – January. On the other hand, the reason for differences existing between plants within a sampling time (for example, plants 1 and 5 at both time points) is less clear. Within each sampling time it was noted that there were some differences in the appearance of the plants, particularly leaf colour, and, to a lesser extent, leaf size and shape, however, it was not possible to associate appearance with presence of anthelmintic activity.

To further examine variation between plants of the same species we sampled from 94 *R. preissii* plants growing in a line over a distance of approximately 400 metres in a field at the Badgingarra field site in September 2007. Figure 7A shows that activity varied markedly between the plants. Significant activity was detected in approximately 70 % of the plants (larval development < 40 %) with the remainder showing only low or negligible anthelmintic activity. There were some differences in the appearance of the plants, however it was not possible to correlate this with anthelmintic activity. No obvious pattern of activity occurred with movement along the line of plants (Figure 7B), indicating the absence of a gradational change in activity that may be expected if soil type changed gradually along the line of plant sampling.

We also examined differences between plants sampled from a number of sites in South Australia. The plant species sampled had shown activity in our initial screens, except for *R. candolleana* which was collected here to further test the earlier observed presence and absence of activity in different species within the Rhagodia genus. The collection sites were spread over a distance of approximately 230 km in the Yorke Peninsula and mid north of South Australia, as well as several sites in the lower Murray region and near the Waite campus. Table 2 shows that significant variation existed between the plants in terms of their anthelmintic activity. The soil type at the collection sites showed a great deal of variation, with the following types recorded: sand, sandy loam, loam, and clayey sand. Soil pH was acid in some cases, and alkaline in others. The presence or absence of activity did not appear to correlate with soil type or pH. However, in several of the species there was a clear relationship between the stage of plant maturity and the presence of anthelmintic activity. The two *R. crassifolia* plants which showed toxicity were at the fruiting stage, while the two plants that were not active had not fruited, with one having flowered only and the other not yet flowering. The three *E. longifolia* plants showing activity were at the fruiting stage, while the two inactive plants had not yet commenced fruiting. There was no obvious discrimination in maturity within the ten *R. parabolica* plants as all were flowering but had not yet commenced fruiting. Table 2 also provided some confirmation of the different solubility patterns noted earlier in comparing water- and ethanol-extracted samples. *R. crassifolia* only showed activity in ethanol-extracted samples, while *E. longifolia* was only active in water-extracted samples.

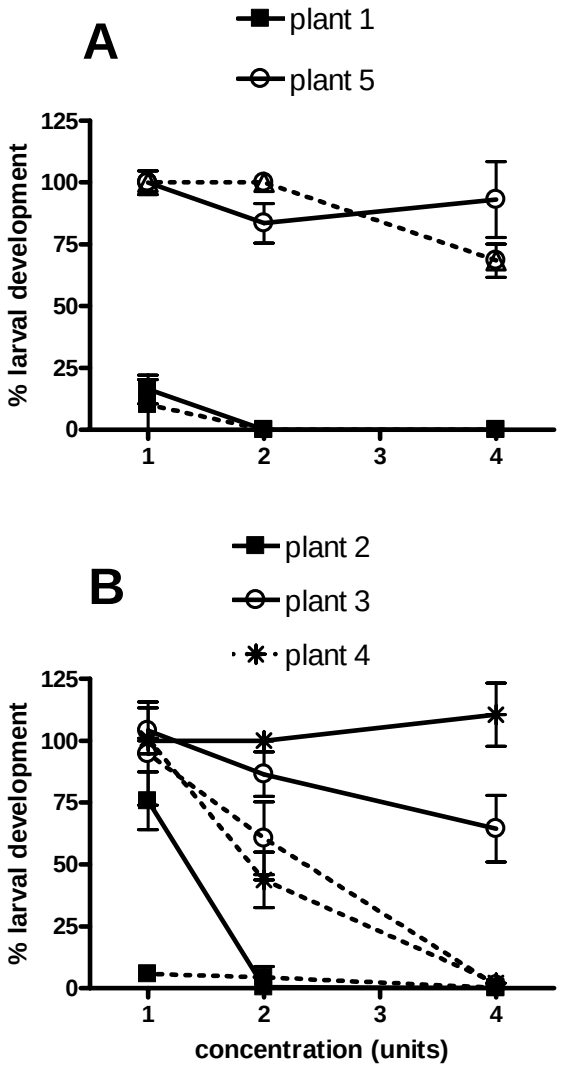

Figure 6. Anthelmintic activity in water extracts of 5 individual *R. preissii* plants sampled in April 2007 (solid lines) and again in August 2007 (dotted lines) at the Badgingarra field site in WA. For clarity, the plants are shown in separate panels, A and B

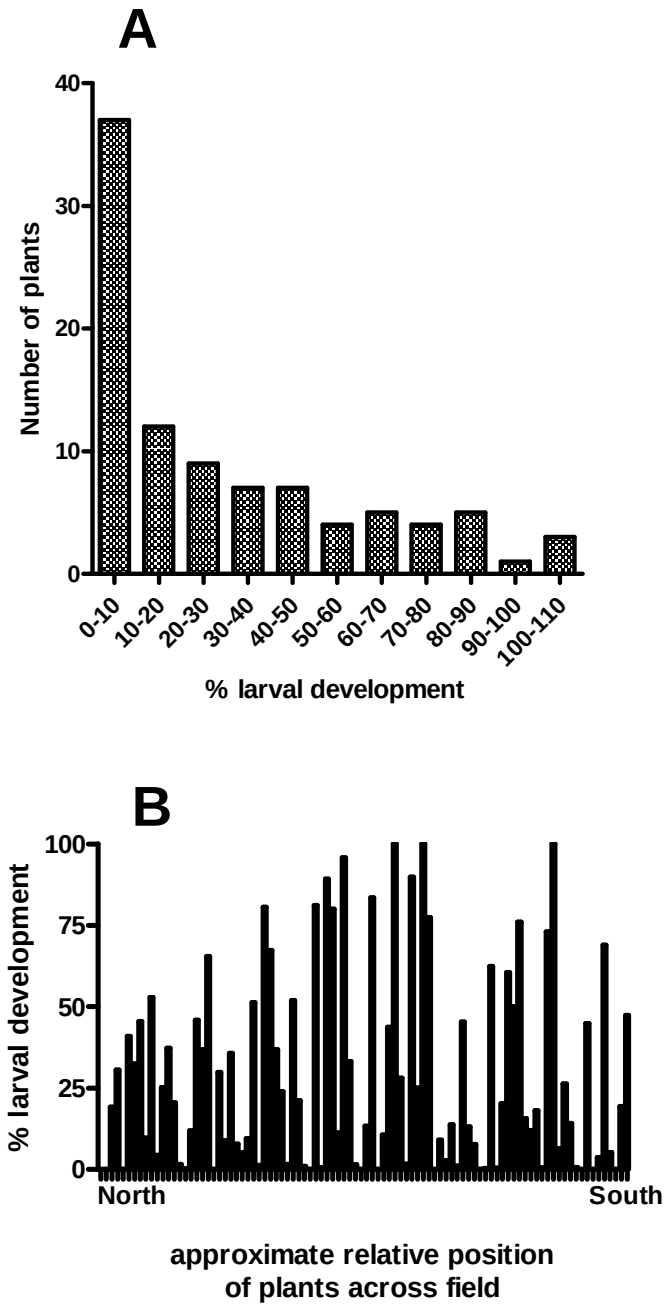

Figure 7. Anthelmintic activity in 94 *R. preissii* plants sampled in September 2007 at the Badgingara field site in WA.

A: frequency distribution of activity in individual plants

B: activity in plants collected in sequence in a line across the field from North to South

Table 2. Presence or absence of anthelmintic activity in extracts from 23 plants sampled in April 2007 in South Australia.

Species	Toxicity expected based on initial screening		H_2O extracts		EtOH extracts	
	H_2O extract	EtOH extract	Number toxic	Number non-toxic	Number toxic	Number non-toxic
R. parabolica	Yes	Yes	5	5	3	7
R. crassifolia	No	Yes		4	2	2
R. candolleana	No	No		1		1
E. longifolia	Yes	No	3	2		5
E. glabra	Yes	Yes		3		3

Given the variation seen in *R. preissii* plants in September 2007 (Figure 7), and the indications from Table 2 that plant maturity may be a factor in determining anthelmintic activity in some species, we further investigated this relationship by sampling a number of *R. preissii* plants at the Badgingarra site in February 2008. The samples were taken from both flowering and non-flowering plants, and from plants grouped at the two extremes of the field. Figure 8 shows that while most plants were highly active in the bioassays (median larval development within each grouping was less than 7 %), a small number of plants showed only low or negligible activity. However this absence of activity occurred in both flowering and non-flowering plants, and in plants from both areas within the field, suggesting that it was unrelated to plant maturity or environmental conditions. Of note was the fact that the median larval development for this combined data set was 3.6 % (n= 24), compared to a median of 18.6 % for the 94 plants which had been sampled earlier in the growing season in September 2007 (from Figure 7), suggesting a greater anthelmintic activity at the more mature plant stage sampled in early 2008.

We examined the effects of several plant extracts on the motility of adult worms in vitro. This is an important step in assessing the anthelmintic potential of the plants as it measures their toxicity towards the life stage of the parasite which actually lives within the sheep. Figure 9 shows that, while worms in control assays maintained motility throughout the 72 hours of the experiment, extracts from 3 plant species significantly reduced the worm motility. This indicates that these plant extracts show significant toxicity to adult worms. Interestingly, despite the Kennedia exemia extract showing toxicity to larvae, it did not affect adult worms. This experiment was only limited in scope, however, it clearly showed that larval toxicity, as indicated by the larval development assay, can in some cases translate into activity against the important adult worm stage. However, as indicated by the K. exemia extract there will likely be cases where activity is limited only to the free living stages.

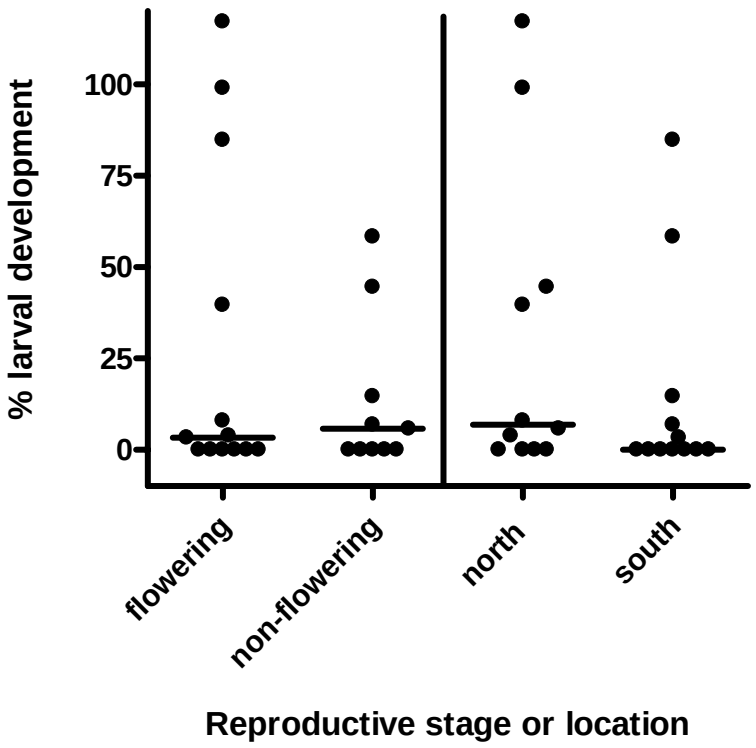

Figure 8. Anthelmintic activity in 24 *R. preissii* plants sampled in February 2008 at the Badgingara field site in WA. Plants are grouped either as flowering or non-flowering (left panel), or as growing at the north or south end of the field (right panel). Horizontal lines represent the median value for % larval development.

Discussion

This component of the Enrich project has indicated that there is significant anthelmintic activity in many of the shrub species examined. This suggests that there is potential for some shrubs to provide a degree of activity against worms in grazing systems.

To consider the principal findings and implications of the study:

1) Nature of bioactive compounds

We conducted experiments using PVPP to determine whether tannins contributed significantly to the observed anthelmintic activities. It was clear that the activity in some species was largely due to tannins, while activity in others did not involve tannins. There are many different types of plant secondary metabolites that could potentially act against nematodes, for example, tannins, saponins, polyphenols, alkaloids, glycosides (Athanasiadou and Kyriazakis 2004, Barrau et al 2005, Hoste et al 2006). The nature of the non-tannin components of the plants studied here is unknown, while the type of tannins present in the extracts responding to PVPP is also unknown. Further studies to identify and characterise the active agents would require some fractionation guided by *in vitro* bioassays.

The comparison of sample preparation and extraction techniques also indicated the presence of bioactives with quite different physical properties in the various plant extracts.

Some plants contained actives which were soluble in both water and ethanol, while some were soluble in only one system. Similarly, some plants contained actives which were stable to oven drying, while the active agents in others were only detected after freeze drying.

2) Variability in activity

It was apparent that the anthelmintic activity of some plants showed variability between samples. Some cases of variability were most likely related to plant maturity:

a. *R. preissii* shown in Figure 6; increasing activity nearer flowering time
b. The *R. crassifolia* and *E. longifolia* shown in Table 2, and described in the text; increased activity in more mature fruiting plants.

It is well known from the literature that levels of some plant secondary compounds may increase as plants mature, and hence it may not be surprising that the compounds toxic to nematode larvae are present at increased levels at flowering and fruiting times.

Examples of variation within a plant species at the one time point were also observed (Figures 6-8). This may relate to the presence of chemotypes within some species of plant. The group of *R. preissii* plants at Badgingara site were certainly not clonal, as reflected by differences noted in their appearance at the time of sampling for anthelmintic assays. It was noted by the plant collector that there were some differences in the appearance of the plants, particularly leaf colour, and, to a lesser extent, leaf size and shape. However, it was not possible to associate appearance with presence of anthelmintic activity. It is well known that chemotypes exist with plant species. A number of reports have described chemotypes which show differing toxicities to insects (Lattanzio et al 2000, Cheng et al 2004, van Leur et al 2008).

Plant variability may also relate to other factors which could affect the plant, for example, soil type, soil pH, recent insect damage, recent grazing. A controlled study in which all such data was recorded alongside anthelmintic activity may be required to provide explanations for the type of plant variability observed in the present study. Identifying the active agent in the plants would allow for a much deeper understanding of patterns of plant variability. An ability to quantify the bioactive would be a more accurate means of monitoring anthelmintic potential than reliance solely on the worm bioassay. Development of simple field tests for detection of bioactive levels would allow graziers to monitor their shrubs and make informed decisions as to the best time to graze stock for maximal anthelmintic benefits in their local environment

It is important to note that the observed plant variability should not at this stage be regarded as a negative that overrides the promise shown by the presence of significant anthelmintic activity in many of the plant species examined in the present study. A deeper understanding of the nature of the variability will enable it to be in fact utilised to maximise the anthelmintic effects of the plants. To consider two likely sources of variation:

a. plant maturity: If the patterns of anthelmintic activity during a season are known, then the use of plants as an anthelmintic component of the grazing system can be effectively managed so as to optimise their impact. For example, a defined pattern of activity associated with flowering and fruiting stages would enable effective use of the anthelmintic activity that those shrubs would provide at certain times through the year.

b. plant chemotype: If more active chemotypes are identified through closer study of the most active species identified in the present study, these could form the basis of seed or cutting populations for distribution to graziers. In this way, inherent variability in different lines of single species could be minimised.

Despite the observed cases of plant variation, the overall prevalence of significant *in vitro* anthelmintic activity seen in groups of plants from some species was quite marked, for example, the majority of *R. preissii* plants sampled in both August 2007 and February 2008 showing significant *in vitro* activity, alongside a smaller number with no, or very little, activity.

3) Translation from free-living worm life stages to adult stages.
The bioassay used for the screening aspect of this study examined the free-living life stage of the parasite. For effective control of parasites within a sheep, a bioactive must also show activity against the adult parasites as these are the life stage which actually infect the animals. The use of free-living larvae in the early stages of a bioactive or drug discovery project such as the present one is necessary due to the difficulty and expense associated with obtaining fresh adult worms for regular bioassays. The use of the free-living stage, is considered appropriate for such studies, and is used by animal health companies and research organisations in drug and bioactive discovery programmes for anthelmintics worldwide. Promising leads are then confirmed either by bioassay with adult worms in vitro, or directly by in vivo trials. Near the end of the current project a small number of adult worms became available, and some in vitro assays with the adult stage were performed (Figure 9). This showed that the larval activity of several extracts was translated to the adult stage, as these extracts showed significant toxicity towards adult worms. In another case (Kennedia eximia), larval activity did not translate into significant adult activity. This translation from larval to adult will need to be tested experimentally for the most promising plant species. Such an experimental path could be a component for the next stage of the Enrich project.

4) Translation from in vitro *to* in vivo
The record of translation of promising *in vitro* anthelmintic activity into useful *in vivo* activity is poor (Athanasiadou et al 2007). Host pharmacokinetics can greatly limit the amount of active material which actually reaches the parasite in the intestinal tract. Components of this include absorption into host and excretion via urine, absorption onto digesta material within the gut, host metabolism in the liver, microbial metabolism in the rumen, and pH effects. However, it should be noted that most cases in which promising *in vitro* activity is then examined *in vivo,* with negative outcomes, are based on examination of the potential for compounds to act as single dose drugs. In these cases the animal health company concerned is looking for a compound which will eliminate the entire (or at least 99%) worm population within a host animal after a single dose. The aims of the Enrich project are however somewhat different. We are looking for bioactives that will provide a degree of anthelmintic effect when ingested over a period by a host animal. Such a need could be satisfied by bioactives showing less intrinsic potency than the compounds required by animal health companies.

The potential for translation to *in vivo* effectiveness of a particular bioactive plant shown to be effective *in vitro* in the present study can only be tested experimentally. For this reason it is proposed that the logical further steps in the present work are to test the most promising *in vitro* candidates in *in vivo* experiments to assess their effects on work burdens in infected sheep.

Next stages in this work

The next stages of the work would involve *in vivo* trials and further *in vitro* assays. The *in vivo* trials would be required to confirm the promise shown by some plant species in the study to date, while *in vitro* assays would also be used to confirm and quantify the anthelmintic activity in plant material used for the trials, and to confirm activity against adult worms. *In vitro* assays would also be used to further examine plant maturity and chemotype variations in activity.

In vivo trials would be of two types:

a. pen trials. These would allow for feed intake to be monitored closely
b. field trials. These would be conducted on existing Enrich field sites in WA and SA, as well as on the properties of collaborating graziers. Several graziers have

expressed an interest in doing this type of work. The advantage of this use of grazier's properties is that the suitability of the plant species for the particular local environment would, in some cases, have already been confirmed by the grazier.

Trials would be conducted on plants which have shown promising *in vitro* results in the present phase of the Enrich project, and which are known to be suited to growing in particular environments. Hence the plant selection would be based on many of the criteria applied to plants by the different research groups involved in the Enrich project (egs, ease of propogation, growth characteristics, rumen bioactives). Such plants could then be assessed for anthelmintic activity *in vivo*.

If significant *in vivo* activity is demonstrated, then further work on that plant species would aim to determine what factors affect the level of bioactive compounds. This will allow for the use of anthelmintic plants to be managed effectively by graziers in order to maximise their toxicity to worms when ingested by sheep.

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Section 6

Grazing behaviour and management in diverse plant mixtures with shrubs

The concept of forage shrubs being successfully incorporated into a grazing system for multiple benefits – ranging from nutrient supply, bioactive effects on livestock, and improved natural resource management – implies, almost by definition, that multiple plant species will be required. This means we will need to understand the key principles that influence diet selection when a potentially diverse array of plants is grazed. The Badgingarra Research site has been established to allow a suite of grazing experiments to be conducted on a paddock scale.

The 'mixed forage site' at Badgingarra Research Station, WA was planted as seedlings in September 2006. Despite a dry spring and a typically dry summer high establishment rates were been achieved. The shrub species were chosen based on the availability of species in sufficient quantities, as the site was established at the start of the Enrich project before new data were available on the alternative species being grown and screened in the Enrich project. Seven shrub/tree species have been established, sown in rows 5 m apart with 5 rows per species (with two exceptions due to limited seedlings being available, see Figure 1): *Atriplex nummularia* (old mand saltbush), *Atriplex amnicola* (river saltbush), *Rhagodia preissii*, *Maireana brevifolia* (bluebush), *Allocasuarina hueglii*, *Acacia saligna* and tagasaste.
The two tree species (*Acacia saligna* and *Allocasuarina hueglii* will be kept to 'shrub size' through grazing and cutting if required. Tagasaste will be trimmed if necessary. To date, the *Acacia saligna* has been cut on one occasion in 2007 as it has grown much faster than all other species. The main experimental site is 11 hectares. 'Training plots' of individual shrub species have been established to one side of the main plot.

Warm-season perennial grasses (Rhodes and Gatton panic) were established in 2 x 25 m strips and are growing well. Annual pasture was sown in winter 2007 past week into the inter-row spaces between shrubs: French serradella (Margarita) and yellow serradella (Charano). Volunteer pasture includes capeweed, wild radish, and annual ryegrass.

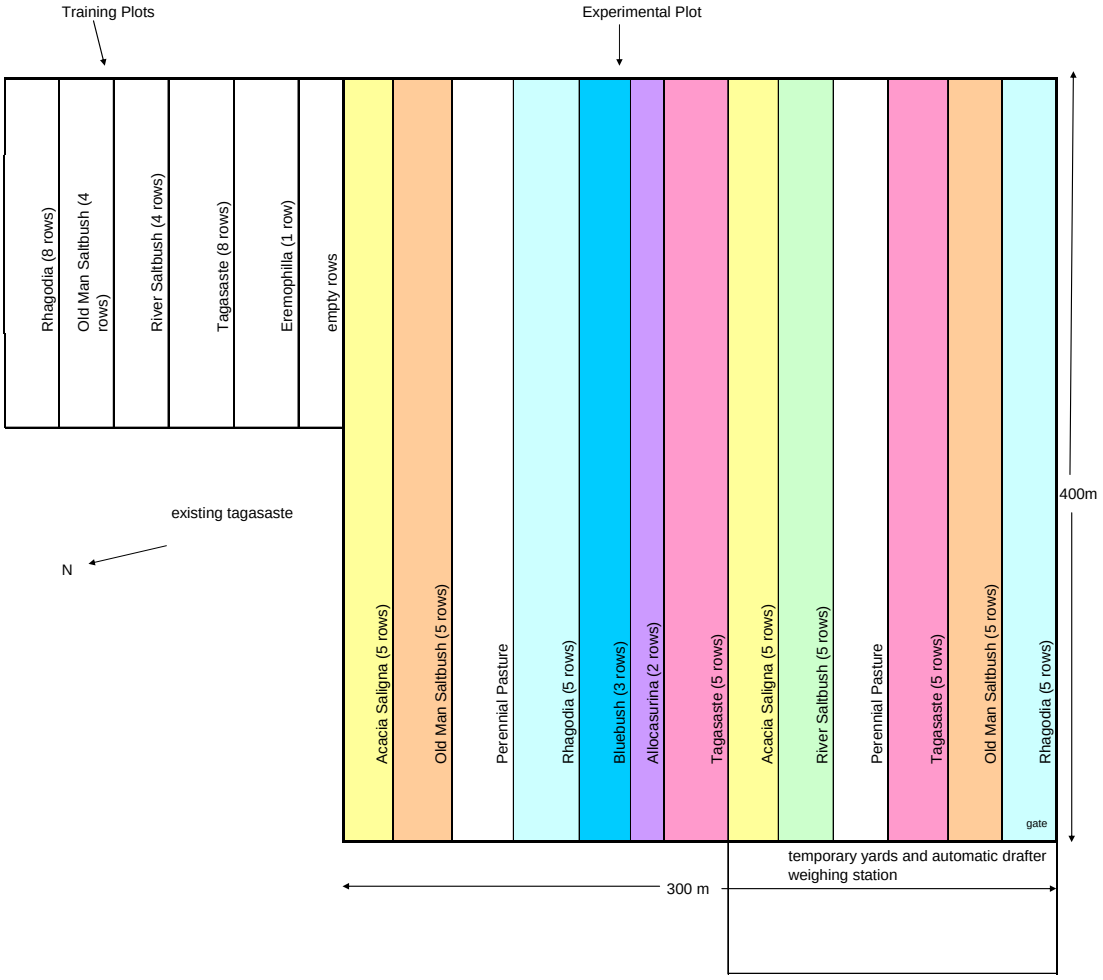


Figure 1. Layout of the Badgingarra mixed forage research site.

October 2006

March 2007

August 2007

April 2008

Figure 2. Development of the Badgingarra research site over about 18 months.

Experiment 1 (February 2008 – April 2008) – Influence of grazing pressure and duration on diet selection and animal performance

Hypothesis
Higher grazing intensity will increase the breadth of shrub species consumed in the diet of sheep offered a diverse range of species.

Rationale
Forage shrubs should be considered as a component of the feed base rather than the sole feed on offer for livestock. As the economic modelling and producer experiences (documented in section 1) indicate, forage shrubs can complement other pastures (perennial or annual) and help fill critical feed gaps. Also, within a period of time, livestock with a diverse rnage of plants from which to select are more likely to obtain a better balance (and seasonal profile) of nutrients and secondary compounds (Provenza et al, 2006). In both cases (i.e., filling a feed gap or providing diet diversity), it will be important that animals do not exhibit strong preferential grazing at the expense of plant persistence or longer term animal performance. Managers have multiple strategies to manipulate the composition of what is eaten by grazing livestock: stocking rate, grazing duration and providing animals with positive experiences of the forage. This experiment investigates the first two options, whilst the second experiment (reported in the next sub-section) focussed on the third.

Even though 100 sheep for 1 day is equivalent in terms of grazing days as 20 sheep for 5 days, the composition of the diet over time is unlikely to the same in both cases. This has implications to both recovery and persistence of plants in the mixture and animal productivity. We expected that more intensive grazing will lead to a consistently broader selection of plants by the animals because they will have less opportunity to be selective due to competition. With lower intensity, we expected animals to initially be more selective (i.e., have a narrower range of plants in their diet) but, over time when the preferred plants become less available, they will be forced to broaden their selection. If this pattern of diet selection occurs, we wanted to know if there would be an effect on liveweight gain.

Experimental design
220 wethers, born in 2005 (i.e., *c*. 2.5 years of age), were stratified by liveweight and randomly allocated to three treatment groups. All animals had *ad libitum* access to forage (pasture) dry matter, but the three groups differed in the stocking rate and duration of grazing. The sheep had access to all species of plants, pasture and shrubs (see earlier subsection for Badgingarra site layout).

Treatment 1 – 'LOW'. Set stocking, where animals will have an opportunity to exhibit strong selection preferences for particular plants species in the mixture. We chose 10 sheep per hectare, as this represents the district average for the annual average stocking rate. Two groups of 10 sheep each, with each group allocated 1 hectare. They commenced grazing on 4 February 2008 and grazed for 66 days (at which time food on offer was considered to be limiting). The animal's average starting weight was 43.9 kg.

Treatment 2 – 'MEDIUM'. Moderate intensity grazing: 67 sheep/ha. 100 sheep grazed 1.5 ha for 5 days (Monday-Friday), before moving onto a new 1.5 ha-plot the following week, and again in week 3. That is, the sheep experienced three rotations over the period of 4-22 February 2008. The animal's average starting weight 42.3 kg.

Treatment 3 – 'HIGH'. High intensity grazing: 333 sheep/ha. 100 sheep grazed 0.3 ha, moving onto a new 0.3 ha-plot on a daily basis. Animals in this 'HIGH' group had the same pasture allowance on a weekly basis (Monday-Friday) as those in the 'MOD' group. Their starting weight was 42.8 kg.

Results & Discussion

Shrub intake

The composition of the herbage on offer at the start of the grazing period was annual pasture growing in the inter-row spaces between the shrubs (70%) and perennial pasture sown in two 25 m wide strips (25%). Consequently, the total shrub biomass was only about 5% of the total herbage on offer. The composition (% of total shrub edible biomass) of the shrub component at the start of the grazing period was: *Acacia saligna* 49; *Rhagodia preissii* 28; old man saltbush 8; river saltbush 6; tagasaste 6; *Allocasuarina heugli* 2 and bluebush 1.

The total shrub consumption was 250 g DM/head/day in the HIGH group, 100 g DM/head/day in the MEDIUM group, and 50 g DM/head/day in the LOW groups. These levels of consumption represent about 16%, 7% and 3% of the total DM intake, which is a modest portion of the diet in terms of dry matter, but not necessarily such a modest contributor to the intake of particular nutrients or secondary compounds. The animals, especially those in the LOW and MEDIUM groups were not forced to eat shrubs because of limiting pasture dry matter. In fact, it was the availability of edible shrub material that was limiting, especially for tagasaste, old man saltbush, river saltbush and bluebush (Figure 1). This was immediately apparent in the HIGH and MEDIUM groups, whilst for the LOW group (set stocked), the intake of three of the shrub species was limited by the amount of edible biomass after 20 days of grazing: tagasaste, old man saltbush and river saltbush. At the midpoint of their grazing, about half of the bluebush and 30% of the *Allocasuarina* had been consumed and, by the end of grazing at day 66, about 70% of the edible bluebush and 50% of the edible *Allocasuarina* had been consumed (Figure 1).

The intake of *Allocasuarina* was more variable over time than for other plant species, but the reason for this is not known. For the HIGH group, *Allocasuarina* was readily consumed during the first week, with leaf removal representing 70-90% of the amount of edible biomass on offer, but intake of this plant declined to about 50% of the edible biomass on offer during the second week of grazing (Figure 1). For the MEDIUM group, it ranged between 10 and 70% of the edible biomass on offer.

Less than 10% of the edible *Rhagodia preissii* and *Acacia saligna* on offer were consumed by the HIGH or MEDIUM groups (Figure 1). A similar situation occurred with the LOW group. Less than 10% of the edible *Acacia saligna* on offer was consumed. At the midpoint of grazing (about 30-35 days), about 10% of the edible *Rhagodia preissii* had been consumed and, by day 66, about 25% of edible Rhagodia on offer had been consumed.

Composition of selected shrubs

An unexpected finding was that the composition of the shrubs selected by sheep, when adjusted for the different amounts of biomass on offer for the 7 shrub species, was remarkably consistent across the three levels of grazing pressure (Figure 2). The composition of selected shrubs only deviated markedly in the LOW group when the 5 more preferred shrub species had been heavily consumed and the animals increased their selection of less preferred plants.

When edible biomass of all species was not limiting, the five most preferred species (tagasaste, old man saltbush, river saltbush, bluebush and *Allocasuarina*) were eaten in approximately equal portions, although the selection of *Allocasuarina* declined over time for the HIGH group and fluctuated over time for the other two groups (Figure 2; $P<0.05$).

Grazing behaviour

Twenty animals in the MEDIUM group and in the HIGH group, and all animals in the LOW groups were monitored for grazing behaviour and diet selection, following the methodology of Altmann et al (1974) that involved observational ‘scan sampling’ every 20 minutes during

the morning grazing period Observations commenced at sunrise and continued until the animals camped after their morning meal(s), typically about 2-3 hours later. On most days, we obtained about 100 observations from each group over about 6-10 time points.

Contrary to estimates of diet selection based on measurable leaf removal, all seven shrub species were grazed throughout the experiment (Figure 3), including Acacia and Rhagodia which we identified as less preferred in the data reported above. This suggests that, for larger shrubs such as the *Acacia saligna* and, to a lesser extent, *Rhagodia preissii*, it is difficult to detect and quantify small amounts of leaf removal. It also suggests that animals may spend time 'inspecting' and sampling all species, which would be recorded as a grazing observation, but not necessary consuming the same amounts, which is reflected in leaf removal data. Consequently, observational data can be an important complementary data set to the more quantitative estimates of diet selection. The use of GPS tracking collars (reported in experiment 2 below) offers another practical tool for monitoring the location and behaviours of animals.

As summarised in Table 1, 2-6% of grazing time was spent with each of the shrub species, and this was consistent across all three grazing pressures. About one-third of grazing observations were of sheep consuming the perennial pasture which, again, was consistent across all three groups. Sheep in the LOW and HIGH groups spent about one-third of their grazing time consuming in the annual pasture inter-row, whilst sheep in the MEDIUM group spent half of their time in the annual pasture inter-row. This indicates an interaction between grazing pressure and consumption of annual inter-row, whereby the shrub and perennial grasses make up a greater portion of grazing observations at both extremes of grazing pressure than at a moderate grazing pressure. This warrants further investigation. The pattern of grazing observations was, in general, consistent across days within each of the three groups (Figure 3), with occasional spikes and troughs for any given shrub species or pasture type, consistent with the concept that livestock vary their intake from day-to-day as they seek an appropriate balance of plant nutrients and secondary compounds.

Table 1. The average proportion of grazing observations for each of the shrub species, perennial grass and annual inter-row pasture.

	Low grazing pressure		Medium grazing pressure		High grazing pressure	
	average	SE	average	SE	average	SE
Acacia saligna	0.02	0.010	0.03	0.009	0.08	0.018
Allocasuarina huegelii	0.05	0.018	0.04	0.017	0.02	0.007
Maireana brevifolia	0.04	0.021	0.03	0.007	0.01	0.004
Atriplex nummularia	0.04	0.015	0.03	0.007	0.06	0.016
Rhagodia preissii	0.02	0.009	0.03	0.011	0.02	0.010
Atriplex amnicola	0.02	0.010	0.01	0.009	0.05	0.014
Tagasaste	0.18	0.079	0.06	0.011	0.09	0.017
Perennial pasture	0.32	0.075	0.27	0.030	0.29	0.044
Annual pasture	0.29	0.055	0.51	0.039	0.37	0.047

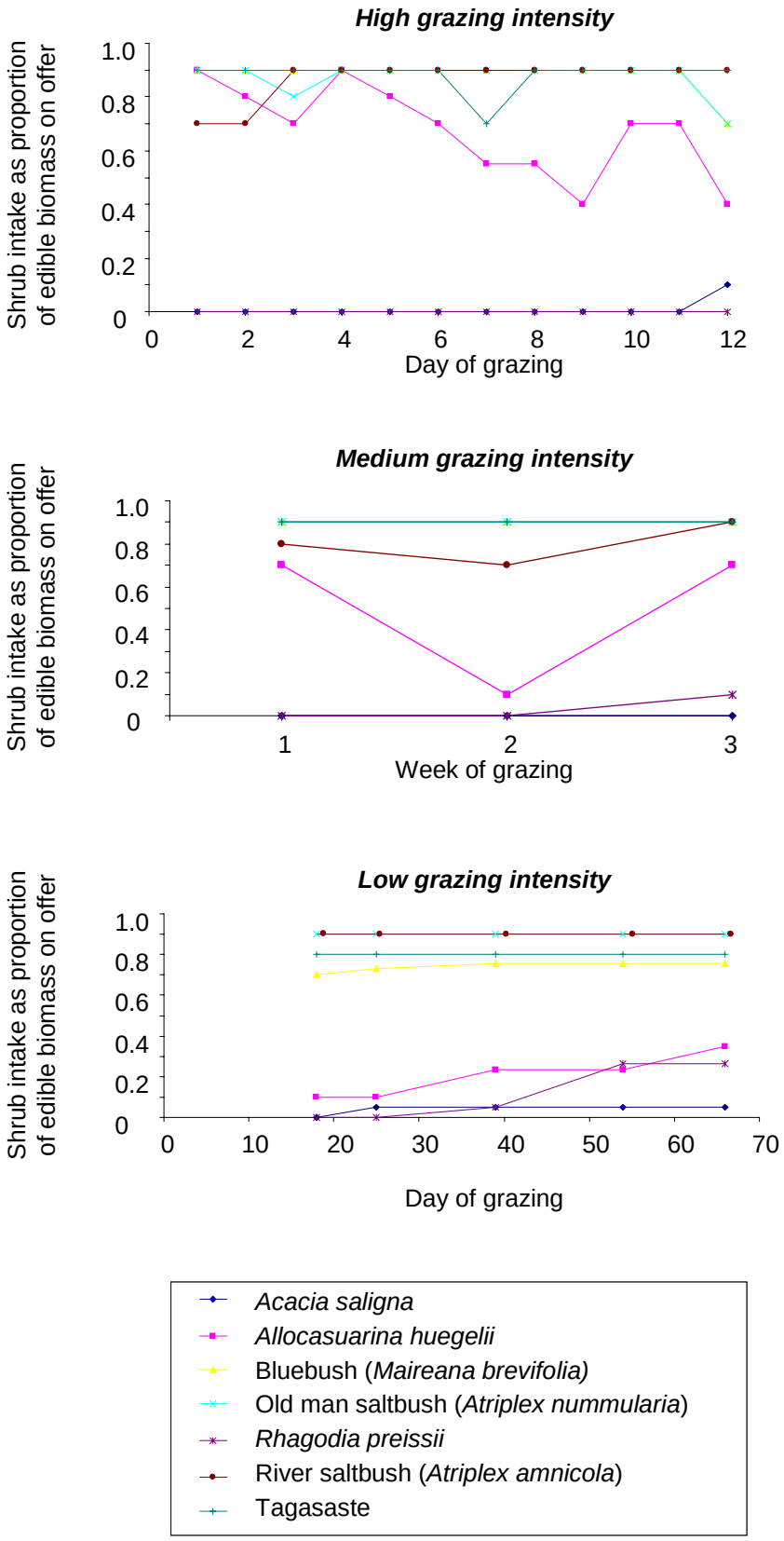


Figure 1. The intake of shrubs, for three grazing pressures, expressed as a proportion of the edible biomass on offer for each species.

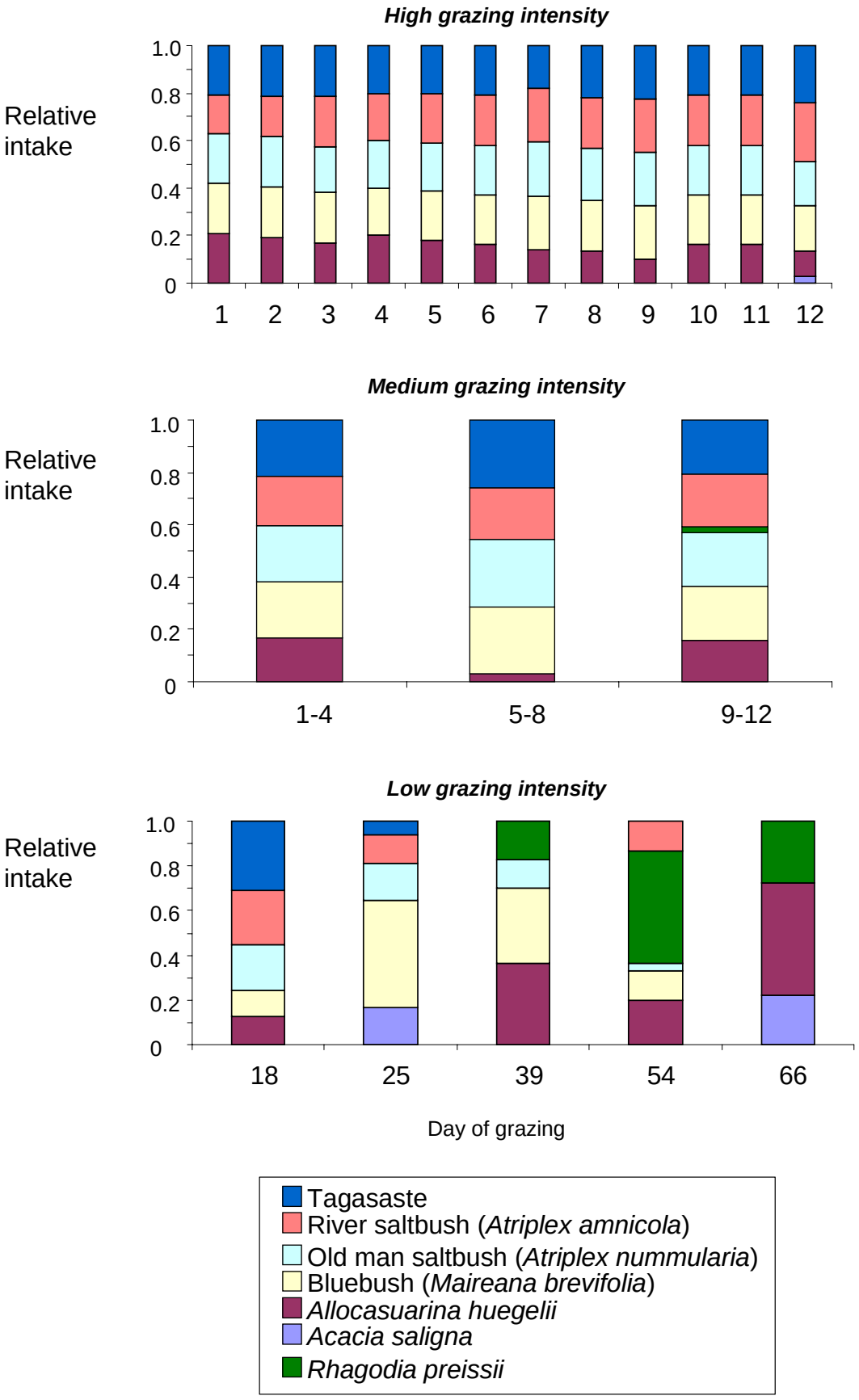


Figure 2. The composition of shrub intake, for three grazing pressures,corrected for the biomass of each species on offer.

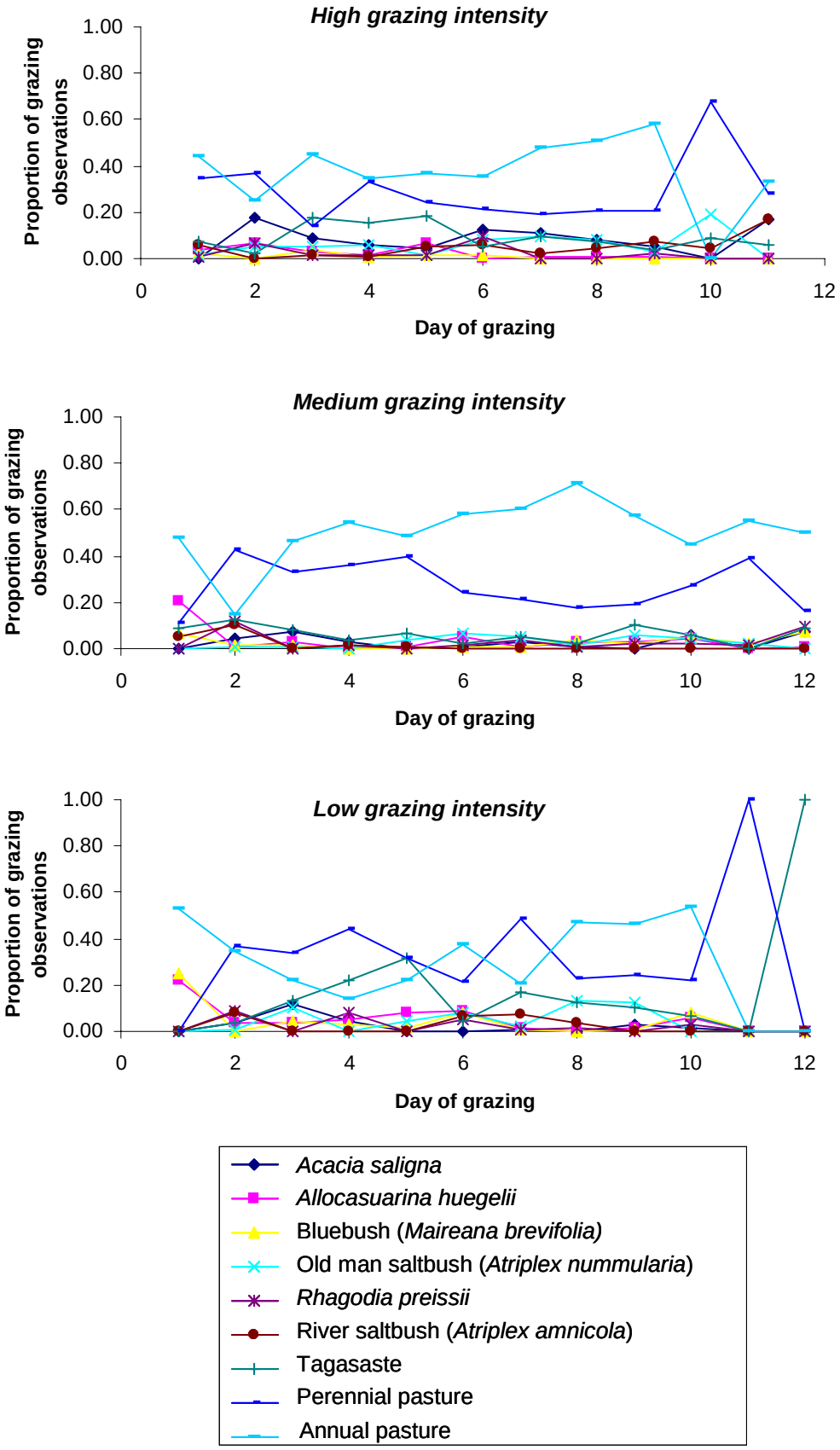


Figure 3. The proportion grazing observations that were attributed to each of the shrub species or pasture (annual and perennial) for three grazing pressures.

Live weight

All sheep initially lost weight when there were moved onto the experimental plots, but by week 2, they began gaining weight (Table 2). This is a typical adaption response to a new environment (in this case, new forages and paddock). We will follow up this finding in autumn 2009 by comparing the performance of experienced animals (i.e., those used in the 2008) with naïve animals.

There was a greater between-sheep variation in live weight in the LOW group than in the MEDIUM and HIGH groups. The co-efficient of variation for the LOW group ranged from 14 to 75%, whilst it was 6-18% for the other two groups. This suggests that when the animals had more opportunity to be selective, they took longer to start consuming adequate amounts of feed. However, after this initial difference, all sheep performed in a similar way.

In the third week, the time of day that the sheep were weighed had to be changed, and the animals almost certainly had a higher 'gut fill' for their third weighing. Overall, there were not large differences in live weight between the three groups, suggesting that different grazing managements (from set stocking to daily rotational grazing) can all be effective in at least maintaining live weight, and possibly increasing live weight in autumn without supplementary feeding. Longer grazing periods (beyond 3 weeks) are required to confirm this finding. We could not graze the MEDIUM and HIGH groups for longer in this case because of limited space, and we needed to leave enough feed for the next two experiments that were scheduled (reported below)

Table 2. Average daily gain (g/day) during the three weeks that all groups were grazing the mixed shrub site at Badgingarra (means and SEs).

Grazing pressure	4-11 February	11-18 February	4-18 February	18-22 February*
Low	n.a.	n.a.	-63 (47.6)	1050 (148.3)
Medium	-147 (24.1)	139 (25.6)		734 (43.8)
High	-229 (34.4)	133 (23.0)		667 (39.3)

*This final weight gain is erroneous because the time at which the animals were weighed accidentally changed between the 18th and 22nd February. However, a comparison across the three groups over this week is still valid.

The two groups of 10 sheep that were set stocked (10 sheep per ha) grazed their allocated plots for 2 months. There were able to maintain live weight throughout tis period. They were moved off the experimental site when feed availability, especially in one of the groups, became limiting. They returned to conventional senesced annual pastures else on the research station, and dropped live weight by 1-2 kg over 3 weeks (Figure 3).

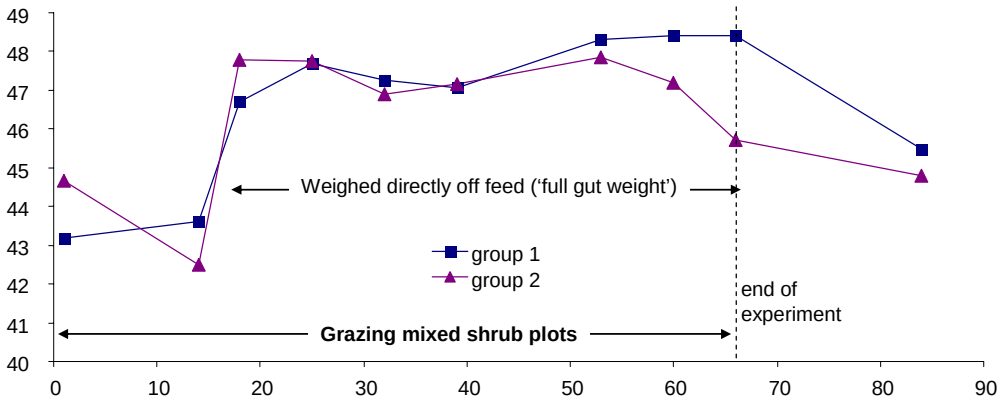


Figure 4. Live weight of sheep managed under 'Low' grazing pressure, which was set stocking at 10 sheep per hectare for about 2 months (early February-April 2008). Two groups of 10 sheep were used.

Concluding remarks and implications

Sheep chose to select a range of shrub species when given the opportunity. In our case, they typically consumed five of the seven shrub species on offer, and only began to consume the least preferred when the availability of the others became limiting. The combination of shrubs selected is not random, as we found that it was consistent across three different grazing pressures when feed on offer is not limiting. The implications of this diversity in the diet for animal performance and health will be further explored in the next phase of the Enrich project. Also, future work is planned to compare animal and plant performance with shrub combinations offered to sheep simultaneously or in rotation (sequence).

Increasing grazing pressure can increase the intake of shrubs. A high grazing pressure and fast rotation maintained a consistent intake pattern from day-to-day, which may have implications to plant persistence and recovery from grazing. A low grazing pressure leads to a more variable pattern of selected shrubs as the biomass of preferred plants becomes limiting over time. Interestingly however, this variable pattern of intake did not negatively affect the live weight of sheep.

After an initial period (1-2 weeks) when animals were first moved onto the shrub-based system), sheep in all grazing treatments approximately maintained live weight without supplementary feed over autumn. This is a particularly encouraging result because with conventional, senesced annual pasture species, expensive supplementary feeding would normally be required. Strategies for avoiding the initial, short-term decline in live weight should be investigated further; it is envisaged that this will be undertaken in Enrich phase 2. The value of shade from plants with a growth habit similar to *Acacia saligna* should also be investigated in subsequent work. The sheep were observed to be spending time amongst the Acacia although they did not consume much of this plant. However, its value in a diverse combination of plants may be through 'collateral' benefits such as improving the thermal environment of animals.

Sheep consuming old man saltbush

Sheep selecting young shoots of tagasaste

Sheep eating *Allocasuarina*

Sheep amongst *Rhagodia*

Fenceline comparisons of post grazing differences in pasture and shrub consumption.

Some individual *Rhagodia* plants eaten in week 3 by the 'Medium' intensity grazing group.

Experiment 2 (May – July 2008) – Modifying the learning experience

This experiment commenced in late May 2008 when we considered there to be sufficient re-growth of the shrubs and perennial pasture to permit another grazing of the experimental plots. The purpose of the experiment was to determine if we could increase the selection of *Rhagodia preissi* by sheep via a 3-week training period. We found in Experiment 1 that sheep did not consume much (if any) *Rhagodia preisii*. (This is consistent with producer experiences, who have noted that sheep reduce their intake of *Rhagodia* when it is flowering, which last season was from October to February.) In the third week, the 'Medium' group did begin to eat *Rhagodia*, but only in modest amounts. The set-stocked sheep began to consume *Rhagodia* during the last 3 weeks (i.e., after grazing for about 6 weeks). By the end of their grazing period, some individual *Rhagodia* plants had been stripped of leaves.

Experiment 2 was designed to modify the learning experience of animals by exposing them to *Rhagodia* in our 'training plots' with a nutritional supplement (200 g lupin grain/head/day). Three groups, each n=20, are being used:

1. TRAINED: The first grazed a stand of *Rhagodia preisii* for 3 weeks with a lupin supplement at 200 g/head/day prior to grazing the mixed forage plots. The reason for this group is our hypothesis that sheep can be trained to consume more *Rhagodia preisii* if they associate the novel plant with positive post-ingestive feedback (in this case, nutrients from lupins).
2. EXPERIENCED: The second group was 20 sheep that had been set-stocked in Experiment 1, thus providing a group 'self-trained' to consume *Rhagodia*.
3. NAÏVE: The third group had not been exposed to *Rhagodia* prior to grazing the mixture of forages in the experimental plot (i.e, a group naïve to *Rhagodia*).

All three groups, each with two replicates, grazed the mixed shrub system after the training period of group 1 was completed. Thirty *Rhagodia preissii* plants in the southern end and another 30 from the northern end of each plot were scored for leaf removal using a 0-5 scale, where score 0 corresponded with no leaf removal and score 5 was full leaf removal.

The timeline for this experiment is shown below:

Week of experiment	Group 1 TRAINED	Group 2 EXPRIENCED	Group 3 NAIVE
Week 1	Grazing *Rhagodia* with 200 g lupins/hd/day	Grazing 'conventional' paddock with 200 g lupins/hd/day	Grazing 'conventional' paddock with 200 g lupins/hd/day
Week 2	Grazing *Rhagodia* with 200 g lupins/hd/day	Grazing 'conventional' paddock with 200 g lupins/hd/day	Grazing 'conventional' paddock with 200 g lupins/hd/day
Week 3	Grazing *Rhagodia* with 200 g lupins/hd/day	Grazing 'conventional' paddock with 200 g lupins/hd/day	Grazing 'conventional' paddock with 200 g lupins/hd/day
Week 4-5	Grazing mixed shrub system – days 1-10	Grazing mixed shrub system – days 1-10	Grazing mixed shrub system – days 1-10
Week 5-6	Grazing mixed shrub system – days 11-20	Grazing mixed shrub system – days 11-20	Grazing mixed shrub system – days 11-20

Intake of Rhagodia when sheep grazed the mixed shrub assembly

Sheep in the TRAINED group did consume *Rhagodia preissii* during their training period, but less than we had anticipated. Therefore, training was more of an 'exposure' to the plant rather than the sheep eating considerable amounts. Further work on strategies to promote the intake of this plant, or other plants with low-moderate palatability, is warranted.

There were no differences in the estimated intake of *Rhagodia preissii* between the three groups when the sheep grazed the mixed assembly of shrubs. On average, each group consumed about 21% of the *Rhagodia preissii* on offer, but this varied from 5 to 40% for individual plots. We also found extremely large variation in leaf removal between individual plants within a grazing plot. This selectivity was not related to the animals' experiences of *Rhagodia preissii* (Table 1). Instead, we found that the location of the plants in the paddock had a profound effect on their selection. Plants in the southern portion of the experimental site were more heavily grazed than those in the northern portion (Figure 1). This emphasises the need for the evlaution of alternative plant species to be made at multiple sites, since the chemistry of the plants may vary from location to location.

Most of the Badgingarra site can be characterised as having a grey sandy topsoil of 0-10 cm, followed by a pale sandy layer of 10-40 cm and then sandy gravel. The pH was 5.0-5.5. A ridge extended across part of the southern end of the plot where the top soil was a sandy gravel with a clay layer 20-40 cm below the surface, and a pH of 4.0 These differences in soil type and pH may account for differences in plant chemistry, an issue that warrants further investigation, especially as it seems that relatively modest soil differences can lead to large differences in shrub palatability.

Table 1. Leaf removal scores for Rhagodia preissii using a 0-5 scale, where 0 corresponds with no leaf removal and 5 is full leaf removal.

	Average	SE
Group		
Trained	0.90	0.410
Experienced	0.69	0.264
Naïve	0.50	0.206
Shrub location in paddock		
South	1.24 [b]	0.257
North	0.15 [a]	0.057

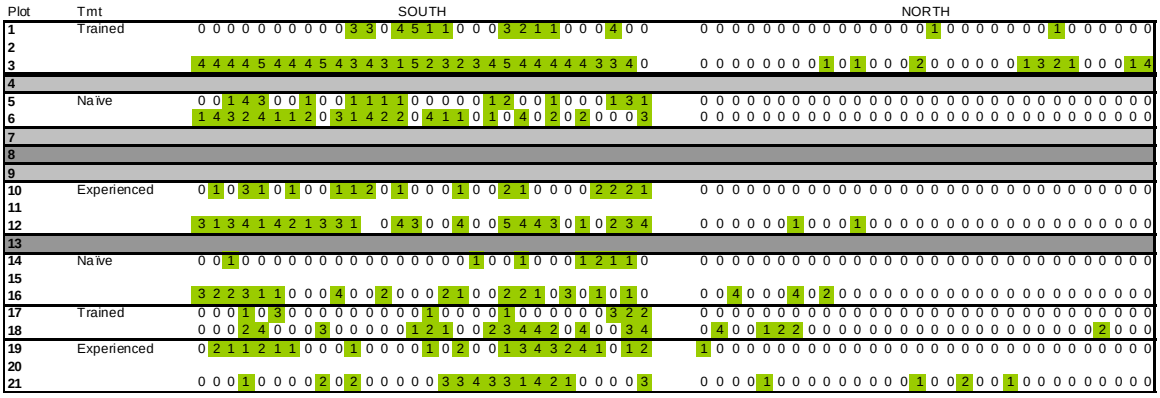
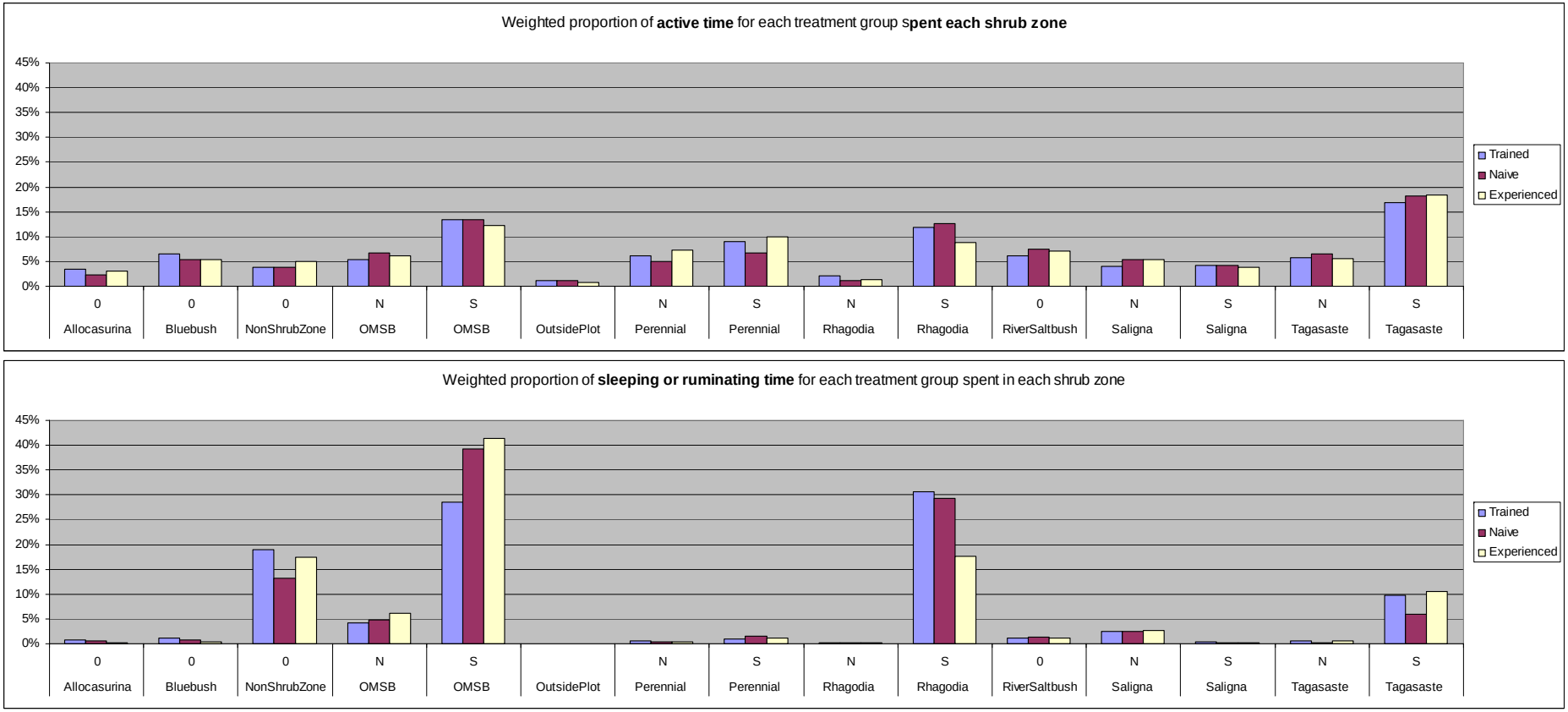


Figure 1. A schematic representation of the leaf removal scores of 30 Rhagodia preissii shrubs in the southern and northern parts of each plot each. A green square indicates some degree of leaf removal (i.e., score 1 or above). Leaf removal was assessed using a 0-5 scale, where score 0 corresponded with no leaf removal and score 5 was full leaf removal.

Additional information

During the grazing of the mixed assembly of shrubs, we fitted GPS tracking collars to 8 sheep, four in the trained group and two each in the experienced and naïve groups. The following figures are an example of some of the data we have compiled. The main purpose of including GPS collars in this experiment was to evaluate their potential for future studies on grazing behaviour and diet selection. The preliminary information is encouraging, and we intend to pursue this approach. The data below show the average proportion of each day that the sheep spent in each shrub zone, with the northern (N) and southern (S) plots demarcated for those species that had two 25 m-wide strips of plants. We have allocated data points as referring to 'active' sheep where successive GPS fixes show the animals have moved. We have allocated data points as 'sleeping/ruminating' animals if successive GPS fixes show the animal had not moved and if the 'pitch and roll' device in the collars indicated only a small movement. Animals sent about 5% of their active time in each of the shrub zones, although showed a preference for the southern plots of old man saltbush (OMSB), *Rhagodia preissii* and tagasaste. These three zones were also favoured for camping, as were areas within the plot that was not planted to shrubs (eg, the ends of the plots).



Experiment 3 (July-September 2008) – Can grazing *Rhagodia* preisii help to reduce intestinal parasites in sheep?

A full description of the experiment and implications is provided in the attached 'paper' prepared by Naomi Zadow as part of the requirements for her Honours degree in agriculture at UWA.

Grazing bioactive forage shrubs (*Rhagodia preisii)* to reduce parasitic burdens in sheep.

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Selectivity and intake of individual shrubs may influence the success of using *Rhagodia preisii* to control parasites in sheep under field conditions.

E. N. Zadow, N. Phillips, A. Toovey, P.E. Vercoe, A. Williams, A. Kotze and D.K. Revell.

Keywords: gastrointestinal parasites, *Rhagodia preisii,* grazing trial

Abstract. *Rhagodia preisii* is an Australian native perennial shrub currently under investigation as a forage shrub in lower rainfall regions of southern Australia. The benefits from including more perennials in these grazing systems include filling the summer-autumn feed gap, providing shelter, reducing soil erosion and dryland salinity. Initial *in vitro* results suggest that the *R. preissii* may have anti-parasitic properties, a trait that would add value to its role in a grazing system. We tested the hypothesis that including *R. preisii* in the diet of parasitised sheep will reduce their worm burden *in vivo*. Four groups of ten Merino wethers were grazed for seven weeks on plots of either *Rhagodia* or annual pasture. All animals received a chemical drench at the start of the experiment to remove existing parasites, and then half of the animals in the treatment and control groups received a single oral dose of 30 000 infective *T. columbriformis* larvae. Faecal egg counts were conducted prior to grazing and at weeks 3, 4, 5, 6 and 7 to measure differences in the worm burden between groups. Feed intake, protein content and animal performance was also measured. Grazing *R. preisii* did not reduce worm burdens of parasitised sheep. Large differences in FEC within the parasitised groups, was attributed to differences in the immunity of individual animals. Parasitised and non-parasitised sheep ate the same amount of *R. preissii* (180 ± 46 and 170 ± 46 g DM/sheep/day). The percentage of shrubs that were eaten ranged from 18% to 47% across plots, with one exception in which 80% of the shrubs were eaten. The *in vitro* toxicity to parasite larvae of *R. preissii* extracts from uneaten and eaten shrubs was not significantly different, indicating that toxicity is not linked to palatability. Overall only 23% of the 120 extracts inhibited larval development to 40% or less of the controls. Protein intake was estimated to be at least 10.4% of daily feed intake for control sheep and 13.7% for treatment sheep and this may have increased immunity, reducing the likelihood of detecting any anthelmintic effect due to *R. preissii*. The likelihood of observing an anthelmintic effect in the field will be dependent on increasing the intake of *R. preissii* which requires a better understanding of the plant's chemistry. This will assist in designing a grazing system that integrates animal selectivity, complementary forages and the best grazing strategies to capitalise on apparent anthelmintic effects of *R. preissii* in a commercial operation.

1. Introduction

Annual pasture species are the basis of grazing systems in low to medium rainfall regions of south-western Australia. This region features a Mediterranean climate, which is characterised by low winter-dominant rainfall, warm to hot summers, mild winters and high solar radiation especially in summer (Cramb 2000). Consequently, pasture quality and quantity fluctuates throughout the year and is lowest during summer and autumn (Purser 1980) making supplementary feeding essential. Other consequences of an annual-based system can include soil erosion associated with a lack of ground cover during the non-growing season, and groundwater recharge that exacerbates salinity problems (Clarke *et al.* 2002). Climate change and increasing input costs may add further pressure to develop more sustainable, lower input systems. Including more summer-active perennial plants may help fill the feed gap in summer and autumn, and provide an environmental management tool through reducing groundwater recharge, stabilising soil structure and reducing soil erosion (Moore 2006). Perennial shrubs may offer particular benefits due to their capacity to tolerate long dry periods and access water deeper in the soil profile (Barrett-Lennard and Malcolm 1999; Knight *et al.* 2002) and they can also provide shade and shelter for livestock (Bird *et al.* 1992).

There are few examples of widespread use of perennial forage shrubs in low-medium rainfall regions of south-western Australia, with the exceptions of tagasaste (*Chamaecytisus palmensis*) and saltbush (*Atriplex* spp.) (Wiley 2006). This is despite the native shrubs of this region being well-adapted to nutrient poor soils (Foulds 1993) and drought conditions (Veneklaas and Poot 2003). The main reason that native shrubs have not been exploited by agricultural grazing systems is that they have traditionally been evaluated solely on their nutritive value and edible biomass production. Consequently, species with lower nutritive values and edible biomass have been overlooked. These plants, however, could justify their place in a mixture of complementary forage species by providing other production benefits attributed to plant secondary compounds, such as reducing worm burdens (Githiori *et al.* 2006) or improving gut health (Durmic *et al.* 2007).

Rhagodia preissii is one shrub species currently being valued for its potential to fulfil a dual or multipurpose role in grazing systems in Western Australia. A range of species of *Rhagodia* are distributed throughout Western Australia but *R. preissii* is the first to be commercially grown and adopted by growers, who are seeking forage plants that are more tolerant to drought than the pasture species they currently use. *R. preissii* performs well in nutrient poor

soils, is drought tolerant and produces substantial dry matter of reasonable nutritive value with up to 23% protein and an estimated digestibility of 60-65% (H. Norman, personal communication). Of particular interest however, is its potential activity against sheep gastrointestinal parasites. Initial results from an *in vitro* larvae mobility assay, which tested extracts of *R. preisii* against gastrointestinal worm larvae, indicated a positive antiparasitic effect (A. Kotze and J. O'Grady, personal communication). With the advent of drench resistance in Australia (Besier and Love 2003) and world wide (Jabbar *et al.* 2006), forage plants with antiparasitic properties may offer one practical solution in an integrated management plan to control gastrointestinal parasites in livestock (Anthanasiadou and Kyriazakis 2004; Jackson and Miller 2006). The aim of this study was to determine whether the positive antiparasitic effect observed *in vitro* could be replicated *in vivo* by grazing *Rhagodia preisii* in a field situation. We compared parasite egg counts in sheep that grazed *R. preissii* with sheep that grazed conventional annual pasture species and hypothesised that including *R. preissii* in the diet of parasitised sheep will reduce their parasite burdens *in vivo*.

2. Materials and Methods

2.1. Experimental design

The experiment was conducted at the Badgingarra Research Station, Department of Agriculture and Food Western Australia (approximately 200 km north of Perth; 30º 19' S, 115º 32' E) from July-September 2008. Stands of *R. preissii* shrubs were established in September 2006. Treatment groups grazed plots of *R. preissii* with an inter-row of annual pasture species, and the control groups grazed plots of annual pasture only. On day 1 of the experiment sheep were randomly allocated by body weight (obtained 2 weeks prior) into four groups of 10 sheep. Sheep were rotationally grazed for seven weeks, with a new plot every week. Two groups grazed plots of *R. preissii* with an annual pasture inter-row (treatment) and two grazed plots of annual pasture (control). Half of each grazing treatment group received a single oral dose of 30 000 infective *Trichostrongylus columbriformis* larvae on day 4. We designed the experiment so that the sheep in the treatment group would be consuming *R. preissii* when the parasite larvae were administered.

2.2. Animals

Forty two-year-old merino wethers (mean liveweight 52.35 ± 0.04 kg) were selected from a larger flock of sheep that had experience in grazing *R. preissii* from a previous experiment at the same site. Sheep were drenched orally with 13 mL of Q-drench (Abamectin

1g/L, Albendazole 25 g/L, Closantel 37.5 g/L. Levamisole 40 g/L; Jurox Pty Ltd, AUS; 1 mL/5 kg liveweight) and placed in a clean paddock for two weeks prior to the experiment (21st of July) to remove any existing parasite burdens.

Weekly liveweights and body condition scores were used to monitor animal performance throughout the experiment. This was done at the same time of day each week to minimise variation in live weight due to gut fill.

Individual faecal samples were collected *per* rectum for faecal egg counts (FEC) on day 0 to ensure all sheep were parasite free and then weekly from week 3 to week 7 to assess the parasite burden of the four groups. Sheep were removed from their plots at the end of week 7 and were drenched with 12 mL of Cydectin SE (Moxidectin 1g/L + sodium selenate; Fort Dodge Pty Ltd, AUS; 1 mL/5 kg liveweight). From week 2 onwards the control groups were fed 100 g of lupins per day and the treatment groups 200 g/d, to correct for a decline in liveweight caused by a limited supply of pasture in the plots.

2.3. Pasture and shrubs

The composition of the annual pasture consisted mostly of capeweed with smaller quantities of radish, ryegrass, perennial grass species, serradella and blue lupins. The plot size of the control plots varied from 525 to 880 m^2 and treatment plots from 645 to 1400 m^2. The size was altered to provide enough pasture for a week in the control plots, but was restricted in the treatment group to encourage the sheep to consume *R. preissii*. Each plot was fenced with a combination of wire and electric fences and fresh water was supplied *ad libitum*.

The annual pasture biomass in the control plots and the inter-row of treatment plots was estimated by randomly cutting five quadrats (0.33 x 0.33 m) in a W transect before and after grazing. Hand shears were used to cut all above-ground vegetable matter. The growth rate of the annual pasture in the control and treatment plots was estimated from quadrat cuts taken within five pasture exclusion cages, placed in a W transect, in the control and treatment plots.

Each treatment plot contained an average of 114 Rhagodia shrubs. The biomass of eaten and uneaten shrubs was estimated using a modified 'Adelaide technique' (Andrew *et al.* 1979). The growth rate of *R. preissii* from the beginning (week 1) and end (week 7) of the experimental period was estimated using this technique on 40 bushes on an adjacent block of ungrazed *R. preissii*. Leaf samples of *R. preissii* from five eaten and five uneaten bushes in

each plot (each week) were collected to determine if there was a difference in *in vitro* antiparasitic activity.

All pasture and *R. preissii* samples were dried in a 65˚C oven for 3 days, sieved to remove sand, and weighed. Eaten *R. preissii* and weekly bulked pasture samples from the control and treatment plots were ground through a 1 mm sieve for nitrogen analysis and eaten and uneaten Rhagodia samples for a larval development assay.

Dry matter intake was estimated by subtracting the biomass (pasture or *R. preissii)* remaining at the end of each grazing period (weekly) from the biomass on offer at the start, corrected for the growth rate of the pasture or *R. preissii* shrubs. Samples of pasture collected in weeks 1, 4 and 7 from the control plots and weeks 1, 4, 5, 6 and 7 from the treatment plots were bulked and sub-sampled for analysis of nitrogen content. More samples from the treatment plots were analysed to better represent the variation in pasture used by this group over the course of the experiment. Sub-samples from eaten *R. preissii* shrubs collected in weeks 1, 4, 5, 6 and 7 from the treatment plots were also bulked, and sub-sampled for nitrogen analysis. Nitrogen content was determined in duplicate samples using a combustion technique with an Elementor instrument (Waite Analytical Services, Adelaide). Crude protein (%) was calculated by multiplying total N (%) by 6.25, and CP intake estimated by multiplying with estimated dry matter feed intake. The average protein content of *R. preissii*, control annual pasture and the inter-row annual pasture in the *R. preissii* plots was 13.8% ± 0.21, 8.5% ± 0.48 and 8.8% ± 0.15.

The parasite load of the annual pasture in the control and treatment plots and the Rhagodia bushes in the treatment plot was assessed before grazing commenced (16th July). A 500 g sample of the control pasture and 250 g sample of both the treatment inter-row pasture and *R. preissii* leaf matter were collected following the technique of (Taylor 1939). An ‘N’ transect was traversed plucking pasture samples close to the ground from in front, behind and to the right and left at each stop. When sampling the Rhagodia bushes, 4 samples were plucked from different places around the bush. Samples were placed in paper bags and refrigerated until they were analysed 7 days later by Albany Animal Health Laboratories (Department of Agriculture and Food Western Australia).

2.4. Parasites and faecal egg counts

Stage three (L3) *Trichostrongylus columbriformis* larvae were sourced from Dr Malcom Knox, CSIRO Livestock Industries, Armidale, NSW. The solution was diluted with water to make a concentration of approximately 1000 larvae/mL. This was administrated orally in three x 10 mL doses using a 15mL syringe with a T attachment on day 4. The dosage of larva (30 000) was selected based on previous work (Miller *et al.* 2000; Steel *et al.* 1980) that induced a moderate worm burden of 400-1200 and 5000-10000 eggs per gram (epg) faeces respectively.

Faecal samples were refrigerated and faecal worm egg counts (FEC) conducted the next day. The number of epg of fresh faeces were determined using a flotation technique (Whitlock 1948). Two grams of faeces was soaked in water and then mixed thoroughly with saturated salt solution to make the total volume up to 60 mL. After mixing to ensure an even suspension, a sub-sample was pipetted into a 0.6 mL egg counting slide. The number of eggs recorded was multiplied by a factor of 50 to calculate the number of eggs per gram (epg) fresh.

2.5. Larval development assay

2.5.1 Plant material

Dried, ground sub-samples of individual eaten (n = 60) and uneaten (n = 60) *R. preisii* (100 mg) was weighed into 2 ml microcentrifuge tubes, and 1 mL distilled water added, and the tubes were placed onto a roller wheel for 48 hours at room temperature. The mixture was then centrifuged at 10,000 rpm for 30 minutes and the supernatant recovered, and stored at 20ºC for use in bioassays.

2.5.2 Parasite egg recovery

The *Haemonchus contortus* used for this study were from the anthelmintic susceptible Kirby1982 strain, isolated from the field at the University of New England Kirby Research Farm (as described by Albers and Burgess (1988)). Infected animals were housed at the McMaster Laboratories of CSIRO Livestock Industries, Armidale, New South Wales. Sheep faeces in vacuum-sealed bags were sent by courier to the Queensland Biosciences Precinct laboratory at St. Lucia, Brisbane. Nematode eggs were recovered from the faeces by passage through a series of fine sieves (250 μm and 75 μm) followed by centrifugation in a stepwise sucrose gradient (10, 25 and 40% sucrose). The eggs were recovered from the interface

between the 10 and 25% sucrose layers, and washed over a 25 μm sieve with water to remove residual sucrose. They were agitated gently in a solution of 8.4 mg/L sodium hypochlorite for 12 minutes, and then washed again with copious amounts of water. The eggs were diluted in distilled water at a concentration of 50-60 eggs per 30 μL after the addition of amphotericin B (final concentration 25.0 μg/mL) and tylosin tartrate (final concentration 800 μg/ml) , and used immediately for larval development assays.

2.5.3 Nematode larval development assay

Agar (200 μL of 2% (w/v)) was added to each well of 96-well assay plates, and allowed to solidify. Worm egg solution (30 μL) was then added to each well, followed by aliquots of plant extract (10 μL) (control wells received water only).

The plates were placed into bags to prevent drying, and incubated overnight at 26^{o}C. The next day, 2 μL of a growth medium, consisting of Earle's salt solution (10% v/v), yeast extract (1% w/v), sodium bicarbonate (1 mM) and saline solution (0.9% sodium chloride w/v) as described by (Hubert and Kerboeuf 1984) was added, along with 2 μL of a solution of *E. coli* cells (XL1-Blue® Stratagene) in LB broth (Maniatis *et al.* 1982), and 6 μL of water. The plates were incubated for a further 5 days, and then the larvae were killed using Lugol's iodine solution (10 μL per well). The number of fully developed infective stage larvae (L3) present in each well was counted. Percent larval development was calculated by expressing the numbers of larvae in treated wells (mean of 3 samples/plant extract) as a percentage of the mean number in control wells.

2.6. Statistics

All statistical analyses were conducted using Genstat 10.2 (Lawes Agricultural Trust, 2007) with a significance level of 95%. Liveweight and body condition scores (BCS) are reported as arithmetic means with SE and were analysed with a mixed model (REML) with repeated measures with the treatment (*R. preissii* or annual pasture), parasite dose, individual sheep and time as factors. Faecal egg counts (FEC) were log-transformed ($\log_{10}(x+1)$) before statistical analysis to normalise the data. The effect of grazing *R. preissii* on FEC between week 4 and 7 for the parasitised sheep only was tested with one-way analysis of variance (ANOVA) at individual time points. This analysis was repeated omitting individual animals that had received the dose of parasite larvae but maintained a FEC of less than 100 epg. One-way ANOVA was used to compare non-parasitised with parasitised sheep for the estimated daily intake of *R. preissii* and the percentage of *R. preissii* shrubs that were grazed, using plots as replicates. The toxicity of eaten and uneaten *R. preissii* extracts to parasite larvae

(LDA) were analysed with a 2-way ANOVA, using plot number and plant selectivity as factors, and T-tests were performed to compare the eaten and uneaten shrubs from each plot. Transformed data are reported in the results as backtransformed means and S.E., while all other data are reported as arithmetic means with S.E.

3. Results

3.1 Parasite Burden

Faecal egg counts over the course of the 7 weeks of the experiment are shown in Fig. 1. The parasitised groups had a higher FEC than the non-parasitised groups in week 4, 5 and 6 ($p \leq 0.05$) but not in weeks 1-3 or in week 7. Worm eggs were first observed in the faeces of parasitised sheep in high number in week 4 and peaked in week 5, at an average of 1537 epg in the control group and 800 epg in the treatment group. FEC declined by week 7 by between 23-54%. There was a large variation in FEC within the parasitised groups, with a co-efficient of variation for weeks 4 to 7 of 130-205%. There was no difference between the two parasitised groups over time ($p = 0.796$), even when individual sheep that had been dosed with larvae but had low FEC values (< 100 epg) were omitted from the analysis.

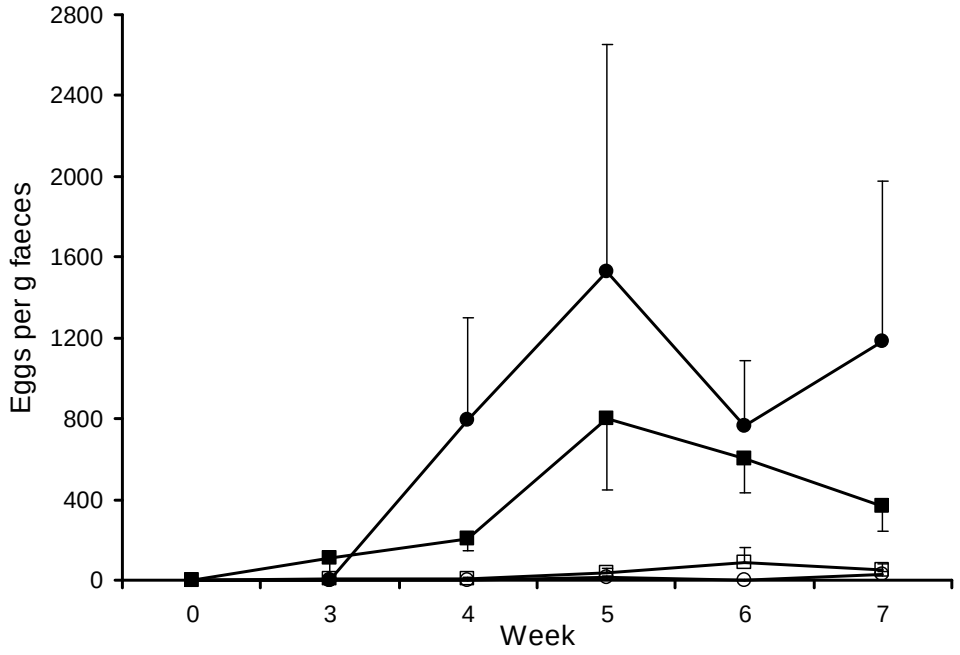

Fig. 1. Backward transformed means of faecal egg counts (eggs per g faeces ± SE) of sheep (n = 10) grazing control annual pasture (circles) or *Rhagodia preisii* (squares) and with (closed symbols) or without (open symbols) receiving a single oral dose of 30 000 *Trichostrongylus columbriformis* larvae.

3.2 Parasite load of pasture

The parasite load of the control and Rhagodia plots inter-row pasture of the Rhagodia bushes are shown in Table 1. No parasite larvae were found on the control pasture or the Rhagodia bushes, but 12612 larvae/kg DW was present on the Rhagodia inter-row pasture prior to the commencement of grazing. Fifty percent of the larvae found were *Trichostrongylus*, 40% *Ostertagia* and 10% *Nematodirus*. This represents 630 larvae/m^2, being present on the Rhagodia inter-row pasture from weeks 0 to 4.

Table 1. Estimated total larvae load (per kg DW) of the control annual pasture, Rhagodia inter-row annual pasture and Rhagodia bushes sampled 5 days before grazing commenced.

Forage	***Ostertagia*** per kg DW	***Trichostrongylus*** per kg DW	***Nematodirus*** per kg DW	**Total Larvae** per kg DW
Control pasture	0	0	0	0
Rhagodia inter-row	5166	6382	1064	12612
Rhagodia preisii	0	0	0	0

3.3 Pasture and Rhagodia preissii biomass

Weekly estimates of dry matter intake of annual pasture, *R. preissii* and lupin supplements were combined to calculate the average daily feed intake (kg DM/sheep/day) of the four groups of sheep. Over the duration of the experiment, control sheep who were unparasitised and parasitises, and treatment sheep who were unparasitised and parasitised, consumed on average 1.2 ± 0.11, 1.6 ± 0.09, 1.2 ± 0.11 and 1.0 ± 0.10 kg DM/head/day. The daily protein intake of the control and treatment groups was 10.3% and 13.7% of the daily feed intake.

There was no difference between the parasitised and non-parasitised groups in the amount of *R. preissii* consumed (kg DM/sheep/day) ($p < 0.835$), however the amount eaten varied depending on the plot in which the animals were grazing (Fig. 2). The minimum quantity consumed was 0.035 kg/sheep/day and the maximum consumed was 0.4 kg/sheep/day.

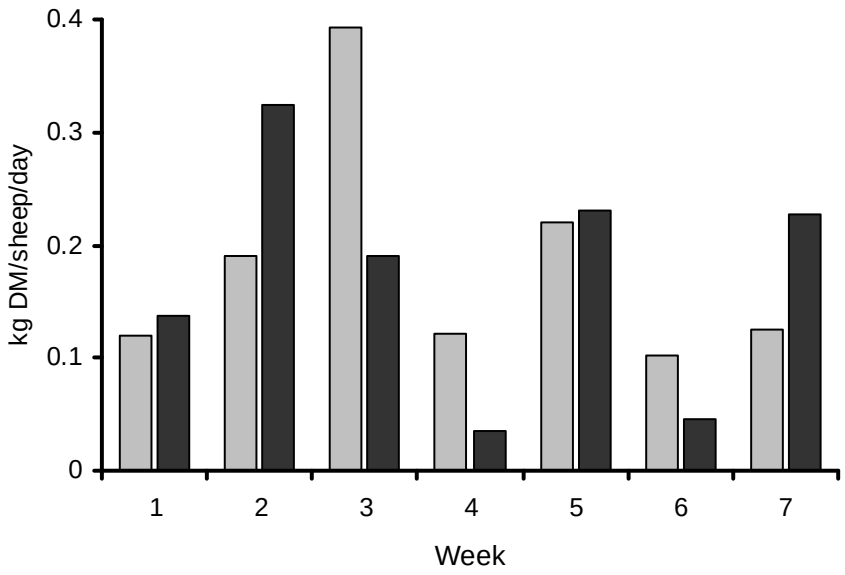

Fig. 2. Estimate of daily intake of *Rhagodia preissi* (kg DM/sheep/day) in each plot for treatment sheep (n=10) that were unparasitised (grey bars) or parasitised (black bars).

The percentage of *R. preissii* shrubs on offer that were eaten ranged from 18% to 47% across all of the shrub plots grazed, apart from one plot used in week 7 in which 80% of shrubs were eaten (Fig. 3). No difference was found between the unparasitised and parasitised groups ($p = 0.56$) or time (plot) ($p = 0.45$).

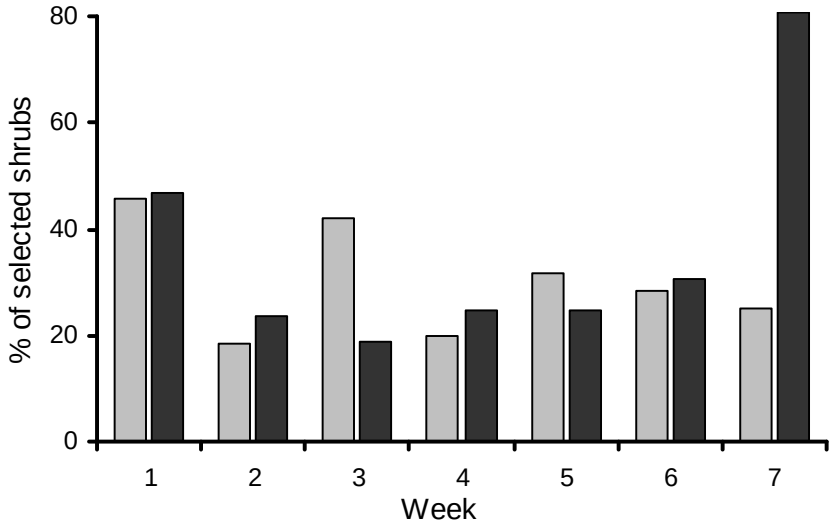

Fig. 3. The percentage of *R. preissi* shrubs eaten in each plot for sheep (n=10) that were unparasitised (grey bars) or parasitised (black bars). Shrubs were classified as eaten if it was obvious that foliage had been removed.

3.4 Larval Development

Water extracts from 60 selected and 60 unselected individual *Rhagodia priessii* shrubs were tested for their toxicity against parasite larvae (% larval development) as shown in Fig. 4. Twenty two of the uneaten and 16 of the eaten plants inhibited parasite larvae to 40% or less of the controls. No difference was found between the eaten (61.0% ± 0.09) and uneaten (58.4% ± 0.09) plants in their toxicity to parasite larvae ($p = 0.64$).

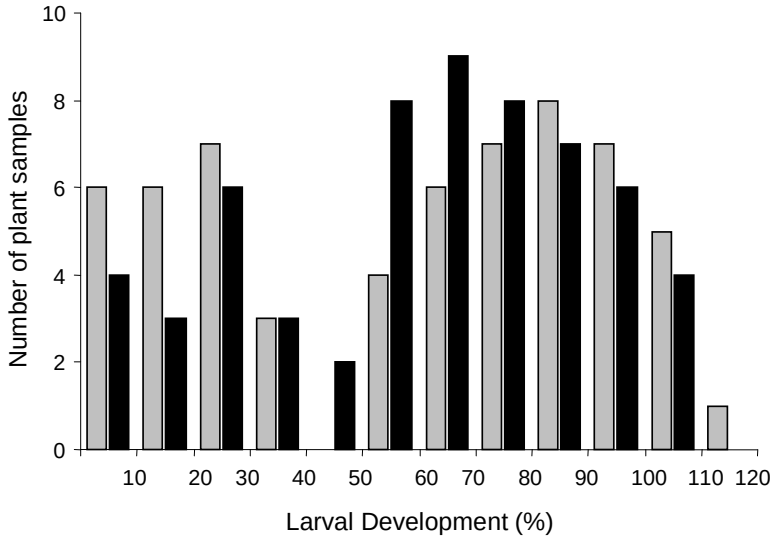

Fig. 4. Frequency distribution of toxicity to parasite larvae of water extracts from individual plants (n = 60) of selected (black bars) or unselected (grey bars) *Rhagodia priessii* shrubs. Shrubs were classified as 'eaten' if it was obvious that foliage had been removed. Larval development inhibited to less than 40% of larval controls is considered 'toxic to larvae'.

3.5 Animal Performance

Live weight initially declined in the first week of grazing in all groups from an initial weight of 50.3 kg ± 0.04, but increased in the control groups from week 1 and the treatment groups in week 2 (Fig. 4) after we began to supplement with lupins. There was a significant difference ($p < 0.005$) between control groups (51 kg ± 0.01) and the treatment groups (48.1 kg ± 0.01) over the 7 week period. No difference in live weight was found between sheep that were parasitised compared to unparasitised ($p = 0.614$). The same trend was found in the body condition scores, where there was a significant difference ($p < 0.05$) between sheep grazing control pasture (2.76 ± 0.00) and Rhagodia (2.59 ± 0.00), due to a greater decline over the first two weeks, but there was no difference between parasitised and non-parasitised groups ($p = 0.234$).

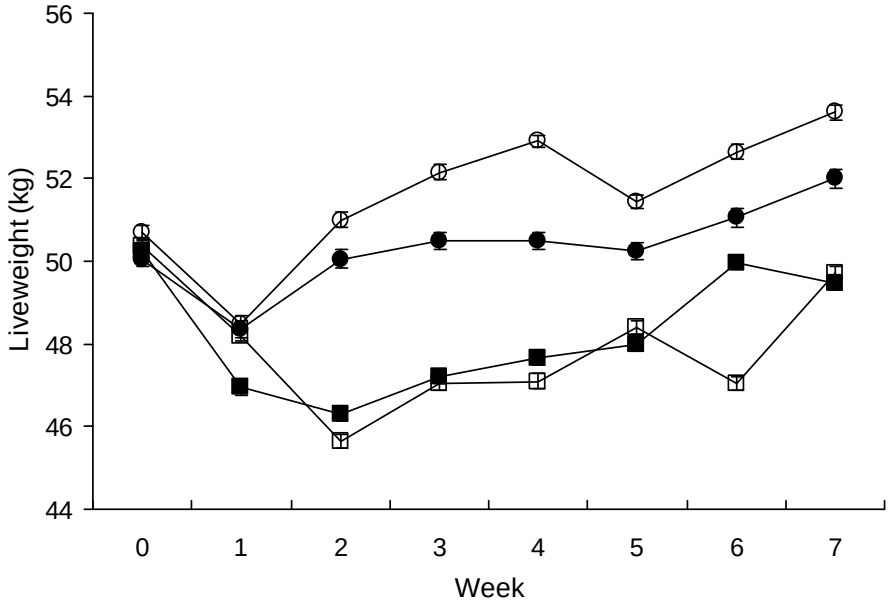

Fig. 4. Average live weight (kg ± SE) of sheep (n = 10) that were unparasitised (open symbols) or parasitised (closed symbols) rotationally grazing annual pasture (circles) or *Rhagodia preisii* (squares) for a period of 7 weeks.

4. Discussion

Including *Rhagodia preissii* in the diet of parasitised sheep did not reduce their parasite burdens as assessed by FEC relative to sheep grazing annual pasture. The complex interaction between variation in host immunity to parasites, individual intake of *R. preissii* and the range of palatable shrubs may be responsible for this outcome. The main finding from this experiment was that both parasitised and non-parasitised animals were highly selective in which bushes they consumed, and that this selectivity was not linked to the toxicity of individual plants to parasites. As some palatable plants showed toxicity to parasites *in vitro*, it suggests that it could be possible to selectively breed *R. preissii* to be both acceptable to sheep and possess anthelmintic (antiparasitic) effects against sheep parasites. There are several possible reasons why *R. preissii* did not have the anthelmintic effect in this experiment that we expected from the promising *in vitro* results against parasite larvae. First, the high degree of plant selectivity and low intake of *R. preissii* may have limited the amount of 'toxic' material that was consumed. Second, the sheep used were healthy adults with a developed immune system and there was a large degree in variation in FEC within the parasitised groups. Third, immunity may have been bolstered by the adequate protein content of the diet in all groups, thus reducing the chances of detecting an effect due to *R. preissii*. Each of these explanations will be discussed below.

The high degree of plant selectivity and the subsequent low intake of *R. preissii* may have limited the amount of 'bioactive' leaf material that was consumed and thus any anthelmintic effect. On average about one third of *R. preissii* shrubs in each plot showed evidence of being eaten. The remainder were left completely untouched (Fig. 5). The same behaviour of selectivity for individual plants has been observed with sheep grazing saltbush (Norman *et al.* 2004). The average daily intake of *R. preisii* was approximately 170 g/day for parasitised sheep and 180 g/day for non-parasitised sheep but it is unlikely that all individuals in the groups would have eaten the same amount. As no grazing observations were included in this experiment, it is not possible to know how much variability existed between animals in shrub intake.

Overall only 23% of the plant extracts from 120 individual *R. preissii* plants inhibited larval development to 40% or less of the controls. A higher proportion of plants from an adjacent site were found to be toxic in earlier larval development assays (A. Kotze, pers. comm.). This difference may be associated with differences in soil type, topography, or plant maturity, all of which can influence plant chemistry. The samples collected for this experiment were from more mature shrubs than the previous collection and were not flowering or fruiting, which some of the previous samples may have been. The effect of the plant's physiological stage (growing or reproductive) on the palatability or anthelmintic properties of *R. preissii* is unknown but should be a key area for future research.

Fig. 5. Photograph of an uneaten and eaten *R. preissii* bushes in a plot that has been grazed for one week.

For *R. preissii* to have an anthelmintic effect in a commercial situation we may need to increase the amount or the frequency that is eaten. Palatability is a function of a food's odour, taste, and texture with post-ingestive consequences of nutrients and toxins from the food (Provenza *et al.* 2007). All plants contain chemicals that are beneficial in the right dosage and toxic in greater quantities. Intake of *R. preisii* would have been influenced by the palatability of individual shrubs and the complementary chemicals of the inter-row pasture and lupin supplement. Animals may choose to eat more *R. preissii* if it is offered in different combinations of plants or supplements, an aspect requiring further study. In the longer term, selective breeding of palatable plants that are also toxic to parasites may be a possibility as the two traits do not appear to be linked. In the short term we may be able to encourage sheep to consume greater amounts through training techniques and grazing strategies; for example, by providing restricted access to a familiar food that helps offset any toxins or negative feedback responses (Shaw *et al.* 2006).

It has been shown recently that lambs can be conditioned to self medicate in response to an illness from consuming excess amounts of grain, tannins or oxalic acid (Villalba *et al.* 2006). Once the animals had had the opportunity to associate the benefits of a remedy to a particular illness, they voluntarily increased their selection for the correct remedy (sodium bentonite, polyethylene glycol or dicalcium) when given a choice of all three options. It may be possible then to condition parasitised animals to associate a reduction in worm burdens with the ingestion of forages containing secondary compounds that have an anthelmintic effect. Once conditioned, parasitised animals may choose to consume a greater quantity of that forage when it is presented in a mixture of species to alleviate their worm burden than non-parasitised animals. If this is possible, producers could allow individual animals with high worm burdens to 'drench' themselves (Villalba and Provenza 2007).

Providing a mixture of plant species with different nutrients and secondary compounds allows individuals to better meet their nutrient requirements and manage their intake of plant toxins (Provenza *et al.* 2003). Livestock can be forced to broaden their palate of what plant species they eat through short duration, high density grazing periods to minimise selective grazing behaviours (Provenza *et al.* 2007). The knowledge of what foods to eat is passed from mothers to their offspring (Mirza and Provenza 1990; Thorhallsdottir *et al.* 1990), so in a self-replacing flock training only needs to occur once before it becomes part of the flocks grazing culture. It is conceivable that *Rhagodia preisii* would be of most benefit if grazed with a mixture of plant species at high grazing intensity.

The second explanation for the lack of a significant reduction in FEC with grazing *R. preissii* could be due to large between-animal variation in immune competence. The large variation in FEC may have made it made it difficult to detect any anthelmintic effect from *R. preissii* because it was the largest source of variation. For example, one individual sheep from the control group had FEC 2000 to 8000 epg higher than other sheep and two individuals in the control pasture group and three in the treatment (Rhagodia) groups that had received the dose of infective larvae had very low FEC, which suggests that they had better immunity to *T. columbriformis* than their counterparts. The non-parasitised sheep grazing *R. preissii* did build up a small worm burden over time, probably from parasite larvae that were found to be on their inter-row pasture (Table 1), but still had significantly lower worm burdens than the parasitised treatment group. These plots had been grazed by other sheep about two months earlier, whereas the control pasture plots had not been grazed for over two years.

A large range in parasite burdens and immune competence is expected in a commercial flock (Hoste *et al.* 2001). Nutritionally stressed adults, lactating ewes and young lambs will have compromised immune systems, but genetic variation in parasite resistance also exists between healthy adult individual animals. Sheep have been bred successfully for high resistance to parasites (Eady *et al*. 1998; Greef *et al.* cited in Besier and Love 2003). This experiment used healthy two year old wethers, which were not nutritionally stressed because, after an initial live weight loss was corrected by supplementing with lupins, live weight stabilised and steadily increased in all four treatment groups. These sheep therefore would have had a reasonable immunity to parasites. Genetic variation in parasite resistance between individual animals is therefore the most likely explanation the large variation in FEC within the parasitised groups.

It may be possible to reduce this variation by altering the experimental design. A reduced variation in FEC may have been achieved by only using sheep with moderate parasite burdens or completing a mature worm count in addition to FEC. Establishing which individuals have a moderate worm burden could be achieved by administrating the parasite larvae to a larger number of sheep to determine their immune response, before allocating only individuals with a moderate worm burden into treatment groups. This would eliminate any outliers that may obscure any positive anthelmintic effect of *R. preissii*, but in doing this we would not be replicating the variation that would normally exist in a flock. A better alternative may be to use a larger numbers of animals per treatment group to account for this variation. Another

option would be to measure the mature worm count in the gastrointestinal tract. This was not done in the current study as it was a preliminary investigation of the potential for *R. preissii* to be used as a bioactive forage. It is possible that differences in worm burdens may be detected in mature adult counts in the gut but not in the FEC, a response that was found with sheep grazing chicory (Tzamaloukas *et al.* 2005). The reverse can also occur, namely a reduction in FEC that does not represent a decline in immature or adult worm burdens in the gut as demonstrated with sheep grazing *Lotus corniculatus;*(Anthanasiadou *et al.* 2005).

The third explanation for the lack of response in FEC in animals grazing *R. preissii* is that protein intake, which is an important factor in controlling parasites in sheep, was sufficiently high in all animals. As parasite infection results in the loss of endogenous protein (Coop and Kyriazakis 2001; Knox *et al.* 2006), protein supplementation can contribute to resistance by influencing the degree and rate of the expression of immunity (Min and Hart 2003). The percentage of total protein in the diet, including the lupin grain supplement, was 10.3 % in control groups and 13.7% in the treatment groups. The parasitised sheep in the current experiment may have had a 'good' immune response due to adequate dietary protein and this may have overridden any anthelmintic effect due specifically to the consumption of *R. preissii.* Using a supplement lower in protein such as barley, instead of lupins may reduce protein influencing immunity.

Although *R. preissii* in this experiment did not reduce the parasite burdens of sheep, there is the possibility that it may be found to have an anthelmintic effect *in vivo* if we can overcome or manage high grazing selectivity and differences in plant chemistry. Currently we have little knowledge of the plant's chemistry, what secondary compounds are responsible for the positive *in vitro* effect against parasite larvae and which compounds influence palatability. The chemicals responsible for toxicity against parasite larvae are unlikely to be condensed tannins but could be phytoecdysteroids, which have been found in high concentrations in *Rhagodia baccata* (Dinan *et al.* 1999). In particular *R. baccata* contained high levels of 20-hydroxyecdysone (20E), which is known to induce abnormal moulting and death in many arthropods. It has been suggested that as nematodes have a similar hormonal regulation of ecdysis, phytoecdysteroids could provide an effective control against free living, plant or animal nematodes (refer to summary by Soriano *et al.* 2004). Phytoecdysteroids may have a similar mode of action as condensed tannins, which interfere with the first phase of parasite invasion, exsheathment (moulting) of L3 stage larvae (Bahuaud *et al.* 2006; Brunet *et al.* 2007; Brunet and Hoste 2006) and possibly the second phase, penetration of exsheathed L3's

into the mucosal lining of the digestive tract (Brunet *et al.* 2008). This mode of action would be difficult for nematodes to develop resistance to as the exsheathment of L3s is a crucial step in their life-cycle (Hertzberg *et al.* 2002).

The results from other studies where the role of using bioactive forages to reduce parasite burdens *in vivo* has been examined, have been inconsistent (Hoste *et al.* 2006). There are currently no set guidelines outlining the best way to conduct *in vivo* experiments. Several suggestions have been made by Athanasiadou *et al.* (2007) in a recent review of this problem to simplify the comparison of studies of different bioactive forages. They recommend that the plant compounds responsible for the anthelmintic activity should be isolated and their concentrations measured and documented as well as animal performance measures, indicators of immunity and behavioural observations. To date, the majority of forages investigated *in vivo* for their anthelmintic effect contain condensed tannins (*Lotus corniculatas*, birdsfoot trefoil, sulla, sainfoin, chicory). It is unlikely that bioactive forages will provide a 'silver bullet' for future parasite control, but they may complement other control strategies including nutritional supplementation, breeding programs and grazing strategies that reduce pasture contamination. Research and development should focus on the integrated management of worms and not be restricted to examining each different strategy in isolation.

Sustainable grazing systems in the lower rainfall regions of south-western systems need to adapt to meet multiple objectives such as the provision of out-of-season feed, natural resource management (erosion and/or water balance) and possibly parasite control. Further work is required to increase the consumption of *R. preissii* to ensure adequate quantities of potentially bioactive material are consumed to justify its inclusion in a mixture of species for its anthelmintic role.

5. Conclusions

Grazing *Rhagodia preissi* was not found to have an anthelmintic effect on parasites in the field, despite positive *in vitro* results. Sheep were found to be highly selective in what shrubs they ate, probably due to variation in plant chemistry, and they did not consume large amounts of the shrub each day. Leaf extracts from eaten and uneaten shrubs were found to have similar ranges of toxicities to parasite larvae *in vitro*. These results suggest that palatability and toxicity are independent traits, indicating that it should be possible to breed palatable plants that also have an anthelmintic effect against sheep parasites. There is already a need to find practical alternatives to chemical drenches and plant breeding programs are

expensive and have a long turn around time. The nutritional and possible anthelmintic benefits of *Rhagodia preisii* in the short-term could be maximised by grazing it with a mixture of complementary forage species and implementing grazing strategies that increase its palatability.

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Benefits of bioactive forage shrubs in low-rainfall pastoral regions: reducing reliance on anthelmintics and improving sheep production

Literature Review

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Abstract. Australian livestock systems need to adopt changes that will increase their financial and environmental sustainability in response to a changing climate, tightening terms of trade, and greater societal demands for 'acceptable' production methods including increased consumer awareness of chemical use. Current practices to control gastrointestinal parasites have relied heavily on the use of chemical drenches however with the advent of anthelmintic resistance, alternative approaches are required. The incorporation of bioactive forages with anti-parasitic properties into livestock grazing systems is one alternative solution currently being investigated. In the medium rainfall agricultural regions of Western Australia, the adoption of a mixed forage system containing perennial shrubs and pastures could provide multiple production and environmental benefits. The benefits of including more perennials, including bioactive shrubs, in grazing systems in the medium rainfall region of south-western Australia are highlighted in this review. The state of anthelmintic resistance in Australia and other alternatives to chemical drenches is also reviewed focussing on bioactive forages and the current problems associated with measuring their effectiveness in grazing studies. The use of bioactive forages to control parasites is complicated by the degree of variation in the concentration of plant secondary metabolites between and within plants of the same species. Accounting for this variation and further research into the mechanisms of these compounds against parasitic nematodes may assist in incorporating bioactive forages successfully into short or long-term grazing systems that reduce the use of chemical drenches. The anti-parasitic potential of *Rhagodia preisii*, a native perennial shrub is also introduced as an example of a plant that has shown *in vitro* bioactive potential.

2.1 Introduction

Livestock grazing systems in Australia need to change. The reliance of current practices on annual pastures has contributed to environmental degradation, including rising water tables and soil erosion. In addition, this system is not well adapted to utilise summer rainfall events, which are becoming more frequent. The adoption of a mixed forage system, containing perennial shrubs and pastures, has multiple benefits from improving land management practices and plant water use to providing livestock with shelter and valuable green feed during summer. Villalba and Provenza (2004) and Villalba *et al* (2007) have shown in grazing behaviour studies that offering livestock a choice of species with different nutrition and chemical compositions allows for self selection for nutritional and health requirements that could improve productivity. Shrubs are an economically viable option in median rainfall agricultural regions of Western Australia, which has more limited annual and

perennial pasture options than higher rainfall regions due to shorter growing seasons and a higher tendency for drought.

The reliance on chemical anthelmintics to treat gastrointestinal parasites has lead to drench resistance worldwide. Resistance to benzimidazole (BZ) and levamisole (LV) drench groups is widespread throughout Australia and drench efficiency is now below a useful level (Besier and Love 2003). Consumers are also driving the agricultural industry to reduce its use of synthetic chemicals for plant and animal production. Bioactive forages with anti-parasitic properties offer a natural alternative to chemicals in an integrated pest management strategy to control gastrointestinal parasites in ruminants. Forage plants containing condensed tannins have been the most extensively researched for their anthelmintic properties with some promising results; however, other plant compounds yet to be investigated could have the same effects. In Australia, native shrubs like *Rhagodia preissii* are currently being investigated for their anthelmintic potential in a shrub-based grazing system for lower rainfall regions of south-western Australia. Including bioactive plants in a mix of perennial species could lead to improvements in animal health and production, while reducing reliance on chemical drenches and assisting in land rehabilitation.

This review will put forward a case for the inclusion of more perennials, including bioactive shrubs, in grazing systems in the median rainfall region of south-western Australia. In doing so, the state of anthelmintic resistance and alternatives to chemical drenches will be reviewed focusing primarily on the use of bioactive forage plants and how they could be incorporated into sheep production systems.

2.2 Improving forage supply in WA grazing systems

The agricultural region in South-Western Australia features a Mediterranean climate, which is characterised by low winter dominant rainfall, varying from 1200 mm per annum on the south and south-western coasts to 250 mm at the inland limit, warm to hot summers, mild winters and high solar radiation especially in summer (Cramb 2000). Grazing systems are therefore based on fluctuating pasture quality and quantity. The situation is further compounded by some serious environmental problems including salinity, soil erosion and acidity and Australia's inherent fragile nutrient-poor soils. Medium rainfall pastoral regions have limited pasture species options as they are constrained by all these factors, in order to remain sustainable, producers need to consider modifying and improving forage supply.

2.2.1 Positive attributes of perennials

Rainfall patterns have altered recently with an increase in summer rainfall events and a reduction in total rainfall (Smith *et al.* 2000). Summer rainfall is not utilised efficiently by annual pastures and rainfall events contribute to a decline in dry pasture quality. In addition, on the south coast of WA 70% to 90% of dry matter (DM) and digestible DM that is accumulated in spring pasture growth is lost from late-spring to autumn without being consumed by sheep through natural decay and trampling (Doyle *et al.* 1996). Perennials offer many production benefits by more efficiently filling in this feed gap in summer and autumn by utilising this summer rainfall. These benefits include increasing carrying capacity by improving the seasonal distribution of feed and reducing supplementary feed costs, improving the flexibility to turn-off animals at target live weights year-round and providing opportunities to rest annual pasture paddocks at the break of season (Moore 2006). As an environmental management tool, perennials reduce groundwater recharge, stabilise soil structure and prevent soil erosion (Bird *et al.* 1992). A six year phase of native woody perennials grown in belts was found to restore C and N in the top 0.1 m of the soil profile to 95% of that found in a 25 year phase of subterranean and annual ryegrass pasture (Mele *et al.* 2003), suggesting that perennials could be used to increase soil fertility for future cropping or pasture programs. Increasing species diversity could also have flow-on production benefits by allowing livestock to select a diet that meets their nutritional and well-being requirements using post-ingestive feedback (Villalba and Provenza 2007).

2.2.2 Reducing the reliance of chemicals in agriculture

Public awareness of the potential health and environmental risks associated with synthetic chemical use in agricultural industries has put pressure on regulatory bodies to reduce the use of chemicals. The European Union in 1996 banned the use of antibiotics in animal feed and other countries are set to follow their example. This move has ignited new research into finding natural alternatives to chemicals, which includes the use of bioactive plants to improve animal health (Rochfort *et al.* 2008). Alternative fodder plants may offer less in terms of annual biomass production in comparison to the annual pasture species currently used in grazing systems. However, other valuable attributes including plant secondary compounds (PSM) that improve gut health (Durmic *et al.* 2007) or reduce worm burdens (Githiori *et al.* 2006), could make them a useful component in a grazing system.

2.3 Anthelmintic resistance

2.3.1 Parasite infections in Australia

In 1995, sheep roundworms were estimated to be the largest constraint to the Australian sheep industry, costing producers $221 million per annum in production losses and control measures (McLeod 1995). The cost of parasite infections would only have increased over the years since that study. Barber's pole worm (*Haemonchus contortus*), black scour worms (*Trichostrongylus spp.* chiefly *T. columbriformis* and *T. vitrinus*) and brown stomach worm *Ostertagia* (*Teladorsagia circumcinta*) are the most important sheep roundworms in Australia. Parasite infections are usually a mix of these species, with dominant species in the mix being determined by climatic zones. In temperate regions of south-western Australia *Trichostrongylus* spp. and *O. circumcinta* are the most dominant species as they are more tolerant of cold and desiccation (Besier and Love 2003).

2.3.2 Effect of gastrointestinal parasites on sheep

In severe cases, parasitism can impair the health of ruminants by causing inappetence, diarrhoea, anaemia and death, but the effects are usually subclinical. The inhibition of nutrient utilisation and normal production is caused by several factors. Briefly, heavy infestations of gastrointestinal nematodes can reduce voluntary food intake (VFI), result in losses of endogenous protein, can disrupt the retention of nutrients and diverts nutrients to compensate for losses and immunological response to infection. It is estimated that an additional 17 g/day of metabolisable protein, above maintenance requirements, is necessary to compensate for losses due to infection (Liu *et al.* 2003). For a more detailed account of these factors refer to Knox et al (2006).

2.3.3 Process of infection

The infective stage of the parasites lifecycle is the L3 stage larvae, which are picked up from the pasture when grazing. Stage 3 larvae are highly resistant to physical and chemical factors due the presence of a protective sheaf. Temperature and moisture are the dominant influences on the free-living survival stage of *H. contotus, T. circumcinta* and *T. columbriformis,* with pasture conditions playing an important modulating role. Temperature determines the length of the development cycle, with warmer temperatures increasing the rate of development; however, moisture is required for development to the infective stage. The

infective stage (L3) is less influenced by temperature and moisture than the free-living stage larvae, although they are inhibited by drought and frost (O'Connor *et al.* 2006).

2.3.4 Anthelmintic resistance

The cornerstone of current worm control has been the use of anthelmintics (chemical drenches) from a limited number of chemical groups. Resistance can be defined as the ability of parasites to survive doses of chemicals usually lethal to parasites of the same age and species (Besier and Love 2003). Several recent reviews have reported the extent and mechanisms of anthelmintic resistance (Besier and Love 2003; Jabbar *et al.* 2006; Papadopoulos 2008). In many countries resistance against drenches from all three broad-spectrum groups, the benzimidazoles (BZs), levamisole (LV) and macrocyclic lactone (ML) has been recorded as well a large list of narrower spectrum drenches (Jabbar 2006). Through various mechanisms discussed later, there is some degree of resistance in worms of all livestock species worldwide to these drench groups, which is a serious concern and has prompted the need to find new alternatives and to change current practices to prolong the use of anthelmintics. This is especially important as no new anthelmintic groups have been developed for many years as animal health companies seem unconvinced of the commercial justification for new compounds (Besier 2007).

Almost all sheep farms in Australia have resistance to the benzimidazoles and levamisole drench groups, and on most farms drench efficiency is now below a useful level (Besier and Love 2003). In Western Australia, 60% of 570 resistant tests showed an efficacy of BZs and LVs administrated singly of less than 60%. Furthermore resistance to a combined administration of the two groups was 45% (*Trichostrongylus* spp.) and 71% (*O. circumcinta*) on WA properties (Palmer *et al.* 1998 in Besier and Love 2003). Resistance to macrocyclic lactones has also appeared and is increasing (Besier and Love 2003).

2.3.5 Contributing factors to drench resistance

In most situations worm control is based largely on drench treatments (Besier and Love 2003). Modern drenches were developed to be used at a high efficiency, i.e. > 90% reduction of adult and/or larval parasites in the host animal (Ketzis *et al.* 2006). However, resistance has developed from the small number of parasites (refugia) that survive drench treatments, which over subsequent generations increase in number on contaminated pasture, thus increasing selection pressure for drench resistance (Papadopoulos 2008). The rate of selection is dependent on several factors, including grazing management regimes, dosage

rates and frequency of treatments, single-drug regimes and the targeting and timing of treating large groups of animals (Jabbar *et al.* 2006).

Under-dosing and the frequency of dosing have been largely attributed as the main causes of resistance. When treatment intervals are close to the minimal period required for new larvae to develop into mature adults, a greater proportion of the population are derived from refugia worms. Many producers, before the development of epidemiology-based strategic programs, treated sheep at frequent intervals to prevent unforeseen outbreaks of parasitism. In Australia producers underestimating bodyweight and therefore dose rate, and using inaccurate drenching equipment, may have contributed to the population size of the resistant refugia by under-dosing their sheep. The implementation of 'summer drenching' programs in winter rainfall regions also increased selection pressure to favour resistant parasites as the population number is already reduced due to the dry conditions (Besier and Love 2003). Population numbers are lowest in summer due to high temperatures and sheep were drenched in an attempt to kill the parasite population and then moved to a 'clean' paddock free of larvae (Van Wyk 2001). It is now recommended to drench sheep only when necessary and to leave them in a 'dirty' paddock before moving to dilute the number of resistant parasites and to mix up drench groups to extend the use of chemical drenches (Torres-Acosta and Hoste 2008).

2.4 Alternative approaches to parasite control

Recognition that chemical-based worm control in sheep production systems is unsustainable has promoted research into alternative mechanisms to control GI parasites in sheep. The need for control methods to provide maximal parasite control (highly efficient) has also been questioned. (Ketzis *et al.* 2006) has suggested that some novel parasite control mechanisms, with lower efficiencies, may be just as effective if the objective is to maintain parasites below their economic threshold. An integrated pest management program tailored for each climate and production system, incorporating several non-chemical approaches and anthelmintics, is likely to be the most successful and sustainable option.

An analysis of the nematode cycle of infection reveals that three broad methods can be applied to "disrupt" its continuity. First, to eliminate the worms present in the host; second, improving the resistance and the resilience of the host; and third, reducing contact between the infective (L3) larvae and the host (Torres-Acosta and Hoste 2008). Alternative control methods fall under these three categories. Additionally nematodes are not equally distributed

amongst a group of sheep, with only a small proportion of the population being heavily infected and the majority will have moderate worm burdens (Hoste *et al.* 2001). This is exploited by several control options.

2.4.1 Breeding for worm resistance

Genetic variation in nematode resistance exists between and within sheep breeds allowing for selection in breeding programs. Nematode resistance is recorded and included in breeding programmes using correlated traits (Dominik 2005). The most commonly used trait is faecal egg counts (FEC), but there is continuing research into identifying a genetic marker to make this process easier and simpler (Dominik 2005; Woolaston and Baker 1996). The heritability of FEC in selected sheep flocks ranges between 0.2-0.4 (Windon 1996). Flocks selected for reduced FEC have maintained consistently lower FEC than control flocks (Eady *et al.* 1998; Greef *et al.* 1999 cited in Besier and Love 2003). Eady *et al.* (1998) found that phenotypic correlations between production traits and FEC were close to zero which was also found for growth traits in Australian Merinos (Khusro *et al.* 2004), suggesting that selecting for reduced FEC has no negative effects on other production traits. In contrast, other studies have found that there is a negative correlation between FEC and wool growth in selected lines (Eady *et al.* 2003; McEwan *et al.* 1992). The addition of FEC to breeding objectives is definitely the way forward in making progress towards producing more resistant sheep, and its inclusion should not affect other production traits.

2.4.2 Grazing management

Knowledge of worm species lifecycle is required to plan grazing rotations that limit exposure to infective larvae and concurrently avoid strong selection for drench-resistant worms. It is also important for young lambs to have significant exposure to worms to develop effective immunity against infection, but avoiding excessive larval intake (Besier and Love 2003; Jabbar *et al.* 2006). It has been suggested that sheep should be dosed with an anthelmintic and put back in the infected pasture for a time period to dilute the number of resistant parasites before being moved onto a clean pasture i.e. crop stubbles (Torres-Acosta and Hoste 2008).

Reducing stock densities reduces larval intake as sheep have the ability to avoid infected patches of pasture around faeces via olfaction cues from contaminated faeces. There is a trade-off however as these patches are often the most nutrient rich and the pasture around them is of a higher nutrient quality (Hutchings *et al.* 2006). In most cases producers want to

maximise pasture utilisation so the benefits in reduced worm burden, gained from reducing stocking rates, may not be an economically viable option. An alternative option is to present feed off the ground, thus avoiding contamination with faeces and limiting the ingestion of L3's, which have a limited climbing ability (Torres-Acosta and Hoste 2008). This can be achieved by grazing forage shrubs, or presenting supplementary feed such as hay in racks and grain in troughs.

2.4.3 Dietary supplementation

The subclinical effects of parasitism are a result of reductions in voluntary feed intake and/or reductions in food use efficiency, particularly the inefficient use of absorbed nutrients (Coop and Kyriazakis 2001). Supplementary feeding parasitised animals, if the limiting nutrient is targeted, can assist in building up resilience to infection (Knox *et al.* 2006). It can also help to develop resistance by increasing the hosts ability to contain and overcome parasitism by limiting the establishment, development, persistence, growth rate and fecundity of a parasite population (Coop and Kyriazakis 2001). Protein is often limiting as endogenous protein is lost to the GI tract through increased sloughing of epithelial cells, secretion of mucoproteins and leakage of plasma protein caused by infection (Coop and Kyriazakis 2001; Knox *et al.* 2006). Protein supplementation contributes towards building up resilience and once the parasite host has acquired immunity it affects resistance by influencing the degree and rate of the expression of immunity (Coop and Kyriazakis 1999). Young animals will therefore benefit from protein supplementation to improving resilience while gaining immunity and adult sheep under stressed conditions (heavily pregnant and lactating ewes) will benefit by improving their resistance to reinfection. Supplementing ewes at parturition, when they relax their immunity due to the high metabolic demand of the foetus and milk production, can restore the lost immunity (Coop and Kyriazakis 2001). If the ewes have a low worm burden, then their lambs will not be subjected to high infection rates and this will assist in their gradual development of resistance. In summary, supplying nutrients that are deficient in the diet using targeted supplementation at times when they are limiting to susceptible groups, i.e. ewes in late pregnancy or lactation or immature animals, can reduce the impact of parasite burdens on production and increase resilience and resistance to future infections. This is also an alternative strategy that is reasonably easy to adopt as no new technology is required and supplementation at the same critical times is also recommended in Australian livestock systems for other benefits i.e. reducing lamb mortality and maximising growth (Martin *et al.* 2004).

2.4.4 Fungi and Vaccinations

Currently no commercial vaccines for GI nematodes are available despite significant research in the field (Torres-Acosta and Hoste 2008). Another control strategy to reduce pasture worm burdens is the use of nematophagous fungi. This diverse group of fungi includes species that are egg parasitic, endoparasitic and predacious nematode trapping (Jabbar *et al.* 2006). As it has been found that fungi can be picked up and excreted by livestock (Larsen *et al.* 1994) they could offer an exciting biological control option, however more research is required into fungi belonging to sheep.

3.3 Bioactive Forages

Bioactive forages can be defined as plants that contain secondary metabolites that are useful for their beneficial effects on animals rather than their nutritional value (Hoste *et al.* 2006). Many plant species have been noted for containing Plant Secondary Metabolites (PSM) with antiparasitic effects (Rochfort *et al.* 2008). Plant species containing condensed tannins (*Lotus corniculatus*, birdsfoot trefoil, sulla, chicory) and Quebracho extract have been the main focus of recent research with some positive results. There is a positive relationship between the concentration of condensed tannins and FEC reduction for several forage types, which is summarised by (Min and Hart 2003). See p. 4 for a recent review on *in vivo* experiments with tanniferous forages refer to (Hoste *et al.* 2006).

2.5 Bioactive forages

2.5.1 Evaluating the antiparasitic effects of bioactive forages

Plants with potential anthelmintic properties are often screened first using *in vitro* assays to determine the effect plant secondary metabolites have on larvae development, mobility and feeding ability (Anthanasiadou and Kyriazakis 2004). Positive *in vitro* results are often hard to replicate in situations closer to real life (*in vivo*) using animals in animal house or grazing experiments (see reviews by (Anthanasiadou and Kyriazakis 2004; Githiori *et al.* 2006). Evaluating the direct effect of extracts of plant material on parasite larvae (*in vitro*) or directly assessing the effect on parasitised livestock (*in vivo*) via a drench or feeding with the plant material is always complicated. Additional plant properties such as its nutritional value, height off the ground and animal factors such as the degree of accumulated parasite immunity or previous experiences in eating novel foods, can compound any effect

that may be due to the PSM itself. Few field studies have been undertaken to investigate the use of bioactive plants in grazing systems.

Several recommendations have been made in previous reviews to improve the likelihood of obtaining positive results from *in vivo* trials. The most important is including a parasitised and non-parasitised group of animals for each treatment group and reducing the length of the trial to help eliminate nutrition as a factor contributing to reduced worm burdens in addition to any anthelmintic activity of the bioactive forage. This is further complicated when assessing a CT-containing forage as it is difficult to distinguish how much of the effect is due to protein bypassing the rumen and how much is due to bioactive mechanisms (Anthanasiadou and Kyriazakis 2004), since condensed tannins 'protect' protein from microbial digestion and can thus increase the post-ruminal supply of protein to the animal (Min and Hart 2003). The only way to eliminate the effect of reduced larval intake due to sward height i.e. shrubs, would be to feed the foliage on the ground or complete an animal house experiment. Using young animals, which have a lower accumulated immunity to parasites, may assist in reducing acquired immunity from conflicting results. However, if the group of animals used has the same background history of management and have similar initial worm burdens, this factor may be relatively insignificant. Inconsistent results between grazing studies may also be attributed to variable concentrations of PSM within and between plants from different sites and the same site.

The number of animals used for each treatment group is often quite small for experiments using FEC as a measure of worm burdens. It is recommended that the number of animals should be at least 10 to account for a wide range in FEC within a group of animals (Cole 1986). FEC may not truly represent the worm burden and total worm counts are required if this is important. For example the FEC of *T. columbriformis*, in an experiment designed to eliminate the interference of direct immunological effects of PSM, were found to be lower for sheep grazing *Lotus corniculatus* than sheep grazing other forages. However, total worm counts revealed that grazing the forages for a 2-week period had no effect on the immature or adult parasite populations of *T. columbriformis* (Anthanasiadou *et al.* 2005). To some extent performing larval cultures in association with FEC can at least attribute the percentage of eggs in the FEC of the parasite species being investigated (Cole 1986).

2.5.2 Mechanisms of bioactive forages

Plants rich in condensed tannins (CTs) have been found *in vitro* to interfere with the first phase of parasite invasion, the exsheathment (moulting) of L3s (Bahuaud *et al.* 2006; Brunet *et al.* 2007; Brunet and Hoste 2006) and in cannulated sheep fed with sainfoin (Brunet *et al.* 2007). The second stage of larval establishment, the penetration of the exsheathed L3s into the mucosal lining of the digestive tract, may also be inhibited by condensed tannins. Incubation with 1200 µg/ml of sainfoin extract reduced the penetration of exsheathed *H. contortus* and *T. circumcinta* into fundic explants (Brunet *et al.* 2008). The effect was attributed to CT in the sainfoin extract as the addition of polyvinyl polypyrrolidine, an inhibitor of tannins, alleviated the effects. No one has yet verified this *in vivo* studies. Both mechanisms have been found to be dose-dependent (Brunet *et al.* 2008), to be effective, it is reasonable to assume that the condensed tannins need to come in contact with the parasites in the digestive tract. Plant material therefore needs to be present in the digestive tract before ingesting the L3 larvae for tannins to be effective in this way. This could be achieved by grazing tanniferous forages with normal pasture or frequent delivery of an extract by drenching or a slow release capsule.

Condensed tannins present in faeces may also inhibit the viability of the eggs and first stage larvae. Extracts of condensed tannins from eight plant species containing 200-500 µg/mL of the monomer units of CTs (flavan-3-ols) and their galloyl derivitives, reduce the proportion of hatched eggs and the larval development of *T. columbriformis in vitro* (Molan *et al.* 2003; Molan *et al.* 2002). Higher concentrations (400µg/mL) of the extracts were more effective than lower concentrations (200 µg/mL). The addition of extracts before hatching was more successful in inhibiting larval development than post hatching (Molan *et al.* 2002). Condensed tannins may be useful in grazing systems in disrupting the cycle of pasture contamination by reducing the number of infective larvae (Molan *et al.* 2003) although this is yet to be tested in the field.

It is also well known that condensed tannins can form complexes with several molecules including proteins, polysaccharides, nucleic acids and minerals. The percentage of *Lotus corniculatus* in a pasture mix was negatively correlated to the percentage of remaining soluble protein when the pasture samples were homogenised. The addition of PEG confirmed that the majority of this effect was due to CTs (Waghorn and Shelton 1997). CT in moderate levels (20 to 40 g of CT/kg DM) binds to protein by hydrogen bonds in the rumen at a pH of 6-7 to form CT-protein complexes. These complexes are dissociated at a pH < 3.5 and therefore

release bound protein in the abomasum (Barry *et al*. 2001, cited in (Min and Hart 2003). Through this mechanism plant material containing condensed tannins can protect dietary protein from degradation by the rumen microbes and thus increase protein supply to the animal to assist it in coping with the effects of a parasite infection as previously discussed in 3.3.

2.5.3 Anti-nutritional effects

Most PSM are toxic to livestock at high concentrations, when they can lead to reduced feed intake, weight loss, toxicity and death (Milgate and Roberts 1995; Waghorn and McNabb 2003). The consumption of condensed tannins has been associated with reductions in food intake, digestibility and impaired rumen metabolism (Barry and McNabb 1999; Min *et al.* 2003). Plant breeders have consequently bred forages to contain low concentrations of PSM. Renewed interest in the anthelmintic effect of PSM may reverse this trend and plants with high concentrations could be highly beneficial in a mixture of forages.

Animals have the ability to maintain homeostasis by selecting feed that meets their nutritional and health requirements through the use of post-ingestive feedback (Villalba and Provenza 2007). If we assume that this is true, it removes the danger of feeding animals plants with high concentrations of PSM, as long as alternative feed is also provided. In fact, supplementing with alternative options increases feed intake of both options even if the alternative choice is another toxin-containing food (Villalba *et al.* 2004), resulting in increased productivity and animal health. The probability of a plant being eaten is dependent on its own chemical profile, nutritive value and those in neighbouring plants and the ability of the foraging animals to learn associations between PSM and nutrients (Villalba *et al.* 2002). For example, experienced lambs consumed greater amounts of toxin-containing food (tannins, terpenes and oxalates) than naive lambs when non-toxic and more nutritious feeds (ground lucerne and barley) were also provided (Villalba *et al.* 2004). Lambs exposed to tannins (*Acacia cyanophylla*) early in life when given Acacia or oaten hay at a mature age, exhibited a higher intake of digestible crude protein, retained more N and excreted more allantoin in their urine than inexperienced lambs (Salem *et al.* 2005). Both examples illustrate that animals can be taught food preferences and there is also a school of thought suggesting that animals also self-medicate themselves to maintain a positive homeostasis (Villalba *et al.* 2006).

Animals can be trained to self-medicate, but it requires conditioning whereby recovery from an illness is associated with the ingestion of a ‘medicine’ (Villalba *et al.* 2006). No studies

have been conducted to test whether parasitised sheep can self-medicate by eating forages with PSM that inhibit worms, however it is highly likely that they may be capable of doing so.

2.6 Alternative bioactive shrubs- *Rhagodia preissii*

South-western Australia is a world diversity hotspot. The native flora is well adapted to nutrient poor soils and water-limiting conditions; however, this is yet to be exploited in agricultural systems. The 'Enrich' research programme in Australia aims to capitalise on these features and evaluate potential native shrubs that could be incorporated into mixed farming systems. *Rhagodia preissii* is one shrub species currently being investigated to fulfil this role. Species of the native perennial *Rhagodia* are distributed throughout Western Australia and *R. preissii* is the first to be commercially grown and adopted by growers as a fodder shrub. It performs well in nutrient poor soils, is drought tolerant and produces substantial dry matter of reasonable nutritive value with 23% protein and an estimated digestibility of 60-65% (H. Norman, personal communication). These traits advocate its use in a mix of perennial species; in addition, initial *in vitro* results have indicated that *R. preisii* may also have an anthelmintic effect on gastrointestinal worms (A. Kotze and J. O'Grady, personal communication), which is worth further investigation.

Previous studies of bioactive forages for worm control have focussed predominantly on pasture species containing condensed tannins (Lotus, sulla, sainfoin) and terpenes (chicory). There have been few extensive studies of forage shrubs. The chemical responsible for the positive antiparasitic results found *in vitro* for *Rhagodia priessii* is currently unknown, however high levels of phytoecdysteroids, including 20-hydroxyecdysone (20E), which induces abnormal moulting and death in many arthropods, has been found in *Rhagodia baccata* (Dinan *et al.* 1999). It has been suggested that as nematodes have a similar hormonal regulation of ecdysis, phytoecdysteroids could provide an effective control against free living, plant or animal nematodes (refer to summary by (Soriano *et al.* 2004). It is proposed that phytoecdysteroids may have a similar mode of action as condensed tannins by inhibiting the first and/or second stage of larval establishment by inducing moulting of the L3 larvae. If the *in vitro* results are supported *in vivo*, the anti-parasitic mechanism of phytoecdysteroids may offer another natural alternative to chemical drenches for which it will be difficult for nematodes to develop resistance as the exsheathment of L3s is a crucial step in the life-cycle.

2.7 Incorporation of bioactive forages in a grazing system

Perennial shrubs including tagasaste and saltbush have been successfully adopted in Australian grazing systems. Increasing the breadth of species included in these systems could increase their value by providing additional complementary nutrients and PSM resources. Research conducted in animal foraging behaviour, suggests that bioactive plants may be best incorporated in a mixture of species to allow animals to self-medicate and to avoid potential toxicity issues (Villalba and Provenza 2007; Villalba *et al.* 2002). Sheep have been conditioned to self medicate by feeding food and toxins (grains, tannins, oxalic acid) that induced a negative internal state and then proving an alleviating substance (sodium bentonite, polyethylene glycol and dicalcium phosphate). Only conditioned animals, when offered a choice of the three foods and 'medicines', preferred to consume the right substance to rectify their negative internal state (Villalba *et al.* 2006). Livestock may need to be trained to eat plants containing PSM to overcome their novelty. Lambs have been successfully taught to overcome food neophobia by adding a familiar flavour association with a known positive food and through repetitive exposure to novel foods (Launchbaugh *et al.* 1997). Once an association is learned it can be passed down to future generation from ewe to lamb (Mirza and Provenza 1990; Thorhallsdottir *et al.* 1990) so training may only be necessary once. Increasing stock densities has also been shown to increase intake of toxin-containing feed (sagebush), providing alternative feed is available (Shaw *et al.* 2006).

2.7.1 Long versus short-term grazing strategies

The question has been raised (Anthanasiadou and Kyriazakis 2004) as to whether bioactive forages are better used for parasite control as a short-term or long-term strategy. Most grazing trials with bioactive forages have been short-term as PSM-containing plants may have toxic effects and thus limit production, despite reducing the parasite burden. As a short-term strategy bioactive forages could be grazed intensely for a short period of time and act as 'de-worming paddocks' before going back onto normal pasture. If this is the case then the carry through benefits of a reduced worm burden gained from grazing bioactive forages may outweigh short-term production penalties. Bioactive forages could be incorporated in a mix of species in a long-term strategy to allow sheep to self-medicate themselves and balance any anti-nutritional effects with other species provided.

2.7.2 Variation in PSM content

Because the compound responsible for the anthelmintic effect of bioactive forages is often unknown, it becomes important to measure the animals performance and condition when conducting *in vivo* trials (Anthanasiadou and Kyriazakis 2004) to meet 'duty of care' requirements for the health of livestock (Revell and Revell 2007). This issue is complicated by the fact that for bioactive plants to have an effect on the worm population, a large enough quantity needs to eaten. Variation in the bioactive value of a plant is likely to exist between plants of the same species at the same site and from different sites due to genetic and environmental differences. Within the same plant, the production of PSM could be influenced by seasonal conditions, plant maturity and grazing pressures. This variation in plant concentrations of PSM creates challenges in designing grazing studies that measure the effect of PSM on parasites and then further implementing the use of bioactive forages in an integrated management program.

The variation in PSM between plants may be useful in selectively breeding lines that contain desirable or more consistent levels of PSM which have an impact on parasites when ingested. Quantifying the variation within and between plants is also useful for ensuring that the right supplements are provided to offset any anti-nutritional or negative performance effects of the bioactive forage. For example, it is well known that sheep can only eat certain proportion of salt-tolerant plants such as old man saltbush, because they may contain salt at more than 20% of dry matter which restricts feed intake (Norman *et al.* 2004). Supplementing saltbush with a moderate or high energy alternative, such as grain or hay, increases the intake of saltbush by assisting the animal to deal with the high salt load (Thomas *et al.* 2007) and boosts total feed intake.

Understanding the factors that cause the variation in PSM i.e. seasonal conditions, stage of maturity, soil type, will assist in designing short or long-term grazing systems that optimise bioactive forages when they are active and incorporate other control strategies when they are not. In the mean time, it is suggested that when measuring the antiparasitic effect of bioactive forages in grazing studies an attempt is made to correlate the concentrations of PSM by collecting plant material over the duration of the experiment. If the compound responsible for the anthelmintic effect is known, measuring the variation in the concentrations should be adequate; if not known, conducting *in vitro* tests on the plant material will help to validate the field results.

2.8 Conclusion

With the advent of anthelmintic resistance and increased public concern over the use of chemicals in agriculture, alternative strategies are required to develop an integrated program to control gastrointestinal parasites. Bioactive forages used as natural alternatives to anthelmintics could play an important role, especially if they are perennial species suited to a particular environment, thereby providing additional production and environmental benefits. *Rhagodia preisii* is just one promising native shrub species which has shown anthelmintic potential *in vitro* and it is hypothesised that its anthelmintic effects can be replicated in a grazing experiment.

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Section 7

Regional group engagement

We have received considerable interest from individual farmers and farmer groups in the project. They have been keen to learn which shrubs species are likely to be suitable performers in their region.Since we have only one shrub evaluation site, at Monarto, it has not been possible for us to provide the necessary information to all groups, especially those with very different soils and rainfall patterns. In November and December 2008 we invited regional groups across southern Australia to co-invest in the establishment and evaluation of 15-20 shrub species in their own localities. Nine groups were able to invest, and 11 sites have been selected and planted across four states (WA, SA, Vic and NSW; Table 1). Each site has 15 species selected by the Enrich team based on favourable traits from the data we have obtained so far. A further three sites will be estbalised in 2009, two more on the Eyre Peninsula and one by the Liebe Group in the northern wheatbelt of WA.

Table 1. The regional groups involved in shrub evaluation with planting to commence in June-August, depending on the site and seasonal conditions. :

Group or Organisation	Contact	#Sites
South Australia		
Upper North	Charlton Jeisman	1
EP NRM	Neil Ackland	1
EP NRM	Tony Zwar	
Western Australia		
DAFWA Geraldton	Tim Wiley George Woolston	3
Evergreen Farming Group	Phil Barrett-Lennard	1
South Coast NRM	Samantha Rayner Glenn McDonald	1
Victoria		
Mallee CMA	Karen Nalder	1
Vic North Central CMA	Dan Mudford	1
	Lindsey Ezard	1
NSW		
Hay Trees on Plains Landcare	Ian Auldist	1
AWI FMFS	Mary Goodacre, Alistair Donaldson, John Murray	1

The Enrich team interacts closely with each regional group. A protocol outlining the requirements for the collaborative work is sent to each group as the basis for the partnership. A copy of this document is provided below.

Enrich Small Plot Species Field Evaluation Protocols

The opportunity that has been created between the Enrich research team and various collaborating groups offers both parties the chance to broaden their knowledge and outlook whilst gaining a greater understanding of the potential of forage shrub species. Each party has an obligation to fulfil the requirements listed below.

The Enrich research team will:

- Undertake initial species weed risk assessment processes
- Propagate and organise delivery of tubestock to regional groups
- Develop protocols for site establishment, management, measurement, sampling etc
- Train regional groups in applying establishment and measurement protocols
- Arrange for plant samples to be processed and analysed (eg for nutritive value)
- Provide a contact person to oversee and coordinate activities across the trial sites
- Provide additional technical advice to regional groups
- Inform regional groups of the results of the shrub assessment from both their and other collaborating trial sites

It is envisaged that in addition to investing the 'buy in' amount, participating regional groups will, at their cost:

- Establish and manage the site, according to protocols
- Monitor and measure plant performance, according to protocols
- Sample plant material for analysis, according to protocols
- Forward plant samples to the Enrich team
- Provide a contact person to liaise with the Enrich site coordinator
- Participate in Enrich meetings including teleconferences and face-to-face meetings

The protocols mentioned above have been developed to guide the establishment of sites at which the growth, development and productivity of a set of woody perennials from the Enrich program will be assessed. The sites, among which shrub management and measurements are consistent can form a national evaluation network, which can yield a greater understanding of the adaptive range of these species than that from individual, inconsistent sites. Hence, these protocols are intended to standardise the measures and management of shrub performance, in each replicated evaluation trial, in order that the collected data can be consolidated into a larger consistent set.

Site Selection

Enrich is focussed on non-saline low-medium rainfall (250-650mm) environments. Sites should reflect the environmental challenges that are to be addressed or are the most applicable to the wider region. Examine why your group has engaged with Enrich. It may help to develop an aim of what you want shrub based grazing systems to deliver to your region. This may help you focus on where the trial should be located. For example, if production on a particular marginal soil class is a pertinent issue in the region, the trial may have most impact located on this soil type.

Attention should be paid to accessibility and the distance from the office. Sites which receive high passer-by traffic may be advantageous from a community interest viewpoint. Sites with a low weed burden will be particularly desirable. Sites should be as uniform in soil type, aspect,

and slope as possible. Use dimensions of 120m x 120m as a guide for an appropriate area. Depending on the site these dimensions may have to be modified to incorporate an appropriate statistical trial design.

No site should be located in the vicinity of natural vegetation. Where possible, locate the trial site at least 1km from any natural vegetation. Experimental site locations should not be prone to seasonal flooding. Seasonal inundation with water may provide an opportunity for seed to be dispersed long distances, which is highly undesirable.

Site Description

It is desirable to capture as much site information as is possible.

Site location
The site name, distance from nearest town, latitude and longitude as well as the altitude should be recorded.

Soil analysis
Collect 20 soil cores (to a depth of 15cm) evenly across the whole site. Bulk the samples together and submit to a laboratory for complete soil analysis. A minimum of pH, EC, organic carbon, major (P and K) and trace elements and exchangeable cat ions is required. Soil texture is also essential.

Soil profile description (optional)
If possible, a complete description of the soil profile obtained from a soil pit is desirable. This is best achieved with the use of professional services.

Meteorological Information
Unless a weather recording station is on site, capture the actual daily rainfall, maximum and minimum temperatures and evaporation for nearest weather station. The long-term means of each should also be recorded. Projected long-term measurement of these variables for many sites can be obtained from the Bureau of Meteorology weather database: SILO.

Soil Preparation

In most cases a deep ripping of the soil is necessary to begin with as early as possible. If deep ripping is done too close to transplanting, the soil will be very lumpy and full of large air pockets which dries seedling roots. An Agrowplow type implement, put into the earth 30 - 50cm is ideal. This shatters any compacted layers without soil inversion. Ripping only needs to be done in straight rows 3m apart into which the shrubs will be planted. Actual length and layout will depend on each group’s trial design (provided by Enrich).

The object is to have soft, weed-free conditions at planting. Multiple weed kills before planting will be desirable if seasonal conditions allow. Be mindful of herbicide resistance as non-selective herbicides will be used for between row weed control during the life of the trial. The use of an autumn tickle (light cultivation to stimulate germination followed by a non-selective herbicide) approach may be useful to lower weed numbers before planting. However, cultivation of the whole area makes the rows which have been ripped more difficult to find when planting. A balance needs to be found between weed control and the risk of late planting and subsequent hand watering for each region.

Sites do not need to be fertilised.

Germplasm

All tubestock will be supplied by Enrich. Germplasm will be covered by a material transfer agreement (MTA) which recognises Enrich as the owner of the germplasm and the IP associated with it. Further details will be listed in the MTA which will accompany the tubestock.

Planting

Once the tubestock has been received they will need to be kept moist. Tubes can dry out very quickly and should be inspected regularly. It is best if they are left outside until they are planted. Take care when transporting tubestock to the site. They should be transported in a covered trailer or tray to be protected from the wind. Tubestock should be well watered just prior to planting.

The layout of the trial will be provided by the Enrich team. It is important to follow the layout provided. The trial will consist of 15 species replicated 4 times. Plots contain a single species and consist of 6 rows by 6 plants within the row. Rows will be 3m apart with 2m between plants within the row. Due to the differing growth habits and productivity of the 15 species a standard plant density will be used throughout the trial.

Accurate spacing between plants is essential. Keeping rows straight will also help in accessibility for weed control and other operations. A simple and inexpensive method is to buy a number (up to 6) of double sided metric tape measures. Loop one end of the tape measure (cable ties or a U-bolt work well) to the peg placed at the end of each row. Roll the tape measure out and wrap it around a peg at the other end of the row so the tape is tight. This allows accurate and consistent spacing between plants. Always keep the zero end of the tape measure at the one end of the site for the duration of planting. It may help to mark every 1.5m on the tape measure using a texta. When the row is planted pick up the tape measure and move to the next row to be planted.

Planting with hand-planters will be the most flexible method. The best type is a Potti Putki design. These are a tube style planter with a beak that opens at the bottom, so no bending over is needed. Soil can be firmed around the plant after planting carefully using the soles of the planter’s boots. New plants should be placed deep enough so that the potting mix of the tube is covered with the ground top soil.

Planting should commence relatively soon after the break of season, so that adequate moisture is available to allow plants to establish before summer. Whilst the risks of frost, greater weed germination and slow plant growth are disadvantages to late autumn-winter planting, it is more preferable to spring planting where watering may be needed. In areas prone to frosts, the use of tree guards may need to be used to protect young seedlings.

Rabbits and hares can destroy seedlings and a high risk of failure may result without some form of protection. Tree guards may give some protection to young seedlings. Vermin proof fencing may need to be considered where it is known rabbits and hares are likely to cause damage.

If planting occurs late or into dry conditions a commitment to inspect seedlings every day for the first week is absolutely necessary to ensure adequate seedling survival. Feel the soil around a sample of the seedlings to gauge the soil moisture content. Look at the seedlings and visually assess their condition. The frequency of watering will depend on the current weather

conditions-the higher the air temperature and the stronger the winds the more water applications are needed. Continue to monitor the seedlings regularly over the first six weeks to determine whether watering is needed. Once the roots reach the stored soil moisture the plant will be self-sufficient and applied watering is no longer necessary.

Weed Control

Weed control is critical in the establishment year. Weeds growing between the rows need to be controlled whilst protecting the seedlings from spray drift using a shielded sprayer. Non-selective herbicides are the most suitable chemicals to use, however, it is important not to allow spray drift onto the plants. It is important to be mindful of herbicide resistance.

Information regarding the species tolerance to herbicides is extremely limited. As new information becomes available it will be disseminated to collaborators. Regular contact with the wider Enrich team will help in keeping abreast of new developments.

Slashing can be a useful operation in spring to clean the site and control weeds that escaped chemical control. The use of a ride-on lawnmower can be a more flexible way to get between shrubs both between and whilst young, in the row.

Shrub Measurements

Data booklets will be provided to groups and all measurements should be completed in the format provided by the Enrich team and forwarded to the relevant member after each main measurement activity.

Canopy Dimensions
Using a 2m ruler measure the maximum height of the canopy of each individual plant. Also measure the maximum width of each individual plant in both directions (i.e. along and perpendicular to the row). Record all measurements against the correct individual. Measurements should commence in the year after planting. Canopy dimensions should take place in between the 2nd and 3rd week in April and again in late October in each year.

An easy way to construct a ruler is to buy two lengths of PVC pipe (1x 2m & 1x1m) and a T-piece joiner. Cut the 2m piece in half and insert the joiner in the middle. In the open end place the other PVC length so that it is perpendicular to the 2m length. This is the handle. Cut a tape measure and stick it to the 2m length positioning it so that it can be viewed whilst holding the handle.

Plant Survival
In the November after planting record any plant deaths. Thereafter, record plant death when measuring canopy dimensions.

Plant Health
Record any incidence of pest, disease or environmental constraint (e.g. frost). Try to identify the pathogen if possible. Record the extent of the damage. Use the following subjective scoring system:

Score	**Description of plant**
0	no damage
1	minor visual damage/discoloration to leaves
2	significant loss of leaf material
3	plant structural damage
4	death

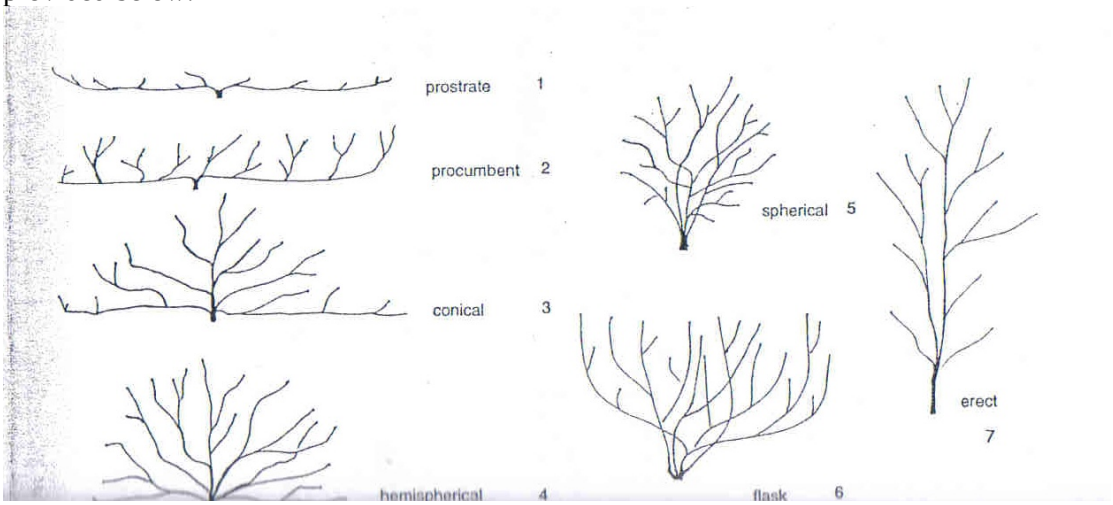
If damage is severe and there is a risk of the loss of the site contact the Enrich co-ordinator to discuss control opportunities.

The site should be inspected at least monthly in the absence of other activities to monitor for pest and diseases, weed burden and general status of the plants.

Flowering and Fruiting
Record whether the individual plant has flowers and or fruit. Record the location of the fruit i.e. on the plant or ground, its maturity and whether intact or not (shattering). This should be recorded in both mid April and again in late October in each year.

Recruitment
Record the number of volunteer seedlings that appear of the planted species, (e.g. 0-10, 10-20), under what circumstances (e.g. amongst weeds, bare ground, in crop, in native vegetation) and of what age-classes they are. See further information below for the importance of this measurement.

Architecture (optional)
Visually assess each individual for their growth architecture. Use the seven categories provided below:



Edible Biomass (optional)
Due to low correlations between canopy dimensions and actual edible biomass for some species, a more preferred method (Adelaide technique) of estimating edible biomass can be undertaken. However, such an approach requires additional time and some initial training. Training support can be provided by members of the Enrich team. Contact the Enrich co-ordinator if this measurement is desired. This should be undertaking immediately before grazing in mid April in the second autumn after planting and subsequently in late October. Thereafter edible biomass can be measured at those times each year.

Leaf Sampling

To test for nutritive value, anthelmintic activity and for effects on rumen fermentation, leaf material will need to be collected. This activity will occur in the 3rd or 4th week of April in the year after planting. Collect approximately an even amount of edible material (leaves and stems less than 5mm in diameter) from each plant within a plot. Collect a total of 100g of fresh material except in the case of *Enchyleana tomentosa* or *Maireana tomentosa* where 200g needs to be collected. Place in 75g in a paper bag (175g for *Enchyleana tomentosa* or *Maireana tomentosa*) and the remainder in a plastic zip lock bag. Keep the material in the

plastic zip lock bag as cool as possible. An esky full of ice should suffice. Record the plot number, growth stage of the plant (see recording sheet), sample constituents and date of sampling. You will also need to record plot number and species on both bags.

As it is important that the plant material in the zip lock bags is kept cool (but not wet), a courier will need to be organised prior to sampling to collect the samples and deliver them to Perth/Adelaide where they will be freeze dried. Overnight delivery will be needed. Co-ordination with Enrich at this time is also essential so that the material can be received and dealt with immediately. It is best to sample early in the week. The material is best packed in a foam esky for travel.

If the group has access to ovens equipped to dry plant material, the samples in the paper bags can be dried at 60°C until fully dry – usually three days. If this is not possible this material can also be sent. It is less important to keep this material cool and thus box packing is sufficient.

Water use (optional)

Perennial species are often valued as they have the ability for higher water use and can act in lowering groundwater. Intensive water use measurements are expensive and laborious so careful consideration should be given before investing in this measurement. A simpler before, during and after sampling using gravimetric methods might be sufficient. Contact the Enrich co-ordinator if this measurement is needed.

Grazing

Once the species have established an opportunity exists to examine how the tested shrub species respond to grazing by livestock. Animal ethics approval from the group's relevant authority will need to be sought before the commencement of grazing.

The site will have to be adequately fenced to hold stock. Adequate water will need to be provided. Due to many species having a high salt content, stock water use will be higher than on conventional pasture. For example in the case of sheep, intakes of 8L water per animal per day should be catered for. If possible multiple water points can be used to minimise the reluctance of stock to graze away from the water source.

A novel, or unfamiliar feed (or plant species) does present an issue for the stock in the short-term as they tend to be cautious with that plant until they have experienced it. Supplementation (hay or grain) will have to be provided if no pasture is present. It is recommended that stock be well fed before entering the site.

The first grazing should take place in their second autumn and after the shrub measurements have been recorded. Grazing in May immediately after the shrub measurements are completed is ideal. Each species should be grazed to a uniform level and also have similar time to re-grow. For this to occur we must minimise selective grazing. Therefore the stocking rate must be high enough to allow for all species to be grazed. Actual stocking rate will be decided by the trial manager as stock availability and plant biomass will influence this. Aim to graze the whole site over a maximum of three weeks. Communication with the Enrich team once an indication of the shrub production is known will aid in a suitable stocking rate to be chosen. The shrubs should be grazed until they reach a desired level of leaf area (i.e. 80% of original leaf area). It is important that the plants are not overgrazed. Record the stock type, breed, age, number of stock and date of grazing.

Grazing for short periods during the growing season may also be possible, mainly for weed control. Unfamiliar livestock will tend to graze the herbaceous layer first before consuming the shrub component. Therefore using stock which were not involved in the autumn grazing (see above) would be useful. A low stocking pressure is necessary (eg 5 DSE). Caution needs to be used to ensure the shrubs are not grazed in any significant amount, as that may impede with their ability to be grazed in the strategic autumn period. This will be particularly likely with highly palatable species (eg *Medicago strasseri*) and this species may have to be protected during a winter/spring grazing.

Note that animals can disperse seed internally and externally. Please see the section below to avoid this occurrence.

Stock Preference

Stock display marked preferences in what feed they will consume. It will be interesting to note which plant species they tend to graze first and if they graze 'species by species' or mix species. Monitoring during grazing will give an indication of this. Preference can be determined using the following method at 5, 10 and 21 days after the start of grazing.

CLASS OF GRAZING	DESCRIPTION OF PLANT	PERCENTAGE DEFOLIATION RATING
1: No sign of grazing can be detected	Un-grazed	0%
2: Rarely grazed	Only a few tips eaten; often a small branch broken but not apparently grazed.	5%
3: Lightly grazed	Uneven grazing of shrub or tree causing some modification in shape; or all soft twigs removed, or certain parts, e.g fruit removed by grazing.	20%
4: Well grazed	Most of plant evenly eaten well back with an occasional branch pulled down. A hedge line is apparent now grazed to the extent of animals grazing height.	50%
5: Heavily grazed	Available forage removed back to stems and trunk.	80% +

Weed Risk Management

Enrich has previously screened all species for their weed history. Species with an obvious weed history will not be planted. There are an increasing number of native Australian species becoming weeds outside their natural range in Australia. This will mean some species will not be planted outside of their indigenous range. Regardless of their origin, the aim is to have no new plant incursions originating from trial sites. The following are a number of recommendations from the FFI CRC to manage the weed risk at field sites.

Minimise fruit/seed set: The genus *Acacia* contains many known weed species, which are often prolific seeders. Seed set should therefore be minimised where possible. Where data regarding fruit/seed set is required, seed should be collected and removed from the

experimental site after counts are made. Ants are also known to disperse *Acacia* seeds, and create caches in the ground. Long-distance dispersal (>100m) by ants is unlikely, but a persistent soil seedbank is likely to form. Fleshy-fruited species may also be attractive to birds, so it may be necessary to monitor bird-feeding habits at each site if these species are planted.

Monitor volunteer establishment: If volunteer seedlings appear, it is important to record how many, under what circumstances and what age-classes they are. How many survive to reproductive maturity? This data is important in a formal weed risk assessment. Many plant species respond to some form of disturbance, such as soil movement, fire, flooding etc. This may result in a flush of germination of propagules, or suckering/vegetative growth of plants. If a particular type of disturbance is noted to cause germination/propagation of new plants, record this information as it could form the basis of a management plan for the species. Also record numbers of new plants and the furthest distance from the parent plant if possible.

Experimental site hygiene: Ensure that shoes, machinery, livestock and vehicles do not inadvertently disperse seed off-site. Seeds of many species can pass unhindered through the gut, pass from the animal and germinate successfully. To ensure seeds are not dispersed in this way from the trial site, livestock should be kept on the trial site or another designated holding area for three days upon completion of grazing. Upon conclusion of the experiments, all plants should be removed and destroyed. It is unacceptable to leave plants in the ground without ongoing monitoring. The cleared sites should also be monitored for several years following the conclusion of the experiments to ensure any seedlings are controlled. Species, such as the *Acacia* spp. produce a persistent soil seedbank that may take many years to exhaust.

Labour Estimation

Based on a site that contains 15 species replicated 4 times, a hectare is required. Working from this scale, it is recommended that provisions are made for at least 175 hours of field labour in the first year. Most of this will involve preparation, planting and weed control. This should decrease to around 100 hours in subsequent years, as weed control will become less important and will be in part managed with grazing. Labour will generally be concentrated in intense periods (e.g. measuring). However, regular site visits will still need to be undertaken at other times.

Budgetary Considerations

Due to the resources and localities of different groups, actual costs will vary but below are a list of costs that should be budgeted for in addition to the tubestock.

- Soil analytical testing/charges
- Field materials (wooden pegs, bags, tape measures, etc)
- Protective clothing
- Chemical purchases
- Transport
- Fuel & lubricants
- Machinery hire
- Postage mail services
- Contractors
- Animal ethics research license

- Livestock requirements - fence
- Livestock requirements - water
- Livestock requirements – supplements
- Administration costs
- Office supplies/costs

Section 8

Publications arising from the Enrich project

Bennell, M., Hobbs, T. Hughes, S. and Revell, D. (in press) Selecting potential woody forage plants that contain beneficial bioactives. FAO/IAEA Special Publication. Ed. P.E. Vercoe

Skaife, J.F. and Vercoe, P.E. (Eds.) (in press) *Harvesting Knowledge, Pharming Opportunities* (Cambridge University Press, Cambridge)

Revell, D.K., Kotze, A. and Thomas, D.T. (2008) Opportunities to use secondary plant compounds to manage diet selection and gut health of grazing herbivores. Proceedings of the International Grasslands Congress/International Rangelands Congress Hohhot, China, July 2008.

Revell, D.K., Z. Durmic, M. Bennell, G.C. Sweeney, and P.E. Vercoe. (2008) "The *in situ* use of plant mixtures including native shrubs in Australian grazing systems; the potential to capitalise on plant diversity for livestock health". *In: Harvesting Knowledge, Pharming Opportunities*, Eds. JF Skaife and PE Vercoe (Cambridge University Press, Cambridge) pp. 36-49

Vercoe, P.E, Z. Durmic, and D. K. Revell. (2008) "Rumen microbial ecology: helping to change landscapes". Options Méditerranéennes, (in press)

Durmic, Z. (2008) "Shrubs could offer a natural alternative". Focus On Perennials. July 2008 Issue

Norman H.C., Wilmot M.G., Thomas D.T., Revell D.K. and Masters DG (2008). Stable carbon isotopes accurately reveal short-term diet selection by sheep grazing mixtures of C3 annual pastures and saltbush or C4 perennial grasses. *Livestock Science* (*in press*).

Durmic, Z., P. Hutton, D.K. Revell, and P. E. Vercoe (2008) "Australian native plant has a potential to protect ruminants from lactic acidosis. 6th Joint INRA-RRI Symposium on Gut Microbiome, Functionality, Interaction with the Host and Impact on the Environment, Clermont-Ferrand, June 18-20, 2008

Hutton P., White, C. L, Durmic Z., Vercoe P. E. (2008) *Eremophila glabra* reduces the accumulation of lactic acid in rumen batch cultures challenged with glucose and has potential to control lactate production in ruminants Journal of Applied Science (in preparation)

Hutton, P.G., C. L. White, Z. Durmic, P. E. Vercoe (2008) Australian plants control induced acidosis *in vitro. ". In: Harvesting Knowledge, Pharming Opportunities*, Eds. J.F. Skaife and P.E. Vercoe (Cambridge University Press, Cambridge) pp. 66-71.

Durmic, Z., S. Payne, P.G. Hutton, and P.E. Vercoe. (2007) Do Australian plants contain secondary compounds that can modulate rumen fermentation? FAO 12th Seminar on Sheep and Goat Nutrition, Thessaloniki, Oct 11-13, 2007

Revell, D.K. (2007) Self-medicating livestock, and how this relates to plant diversity for healthy animals and landscapes. *5th National Native Grasses Conference* (7-10 October, Mudgee, NSW, 2007) Pp. 131-137.

Durmic, Z., P. G. Hutton, F. Kafilzadeh, P.E. Vercoe. (2006) "The effect of Australian native plants on gas production and pH *in vitro*". Short Communication 54. Australian Society of Animal Production 26th Biennial Conference, Perth, July 2006

Masters, D., Edwards N., Sillence, M., Avery, A., Revell, D., Friend M., Sanford, P., Saul, G., Beverly, C. and Young, J. (2006). The role of livestock in the management of dryland salinity. *Australian Journal of Experimental Agriculture* **46**, 733–741.

Revell, D.K. and Sweeney, G. (2004) Aligning profitable grazing systems with reduced water recharge in southern Australia; matching plants, animal grazing behaviour and the environment in mixed forage systems. In: Proceedings of the Conference "Salinity Solutions: Working with Science and Society", 2-5 August 2004, Bendigo, Victoria, Eds: Ridley A, Feikema P, Bennet S, Rogers MJ, Wilkinson R and Hirth J, (CRC for Plant-Based Management of Dryland Salinity: Perth) CD ROM.

Invited presentations

Dean Revell

Invited speaker at the International Grasslands Congress/International Rangelands Congress, July 2008 Hohhot, Mongolia, China. *"Opportunities to use secondary plant compounds to manage diet selection and gut health of grazing herbivores"*

Invited speaker at the national conference on Native Grasslands (Stipa), October 2007, Mudgee, NSW *"Self-medicating livestock, and how this relates to plant diversity for healthy animals and landscapes"*

Invited speaker to the BEHAVE (Behavioral Education for Human, Animal, Vegetation and Ecosystem Management) Conference: "Creating Futures: Behavior in Principle and Practice"; 24-26 October 2006, Utah, USA: *"Recreating pasture ecosystems in Australia"*

Invited speaker to the British Society of Animal Production Special Meeting on Ethnoveterinary Medicine Conference: "Harvesting Knowledge, Pharming Opportunities", 14-15 September 2006 Writtle College, Chelmsford, UK: *"Plant mixtures with Australian native shrubs; The potential to capitalise on plant diversity for livestock health"*

Invited member to FAO/IAEA workshop on plant-derived bioactives for livestock production and health, September 2006, UK: *"Plant mixtures with Australian native shrubs; The potential to capitalise on plant diversity for livestock health"*

Invited speaker at The University of Western Australia Industry Innovation Symposium on Clean, Green and Ethical Animal Production, August 2007: *"Clean, green and ethical production systems with perennial shrubs"*

Invited speaker in symposium at the 2006 conference of the Australian Society of Animal Production, July 2006, Perth: "The use of livestock in the management of dryland salinity"

Philip Vercoe

Expert Consultant and invited speaker at FAO/IAEA Consultants Meeting to screen plants and/or plant products for impact on animal production, health and the environment (July, 2008).

Invited speaker at 12th Seminar of the Sub-Network FAO-CIHEAM on Sheep and Goat Nutrition; 11-13 October 2007, Thessaloniki, Greece: *"Rumen microbial ecology: helping to change landscapes"*

Convenor of FAO/IAEA workshop on plant-derived bioactives for livestock production and health, 12-13 September 2006, UK.

Programme Committee British Society of Animal Production Special Meeting on Ethnoveterinary Medicine Conference: "Harvesting Knowledge, Pharming Opportunities", 14-15 September 2006 Writtle College, Chelmsford, UK.

Session Chair, British Society of Animal Production Special Meeting on Ethnoveterinary Medicine Conference: "Harvesting Knowledge, Pharming Opportunities", 14-15 September 2006 Writtle College, Chelmsford, UK.