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Increase feedbase production and quality of subtropical grass based pastures – NSW component

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Abstract

Tropical grass based pastures in Northern Inland NSW are productive and persistent and provide flexibility to sustainable grazing systems. In Central West NSW, an aseasonal, low-medium rainfall environment, there is also increasing interest in tropical pastures. To maintain productivity of these pastures requires nitrogen and legumes offer the most cost effective and sustainable source. A range of prospective tropical and temperate legumes were evaluated as companion species and component studies addressed specific aspects of their agronomy and ecology. Despite low rainfall during much of the evaluation period, the superior tropical legume was desmanthus. Leucaena was also persistent, however, will not be recommended due to its potential as an environmental weed. Lucerne was the most productive legume evaluated and studies found that to reduce its competitiveness with the grass the pasture species should be sown more closely together. Barrel medic and woolly pod vetch were superior annual temperate legumes in both regions and the dry conditions highlighted that long term persistence of annual legumes requires good establishment and first year seed set. Component studies were conducted to increase knowledge of legume seed banks (hard seed breakdown patterns, optimum sowing time and depth), and soil water dynamics. A tropical grass pasture with 4-9 plants/m^2 was optimum for herbage production and water use efficiency, and importantly provided a 2 year window for establishing a companion legume. The risk of establishment failure of tropical grasses in the Central West was evident and spring determined as the optimum time to establish these species. Additionally, alfalfa mosaic virus was detected in desmanthus cv. Marc; this is the first report of desmanthus as a host in Australia. A number of priority areas for future research were identified and discussed in this report.

Executive summary

Tropical grass based pastures in Northern Inland NSW are productive and persistent. They have become an increasingly important component of grazing systems due to the flexibility they provide and have largely replaced sown temperate grasses throughout the slopes and plains of Northern Inland NSW. There is also increasing interest in these pastures in the Central West of NSW.

Maintaining productivity and quality of tropical pastures is important and can be achieved by applying fertiliser, primarily nitrogen (N). Legumes, however, have high nutritive value and fix N and so are a cost effective and sustainable alternative to fertiliser N. While temperate annual and perennial legumes have traditionally been sown in both regions, previous studies identified tropical legumes as a third group that may be suitable as companion species in tropical grass mixes.

The addition of temperate annual legumes as companion species in tropical grass pastures offers the ability to provide almost year-round forage for livestock. Subterranean clover (*Trifolium subterranean*) has historically been a legume of choice, but with reduced autumn and early-spring rainfall, germination and seed set have been poor resulting in poor persistence. The newer hard seeded legumes (e.g. biserrula, serradella) offer the potential to maintain seedbanks for longer and may be a viable option. To increase success with these species we need to improve establishment and seed set, improve understanding of hard seed breakdown patterns, define optimum plant density of tropical grass pastures that allows the legumes to grow, and develop strategies to build size and robustness of seed banks to insure against adverse years when either legumes or seed set fails.

Lucene (*Medicago sativa*) is the most productive and persistent temperate perennial legume in this environment and has the ability to increase productivity and the length of the growing season of a tropical grass pasture. Lucerne has high early spring growth which increases the grazing period of the pasture, however, it is highly competitive against tropical grasses, so strategies that reduce the competitiveness of lucerne may increase the overall productivity of the pasture. These could include using cultivars with a lower winter activity rating or separating the grass and lucerne in mixed pastures by altering the sowing configuration.

Tropical legumes have not been widely tested in Northern Inland NSW, although earlier studies have indicated that they may have potential, especially desmanthus (*Desmanthus virgatus*) and leucaena (*Leucaena leucocephala* ssp. *glabrata*). Tropical legumes, however, frost in winter like the tropical grasses, so are unlikely to provide a longer growing season, but since they have similar growth patterns as the grasses they may be more equally competitive, and there is potential to replenish soil water in winter, which could be used for spring growth.

This project commenced in 2012 addressing two research objectives:

1. Assess the productivity and persistence of a range of tropical legumes in mixtures with a tropical grass to determine their suitability, and conduct component studies to determine the optimum sowing time for establishment and winter survival, seed softening patterns and soil water dynamics of tropical legume-tropical grass mixed pastures.
2. Identify companion temperate legumes which are persistent and productive in established tropical perennial grass-based pastures, and conduct component studies to determine the most effective time and method of sowing, seed softening patterns, techniques to maximise legume establishment, lucerne sowing configuration and optimum tropical grass plant density to maximise herbage production and water use efficiency

In 2017, following results during the project two additional objectives were added:

1. Conduct targeted research addressing issues relating to establishment and of tropical grasses in low rainfall environments, such as Central West NSW.
2. Establish the causative relationship between alfalfa mosaic virus (AMV) and desmanthus cv. Marc.

Research during this project was conducted during a series of low rainfall years, but has made significant advances in our knowledge of companion legumes for tropical grass pastures in Northern Inland and Central West NSW. This new and increased knowledge of the agronomy and ecology of these species will make quality contributions to agronomy packages for red meat producers and their advisors and will improve both forage supply and quality of the feedbase for the red meat industry.

Tropical legumes

Confidence in desmanthus as a companion legume in Northern Inland NSW. Desmanthus is a productive and persistent legume. A desmanthus-digit grass (*Digitaria eriantha*) pasture has the potential to increase herbage production by 40% compared to a digit grass pasture with a non-persistent legume. In addition, desmanthus did not appear to directly compete with digit grass, is non-bloating, sets large quantities of seed and has a high proportion of hard seed which has a slow breakdown rate in this environment. While the susceptibility of all commercial cultivars of desmanthus to AMV is currently unknown, development of the tropical grass-desmanthus pastures agronomy package has progressed with new knowledge developed in this project.

Leucaena is persistent in Northern Inland NSW. Good preparation is important for successful leucaena establishment and once established plants are highly persistent in Northern Inland NSW and moderately persistent in Central West NSW. While leucaena is not as productive as lucerne and provides forage over a shorter growing season with a grazing window between December and May, it was the only species that produced green forage during extended periods of low rainfall. Leucaena will not be recommended by NSW DPI until sterile lines are available as it has potential to be an environmental weed.

Temperate legumes

Temperate legumes can be effective companion legumes in tropical grass pastures. Barrel medic (*Medicago truncatula*) and woolly pod vetch (*Vicia villosa*) were the best performing legumes in both Northern Inland and Central West NSW showing their broad adaptation. Highest production of hard seeded temperate legumes was achieved by sowing them in autumn, either before or after the tropical grass. Increased knowledge of optimum sowing time to build a seed bank, species seed softening patterns, water use patterns and optimum grass density all contribute to agronomic packages for red meat producers and their advisors.

A tropical grass pasture with a plant density of 4-9 plants/m^2 is optimum for herbage production and water use efficiency. Digit grass pasture with these densities achieves both production and sustainability goals and allows sufficient carryover initial resources to establish a companion legume over the initial 2 years of the pasture.

Lucerne is productive in mixed pastures with tropical grasses. Lucerne was the most productive legume assessed in this project, but highly competitive with the tropical grass. Sowing configurations that keep the grass and legume in narrow bands increased productivity but the optimum plant density and proportion of legume is not understood and potentially also limits long term production and persistence of the pasture.

Establishment of tropical pastures in Central West NSW

Results from this project support anecdotal evidence that tropical pastures can be productive and persistent and have potential in this low-medium rainfall environment, however, the risk of failure during establishment is high. Preliminary studies showed spring as the optimum time to establish tropical grasses.

Desmanthus cv. Marc is a new host for alfalfa mosaic virus in Australia

Plants of desmanthus cv. Marc showing severe yellowing and stunting of plant growth during the 2015-16 summer season were identified as infected with AMV. The cowpea aphid (*Aphis craccivora*) was confirmed as a vector. This is the first report of desmanthus as a host of the virus in Australia. Desmanthus is only one of a large number of susceptible winter and summer crop, pasture and forage legume species grown in Northern Inland NSW, however, AMV could become a limiting factor for the adoption of desmanthus as a pasture legume in NSW.

Priorities for future research

Recommended priorities for future research activities in decreasing order of urgency are:

1. *Effective rhizobia for desmanthus and leucaena*. In 2017, rhizobia strain CB3126 was identified as being ineffective and not nodulating on either desmanthus or leucaena leaving the seed industry and red meat producer without rhizobia. There is a critical need to find a new rhizobia strain for these species and this should be a priority for immediate research. Both legumes are important to the red meat industry so the lack of rhizobia limits effective sowings of both species in Australia and adoption in NSW.
2. *Alfalfa Mosaic Virus in desmanthus*. AMV could become a limiting factor for the adoption of desmanthus as a pasture legume in NSW. In the first instance the current cultivars of desmanthus should be screened for their susceptibility to AMV and the relative productivity losses quantified. Any current and future desmanthus breeding and development programs should incorporate an AMV screening component.
3. *Packages for companion legumes*. There are a number of outstanding gaps in our knowledge to support establishment and management of companion legumes in tropical pastures. These include: establishing legumes into an established grass pasture (as much of the area sown to tropical pastures does not have a productive legume component), herbicide permit (no herbicides are currently recommended for desmanthus in NSW), grazing management of desmanthus to maintain an effective seedbank for long term persistence, seedling survival of desmanthus from late summer and autumn through winter, timing of seed softening and the likelihood of that soft seed remaining viable over winter, the relative competitiveness of seedling tropical legumes and tropical grasses, and the optimum proportion of lucerne in a mixed pasture.
4. *Improving establishment of tropical pastures in Central West NSW*. Desktop and field studies are required to better understand sowing time and seedling survival, and develop strategies that maximise stored soil water prior to sowing and surface soil moisture during germination and emergence.
5. *Impact of diseases and pests on legume productivity*. AMV is only one of a range of potential diseases that affect crop, pasture and forage legume species grown in Northern Inland NSW and Southern Queensland. A comprehensive survey of summer and winter growing crop, pasture and forage species needs to be conducted to determine the diseases that are present, the level of infection and associated productivity loss. Insect vectors should also be considered in this survey.
6. *Tropical legume development* – desmanthus, stylo and leucaena. These three legumes are important legumes in Queensland and also have potential in NSW. Issues which restrict their adaptation in Northern Inland NSW and Southern Queensland include AMV susceptibility (desmanthus), poor cold tolerance and poor seedling vigour. A broad range of germplasm of

Desmanthus and *Stylosanthes* collected from environments and latitudes similar to southern Queensland and northern NSW is held in the Australian Pastures Genebank and warrant investigation to identify lines suitable for Queensland and NSW environments.

Benefits to industry

This project has identified a range of legumes that are suitable as companion species in tropical grass pastures and has significantly increased knowledge of the agronomy and ecology of these species in mixtures.

The development of this new knowledge, that supports both the establishment and maintenance of companion legumes in tropical grass pastures, gives informed options to graziers to increase both the productivity and sustainability of their tropical grass pastures. While this research targeted Northern Inland and Central West NSW, findings apply more broadly to red meat producers in Southern Queensland and Central NSW.

The knowledge of the agronomy and ecology of these companion legumes gained during this project is valuable to inform future systems research, analysis and modelling.

Improving the legume component of tropical grass pastures to supply nitrogen for grass productivity while simultaneously providing high quality forage for livestock, will better enable producers to match forage supply to meet livestock intake requirements in order to reach red meat production targets.

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1 Background

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Sown tropical pastures have made an important contribution to Australian grazing systems for over 100 years. In northern Australia sown tropical pastures have been a component of productive grazing systems since the 1930s, while in Southern Queensland, pasture improvement commenced 1900–1940 and progressed west into the mixed farming and pastoral zones during the 1950s (Walker and Weston 1990). In northern NSW, evaluation commenced in the 1950s (Johnson 1952; Buckley 1959), but it was not until the late 2000s that adoption started to gain momentum and tropical grasses began to become a component of grazing systems due to the productivity and persistence.

In northern NSW, tropical grasses have an important role providing flexible and sustainable grazing systems (Teitzel *et al.* 1991; Boschma *et al.* 2010b). Their favourable attributes include high productivity (Boschma *et al.* 2009), persistence (McCormick *et al.* 1998; Boschma *et al.* 2009), responsiveness to summer storms and fertiliser (Boschma *et al.* 2014a, b), deep rootedness (Murphy *et al.* 2008a), high ground cover and water use efficiency (Murphy *et al.* 2008b).

Prior to the widespread adoption of tropical grasses, temperate grasses, phalaris (*Phalaris aquatic*) and tall fescue (*Festuca arundinaceae*) were the most commonly sown species to increase pasture production and quality in the northern region of NSW (Lodge and Orchard 2000; Harris and Culvenor 2004). However, their inability to persist in the variable and dry environmental conditions on the slopes and plains over several decades (Harris and Culvenor 2004; Boschma *et al.* 2009) forced producers to look for alternative pasture species in their grazing systems. Now, tropical perennial grasses have largely replaced temperate grasses throughout the region (Harris *et al.* 2011).

Northern Inland New South Wales (NSW) region receives 500–800 mm annually and has a summer dominant rainfall pattern, 60% falling over the warmer months of the year (October–March), with high evapotranspiration (Murphy *et al.* 2004). While summer rainfall is higher, it commonly falls in high intensity storms when temperatures are high (Hobbs and Jackson 1977) and so rates of evaporation and runoff can be high. Winter rainfall is more effective, but temperatures are low and frosts frequent with about 50 frosts received over a 145 day period annually (Hobbs and Jackson 1977). Central Western NSW has a lower annual rainfall (400–600 mm), with highest totals occurring in the eastern areas adjoining the slopes and at higher elevations. Rainfall has a near even, or aseasonal distribution throughout the year, and again summer temperatures are high and in winter frosts are common (Central West Catchment Authority 2008).

1.1 Tropical perennial grass in NSW

The first recorded evaluation of tropical grasses in northern NSW was in the 1950s (Johnson 1952; Buckley 1959). Their potential for use in pasture mixes was recognised and recommended at this time, but adoption was low due to poor seed availability and pursuit of grain farming. Since the 1970s a diversity of research has been conducted; species evaluation on the North-West Plains in the 1970s (Watt 1976) and Central West during the 1980s (Muldoon 1986), establishment technologies in the 1980s and 1990s (Bowman 1990; Campbell *et al.* 1993), and, NSW Department of Primary Industries (DPI) conducted a wide scaled evaluation of tropical grass species and cultivars across half of inland NSW during the 1990s (McCormick *et al.* 1998), with further evaluation and selection studies conducted in 2000s (Boschma *et al.* 2009; Harris *et al.* 2009). Studies conducted on the North-West Slopes during the 2000s clearly showed the superior persistence and production potential of tropical grasses compared to the temperate grasses (Boschma *et al.* 2009).

1.2 Barriers to adoption

Some producers have been successfully using tropical grasses in their grazing systems for many years (e.g. Murray 2004; Bowman 2008; Case studies in Harris *et al.* 2014), but it is only since the 2000s with extended periods of dry conditions and continual highly variable seasons that tropical grasses have shown their advantage as a responsive and persistent pasture option for livestock production. There was interest by producers in this suite of grasses, but concerns about sowing them, so a series of workshops were conducted to investigate producer perceptions and to identify issues affecting adoption. Issues raised included difficulty establishing the grasses/high risk of failure sometimes resulting from a previous bad experience, cost of establishment, lost production during establishment (slow establishment), perceived poor forage quality compared with temperate grasses, and a general lack of information or 'agronomic package' (McCormick *et al.* 2009a).

Initial research targeted barriers to successful establishment by addressing issues such as seed quality (McCormick *et al.* 2009a), optimum time of sowing and resultant seedling survival (Lodge and Harden 2009; Lodge *et al.* 2010a), optimum sowing depth (Lodge and Harden 2009), importance of weed control (Lodge *et al.* 2010a), growth rates (Murphy *et al.* 2010; Boschma *et al.* 2016), response to nitrogen (N) (Boschma *et al.* 2014a), forage quality (Boschma *et al.* 2016), rooting depth (Murphy *et al.* 2008a, 2018), and soil water dynamics (Murphy *et al.* 2018). These were developed in tips for successful establishment of tropical perennial grasses (Lodge and McCormick 2010), and published in extension booklets for producers and advisors (e.g. Harris *et al.* 2014). The success of this work is demonstrated by more than 450,000 ha now estimated to have been sown across northern NSW in the last 10–15 years, with seed sales (2011–15) indicating that at least 25,000 ha are sown annually.

1.3 Maintaining pasture productivity and quality

Tropical perennial grasses are a productive suite of species that commence growth in spring and grow throughout summer, ceasing when frosts commence in autumn (e.g. Muldoon 1986; Murphy *et al.* 2010; Boschma *et al.* 2014a, 2016) Therefore, they can be productive for about 9 months of the year on the North-West Plains, 7–8 months on the North-West Slopes and 5–6 months on the Northern Tablelands. Growth rates are highly variable; dependent on both rainfall/soil moisture and fertility, in particular N (Boschma *et al.* 2014; Murphy *et al.* 2018), which is required to maintain productivity (e.g. Robbins *et al.* 1987; Meyers and Robbins 1991). Research in Northern Inland NSW has shown good response in productivity and forage quality when 50–100 kg N/ha is applied (Boschma *et al.* 2014a, 2016). In this region 3 year old digit grass (*Digitaria eriantha*) fertilised with 100 kg N produced about 15,000 kg DM/ha of forage with crude protein and metabolisable energy of about 16.0% and 8.6 MJ/kg DM respectively, while unfertilised digit produced about 5000 kg DM/ha of forage with a crude protein and metabolisable energy of 10.8% and 8.1 MJ/kg DM respectively (Boschma *et al.* 2014a, 2016). These studies show that improved productivity and forage quality can be achieved by application of N fertiliser, or alternatively N could be supplied by legumes.

1.4 Legumes

Legumes are an attractive option in grazing systems as they can be a cost effective and sustainable source of N for grass pastures (e.g. Peck *et al.* 2011, 2012) and a high quality forage. To fix N a legume must be adequately nodulated with the correct, effective strain of rhizobia (Thies *et al.* 1991; Peoples *et al.* 2012) and while there are multiple factors that can affect nodulation and subsequent N fixation, ultimately, the amount of N fixed by a legume is proportional to the amount of herbage produced (Peoples *et al.* 2012). Reported N fixation values are highly variable, but a general rule of thumb is that temperate legumes fix about 35–40 kg N/ha/t legume DM produced (taking into account N fixed on a whole plant basis; Peoples *et al.* 2012). Published literature suggests that tropical legumes fix less N than temperate species, however this requires further work to quantify. For cereals grown after tropical legume crops, reported differences in soil organic N and improvements in N uptake suggested that more N can be produced (Vallis 1972; Cameron 1996; Jones *et al.* 1996) but there were also issues of variable nodulation (e.g. McInnes and Date 2004), which can significantly impact on N fixation. So, using temperate legumes as an example, of the 35–40 kg N/ha/t legume DM produced by temperate legumes, about 13 kg N/ha/t DM becomes available to plants in a perennial pasture (Herridge 2004). Therefore, if in order to fix a minimum of 50 kg N/ha to maintain productivity in a tropical grass pasture, a legume needs to produce >4 t DM/ha annually.

In order for a legume to fix N year-to-year, it must be able to establish and persist in competition with the tropical grass and through highly variable rainfall seasons with hot summers and cold frosted winters. It must also be able to respond quickly to rainfall which falls during its growing season, withstand grazing, and pest and disease pressure.

During 2009–12, NSW DPI conducted preliminary research to determine the types of legumes that might be effective in tropical grass pastures (Boschma and Harris 2009), that is, legumes that were both productive and persistent. Three legume groups were identified as having potential and warrant further investigation and development: temperate annual, temperate perennial and tropical legumes.

1.4.1 Temperate annual legumes

The main temperate annual legumes traditionally sown in Northern Inland and Central West NSW have been subterranean clover (*Trifolium subterranean*) on Chromosol soils and medics (*Medicago* spp.) on alkaline Vertosol soils (Michalk and Beale 1976; Lodge *et al.* 1991) especially in lower rainfall environments. They have a contrasting growth pattern to tropical grasses; regenerating from seed on autumn rainfall, grow through winter, with peak growth in spring before setting seed in spring. Incorporation of a temperate annual legume into a tropical perennial grass pasture offers the potential to increase the period that forage is available from a paddock from about 7 to almost 12 months a year (Fig. 1.1a).

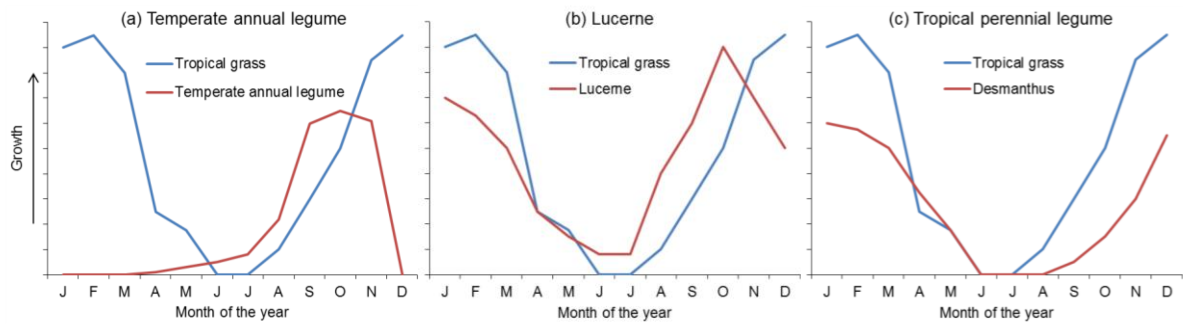

Fig. 1.1. Estimated relative growth curves of (a) temperate annual legume, (b) lucerne and (c) tropical legume in a mixture with a tropical perennial grass (modified from Boschma *et al.* 2014b). Note, actual growth is dependent on climatic conditions and competitive interactions between species.

Preliminary studies in northern NSW identified some species of temperate annual legumes that were both productive and persistent, but also presented a number of challenges. These challenges included initial establishment techniques and an ongoing risk of failure for the legumes to regenerate each autumn because stored soil moisture levels are often low at that time of the year in the established tropical grass pastures (Murphy *et al.* 2018). The lack of stored soil moisture places further risk on the legume and forces it to be dependent on rainfall during the growing season to produce herbage mass and set seed. Improved establishment techniques (e.g. sowing time) need to be developed to improve initial establishment success.

1.4.2 Temperate perennial legumes

Temperate perennial legumes generally have a similar growth pattern to the temperate annual species; growing during the cooler months of the year and potentially offer near year-round forage from a single pasture. Perennials have the advantage of being able to survive multiple years and are not dependent on annual regeneration. In both northern and central NSW, lucerne is the most productive and persistent temperate perennial legume (Boschma *et al.* 2011). Lucerne grows actively when there is soil water from spring, through summer and autumn, with more modest growth in winter, which is dependent on the winter activity rating. In a mixture with a tropical grass, forage production can be increased from 7 to ≥9 months of the year (Fig. 1b).

Studies conducted in northern NSW have shown that lucerne-tropical grass mixed pastures can be highly productive (Murphy *et al.* 2014b), but any soil water accumulated during winter when both species are not actively growing is utilised in early spring by lucerne, leaving little/nil for the tropical grass (Murphy *et al.* 2018). Lucerne is a highly competitive species and actively competes with the grass during establishment (Boschma *et al.* 2010a) and beyond, however this competition could potentially be manipulated by sowing configuration of the two species and/or using lucerne with different winter activity ratings.

1.4.3 Tropical legumes

These summer growing legumes are commonly sown in Queensland but have not been widely tested in NSW. Some published evaluations include Moylan and Crocker (2000) and Boschma *et al.* (2012) in northern NSW, and Muldoon (1986) in Central West NSW. Tropical legumes are frost susceptible and have a similar growth pattern as tropical grasses; growing during the warmer months of the year from spring until autumn (Boschma *et al.* 2014a, 2016; Murphy *et al.* 2018; Fig. 1c). Their synchronised growth means that pasture production is most likely limited to about 7 months (the same as a pure grass; from spring until autumn), however, similar to a pure grass stand, soil water reserves have potential to be replenished over the winter for use in spring (Murphy *et al.* 2018).

Preliminary evaluation conducted in Northern Inland NSW found desmanthus (*Desmanthus virgatus* cv. Marc) and leucaena (*Leucaena leucocephala* ssp. *glabrata* cv. Tarramba) to be both productive and persistent under grazing (Boschma, unpublished data). Sowing these legumes in NSW is outside their recognised area of adaptation, so little is known of their agronomy (establishment and management), productivity, and persistence in this environment. Before these legumes can be recommended in NSW, evaluation of a broader range of species across the region is required to confirm species adaptation, productivity and persistence, and development of agronomy knowledge (establishment and management) to support producers and their advisors.

2 Project objectives

2.1 National project

In 2012, a national project involving NSW and Western Australia (WA) commenced to increase the productivity of tropical pastures by incorporating legumes or other species with high nutritive value, which would therefore also increase the overall quality of the feedbase. The national objective was to enable increased feedbase production and quality of tropical grass-based pastures through improved establishment and maintenance of legumes and other compatible forage components in four targeted southern Australia red meat producing regions. This was to be achieved by:

1. Evaluating establishment techniques, persistence and productivity of prospective species of tropical legumes.
2. Evaluating establishment techniques, times of sowing, and management requirements of prospective temperate legume species into existing subtropical grass pastures on a range of soil types.
3. Evaluating the feasibility of strategies to manage seasonality of forage supply from subtropical grass based pastures.
4. Establishing up to 5 participatory learning sites and engaging at least 100 leading producers twice annually at each site to assist research implementation, data interpretation and product development. Establishing commercial scale integration and evaluation of best practice pasture management will be explored with the producers during development of the participatory sites.
5. Providing project integration across two state agencies and producers involved in the participatory sites and other key networks as relevant.

These national objectives were developed into six activities, addressed by either or both NSW DPI and Department of Primary Industries and Regional Development (DPIRD), Western Australia (WA) (formerly Department of Agriculture and Food WA). DPIRD submitted a separate final report in July 2017 (Sanford *et al.* 2017). The following activities and objectives were addressed by NSW DPI.

2.2 NSW DPI research activities and objectives

2.2.1 Evaluate establishment techniques, persistence and productivity of prospective species of tropical legumes

Objective: Test the suitability of cultivars and elite lines of a range of subtropical legumes identified as having potential (e.g. desmanthus, fine stem stylo, round leaf cassia, leucaena) being sown in mixtures with a tropical grass.

In replicated plots in spatially designed experiments at three core sites across northern and central NSW representing a range of soil types and annual rainfall; conduct component studies including time of sowing to optimise establishment and winter survival; seed production and softening patterns. Water use relationships between combinations of tropical legume-tropical grass pastures and contrasts between grass productivity under nitrogen application will also be examined at one location.

2.2.2 Evaluate establishment techniques, times of sowing, and grazing management requirements of prospective temperate legume species into existing tropical grass pastures on a range of soil types

Objective: To identify companion annual (and/or perennial) legumes which are persistent and productive in established perennial grass-based pastures; and develop reliable methods for establishing annual legumes into perennial grass-based pastures.

Cultivars of a range of hard seeded temperate annual legumes (e.g. biserrula, serradella, bladder clover) sown into subtropical grass pastures at each of the three core sites. Core data collection will consist of grass and legume herbage production (collected throughout the legume and grass growing seasons), legume regeneration and grass persistence (assessed twice a year) in accordance with a protocol. Component studies will also be conducted to better understand specific agronomy aspects of temperate legumes in mixtures with tropical grasses. These studies will include time and method of sowing; seed softening patterns and optimum tropical grass plant density to maximise herbage production and water use efficiency.

2.2.3 Conduct targeted research addressing (i) issues relating to establishment, growth and nutritive value of tropical grasses in low rainfall environments (Central West NSW), and (ii) infection of desmanthus cv. Marc with alfalfa mosaic virus.

Objective: (i) Determine the optimum sowing time for emergence and seedling survival, and determine herbage production and nutritive value response of tropical grass species to nitrogen in Central West NSW. (ii) Establish the causative relationship between the virus and disease on desmanthus.

2.2.4 Establish up to five leading producer-directed participatory research sites, and engage 100 leading producers in planning, implementation and interpretation of the science as well as conducting general field days and communications.

Objective: Evaluate the establishment, productivity and persistence of subtropical based pastures under commercial grazing conditions. (Funding for this activity will be from a separate contract. This may include commercial scale integration of best practice).

2.2.5 Project integration

Objective: Maintain effective communication and sharing of R&D outputs between project partners and researchers towards developing operational plans, package development and communicating messages to industry.

2.2.6 Report

The activities conducted in this program of work were diverse with a series of multisite and component research studies conducted to address the above objectives.

To help communicate the findings from these diverse activities, each study has been prepared as a separate chapter and the chapters ordered into four sections that focus on different areas of the work. The chapters have been drawn together to address each of the project objectives in the general discussion (Chapter 17). The research areas were:

1. *Legume evaluation*. Chapters 4–6 outline findings of studies to determine temperate and tropical legumes that may be suitable companion legumes for sown tropical grass pastures.

2. *Effective grass and legume establishment*. Studies which investigated specific aspects of establishment of tropical grasses or legumes are detailed in Chapters 7–10.
3. *Improving productivity and persistence of tropical pastures*. Chapters 11–14 outline studies conducted to increase knowledge about specific aspects of either pure or mixed sward persistence
4. *Hydrological response of tropical pastures*. Soil water dynamics of tropical pastures in northern and central west NSW are provided in Chapters 15 and 16.

3 The project team

Members of the project team are listed in Table 3.1.

Table 3.1. Name and location of NSW DPI staff who contributed to the project; scientific, technical and/or extension.

Name	Location
Dr Suzanne Boschma, Dr Sean Murphy, Mark Brennan, Steven Harden, Dr Joop van Leur, Zorica Duric, Lester McCormick, Geoff Bevan, Peter Sanson, Jule Helen George, Ivan Stace	Tamworth Agricultural Institute, 4 Marsden Park Rd, Calala NSW 2340
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4 Companion legumes for tropical perennial grass pastures in Northern Inland and Central Western New South Wales. 1. Temperate annual legumes

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4.1 Introduction

Northern Inland and Central West NSW have a summer dominant and aseasonal rainfall distribution respectively, but in both regions winter rainfall is more effective because rainfall intensity and evapotranspiration rates are low compared with summer when rainfall commonly falls in high intensity storms and temperatures are high (Hobbs and Jackson 1977). Sown pastures in both regions have traditionally consisted of temperate annual and perennial species; *Phalaris aquatica* (phalaris) and *Festuca arundinaceae* (tall fescue) the most common grasses (Lodge and Orchard 2000; Harris and Culvenor 2004), *Medicago sativa* (lucerne) the most widely sown perennial legume (Boschma *et al.* 2011), and temperate annual legumes *Trifolium subterraneum* (subterranean clover) and *Medicago* spp. (medics) on the neutral and alkaline soils respectively (Lodge *et al.* 1991). However, over several decades these temperate species have failed to persist (Harris and Culvenor 2004; Boschma *et al.* 2009), such that the temperate grasses have now largely been replaced by tropical grasses in many areas, particularly in Northern Inland mixed farming zone of NSW (Harris *et al.* 2014).

Tropical grasses have a range of agronomic and environmental attributes making them valuable pastures in these regions, including responsive to summer rainfall, highly productive and persistent, and drought tolerant (Harris *et al.* 2014; Boschma *et al.* 2010b, 2014a,b). Good fertility, especially nitrogen, is essential to maintain productivity (Boschma *et al.* 2014a) and while inorganic nitrogen can be applied, productive legumes offer a sustainable, cost effective alternative (e.g. Harris *et al.* 2014; Peck *et al.* 2011, 2012b). Of the traditionally sown legumes, lucerne is still the most persistent and productive perennial legume, but is most commonly sown as a pure sward due to its fit in cropping rotations (e.g. McDonald *et al.* 2003) and competitiveness with grasses (Boschma *et al.* 2010a). In the Northern Inland region, subterranean clover was once the dominant annual legume, however, regular extended dry periods and changing rainfall patterns has resulted in fewer years where it is productive, and seed banks have become depleted in many areas. This demise of traditional legumes has left a large gap in adapted legumes for this region, especially in mixed pastures. In central west NSW, medics have exhibited adaptability and are the most widely sown annual legume, but they have not been evaluated as a companion legume in sown tropical grass pastures. Running parallel to declining persistence of traditional legumes has been the development of a range of new legume species which have favourable attributes including deep rootedness, indeterminate flowering, high seed production, and high hard seed levels (Nichols *et al.* 2012a). These new species have shown potential in southern and central NSW (e.g. Hackney *et al.* 2012a,b,c), but have not been widely tested in either the low rainfall Central West region or the Northern Inland summer dominant rainfall zone, nor in mixes with a tropical grass.

Research has shown that tropical grass pastures use soil water from about September, throughout summer until May when frosts commence in this environment (Boschma *et al.* 2014a; Murphy *et al.* 2018). At this time soil profile water levels are commonly lowest (Murphy *et al.* 2018), and while this has the advantage of providing a dry buffer to conserve soil moisture over the winter period, it means that there is little available for establishment or regeneration of temperate annual legumes. A reliable temperate annual legume to be sown with tropical perennial grass mixes will need to exhibit high seedling vigour for rapid legume establishment, deep rootedness to allow the legume to access deep soil moisture, either early to mid or indeterminate flowering to ensure seed set, and high seed yields of hard seed to maintain a large seed bank (e.g. Hackney *et al.* 2012a,b,c).

A series of studies were conducted 2013–16 with experiments at three locations across Northern Inland and Central West NSW to identify compatible legumes for tropical grass pastures. Three groups of legumes were considered: temperate annual legumes, perennial herbaceous legumes and leucaena. The main objective of this study was to evaluate a range of temperate legume species and cultivars in mixes with *Digitaria eriantha* (digit grass) to identify those suitable for Northern and Central West NSW. The agronomic traits used to determine suitability were establishment and regeneration plant density, grass persistence and legume and grass herbage production over three years. The aims of the study were to compare species and identify those with (1) broad adaptation to a range of environments, but also those with (2) adaptations to specific environments and location.

4.2 Methodology

4.2.1 Study Sites

Three experimental sites were established near Bingara and Manilla in northern NSW and Trangie in Central West NSW. The northern sites represent areas where tropical perennial grasses are currently grown while the Trangie site represents a region with a more marginal environment where interest in tropical grasses is increasing. They were selected as they represent a range of annual average rainfall and seasonal distribution patterns and altitude (Table 4.1, Fig. 4.1).

Table 4.1. Characteristics of the three experimental sites including location, elevation, soil classification (Isbell 1996), soil pH ($CaCl_2$, 0–0.1 m, pH_{ca}) and pre-experimental management.

Characteristic	Bingara	Manilla	Trangie
Location	29°42’39” S, 150°27’07” E	30°42’11” S, 150°30’10”	31°59’45” S, 147°56’18” E
Elevation (m)	297	412	214
AAR (mm)	745	576	500
Soil classification	Brown Chromosol soil	Brown Chromosol soil	Brown Chromosol
Soil pH_{Ca}	5.0	6.1	5.1
Pre-experimental management	During the two winters prior, the area was sown to forage oat and left as a fallow over the summer period. Summer weeds were controlled with 1.5 L/ha glyphosate (450 g/L a.i.). In December 2012, prior to sowing, area was cultivated with a rotary-hoe and levelled with pasture harrows	In the two years prior to sowing, area was sown to forage oat during winter and left as a fallow over the summer period. Summer weeds were controlled with 1.5 L/ha glyphosate. In November–December 2012, prior to sowing, the area was irrigated with about 50 mm water to encourage germination of summer weeds and the weeds were sprayed with 1.5 L/ha glyphosate	During the 12 months prior to sowing the experiment, the native pasture (dominated by *Enteropogan acicularis*) was sprayed three times with 2 L/ha glyphosate and cultivated with offset disks six weeks prior to sowing

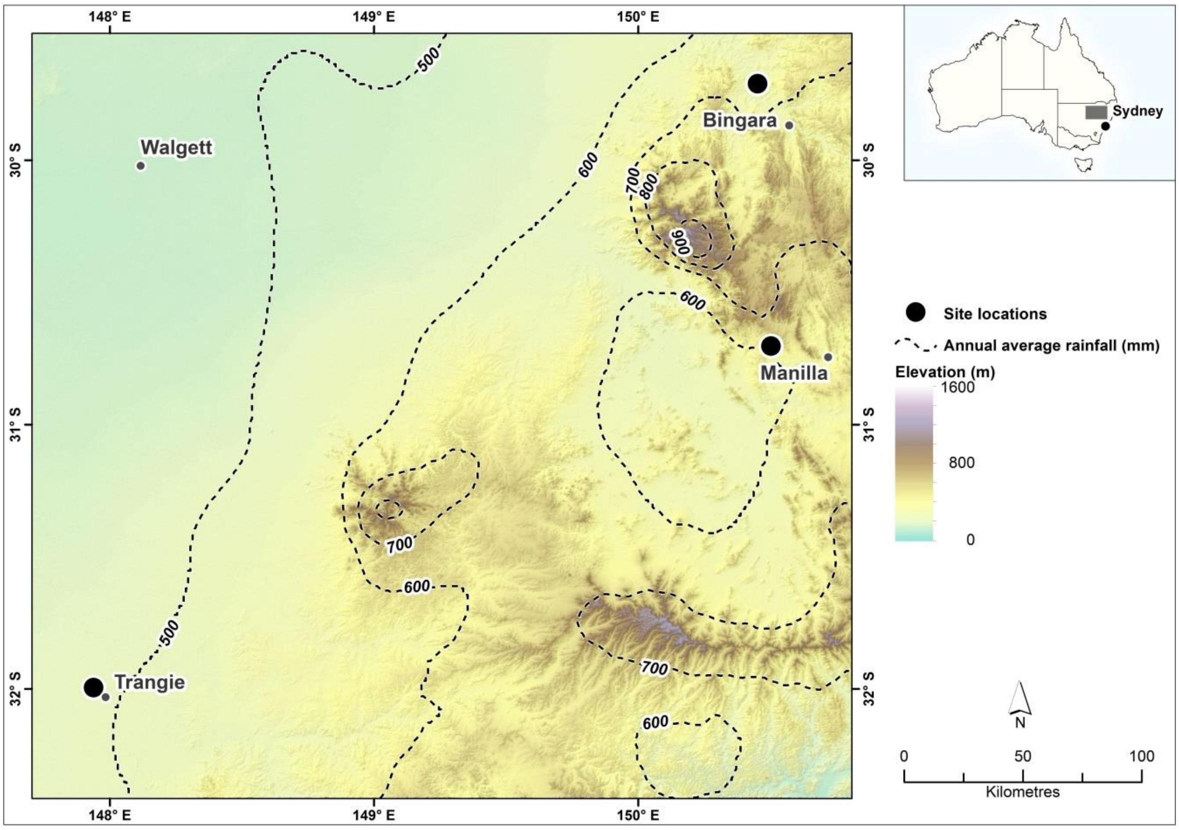

Fig. 4.1. Northern Inland and Central West NSW legume evaluation study area. The area has a rainfall gradient from higher average annual rainfall (>600) in the east, to lower (<500 mm) in the west.

Seasonality of rainfall also changes across the area with southern localities representing aseasonal (even) rainfall distribution and northern localities summer dominant rainfall. Experimental site locations are indicated with ●.

At each of the three study locations, soil cores (0–10 cm, 10–20 cm) were collected at the start of the experiment, analysed for pH (in both water and $CaCl_2$), organic carbon (Walkley and Black), nitrate nitrogen (N), sulphate sulfur (extracted in 1 M KCl at 40°C, KCl_{40}), phosphorus (P, Colwell), potassium potassium (ammonium acetate), also cations and anions and electric conductivity at commercial NATA accredited laboratories (Table 4.2).

Table 4.2. Soil chemical properties (0–10 and 10–20 cm depth) for Bingara, Manilla and Trangie sites.

Property	Unit	Bingara		Manilla		Trangie	
		0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
EC	dS/m	0.12	0.15	0.063	0.14	5.09	5.04
pH ($CaCl_2$)	pH units	5.0	5.9	6.1	7.3	5.1	5.3
pH (water)	pH units	5.8	6.4	7.1	8.0	6.0	6.0
Sulfur (KCl_{40})	mg/kg	3.2	3.0	4.2	2.3	3.0	2.6
Phosphorus (Colwell)	mg/kg	50.0	36.0	36	12	10	11
PBI	L/kg	81.0	96.0	70	88	50.5	55.2
Organic carbon	%	1.4	0.86	1.4	0.99	1.1	1.0
Total nitrogen	%	0.15	0.094	0.12	0.086	0.086	0.076
Aluminium	cmol(+)/kg	<1	<1	<1	<1	<0.1	<0.1
Calcium	cmol(+)/kg	16	20	14	19	6	6
Potassium	cmol(+)/kg	0.73	0.42	0.70	0.43	1.30	1.20
Magnesium	cmol(+)/kg	6.3	8.3	7.4	9.2	1.4	1.4
Sodium	cmol(+)/kg	0.18	0.12	0.49	0.76	0.06	0.05
CEC	cmol(+)/kg	24	29	23	29	8	8

4.2.2 Species, sowing and experimental designs

The study consisted of 18 treatments at each site: 16 cultivars from 13 temperate annual legume species, also two non-legume treatments: plus-nitrogen (+N) fertiliser and nil-nitrogen (-N) fertiliser. The legumes were selected to represent those traditionally sown in either Northern Inland (e.g. subterranean clover) or Central West NSW (e.g. medic species), those developed in recent years that have either not been evaluated or extensively evaluated in either region (e.g. serradella, biserrula, gland and rose clover), and species that had shown potential previously, but not widely sown (e.g. woolly pod vetch). The species and cultivars sown are listed in Table 4.3.

Each experimental design consisted a randomised complete block with three replicate plots (1.5 x 6 m) of each treatment. Digit grass (*Digitaria eriantha*) cv. Premier was sown at 1 kg/ha (viable bare seed) at <5 mm depth into 3 rows (0.5 m apart) in December 2012 sown with a disc seeder. The grasses were defoliated in May 2013 with a rotary mower and herbage removed in preparation for the legume treatments. In May 2013, the temperate annual legumes were inoculated with the appropriate strain of Rhizobia then sown into three rows between the digit grass using the same disc seeder to produce alternate grass-legume rows (each plot consisted of a total of 6 rows each 0.25 m apart). Sowing rates were based on commercial recommendations with some adjustments so that similar viable seed numbers were sown of cultivars of the same species (Table 4.3). Due to dry conditions, the Manilla site was irrigated with 24 mm (5–12 January) to assist establishment of the grass and 43 mm (4–5 September) to assist the legumes to flower and set seed.

Below average rainfall at the Trangie site prevented the establishment of digit grass following two sowing times (late spring 2012 and 2013) and the experiment continued with pure swards of temperate annual legumes.

Table 4.3. Temperate annual legume species, cultivar and sowing rate and their corresponding treatment number (ID), including the -nitrogen (-N) and + nitrogen (+N) treatments.

ID	Common name	Botanical name	Cultivar	Seed coated	100 seed wts (g)	Sowing rate (kg/ha)
1	Biserrula	*Biserrula pelecinus*	Casbah	No	0.2947	1.5
2	Snail medic	*Medicago scutellata*	Silver	Yes	3.4148	7.0
3	Barrel medic	*M. truncatula*	Caliph	Yes	0.9372	5.0
4	Barrel medic	*M. truncatula*	Jester	No	0.3917	2.0
5	Yellow serradella	*Ornithopus compressus*	Santorini	No	0.6564	3.5
6	French serradella	*O. sativus*	Margurita	No	0.2519	1.5
7	Gland clover	*Trifolium glanduliferum*	Prima	Yes	0.1661	2.0
8	Rose clover	*T. hirtum*	SARDI Rose	Yes	0.2257	1.0
9	Purple clover	*T. purpureum*	Electra	No	0.1725	1.0
10	Persian clover	*T. resupinatum*	Nitro Plus	No	0.0776	1.0
11	Bladder clover	*T. spumosum*	Bartolo	Yes	0.5548	3.0
12	Subterranean clover	*T. subterraneum* ssp. *subterraneum*	Dalkeith	Yes	2.0246	10.0
13	Subterranean clover	*T. subterraneum* ssp. *subterraneum*	Campeda	No	0.5201	3.0
14	Arrowleaf clover	*T. vesiculosum*	Cefalu	Yes	0.2192	1.0
15	Woolly pod vetch	*Vicia villosa*	Capello	No	4.8247	6.0
16	Woolly pod vetch	*V. villosa*	Haymaker	No	4.8025	6.0
17	-N	-	-	-	-	-
18	+N	-	-	-	-	-

No fertilisers were applied the year of sowing. Each year from November 2013, 100 kg/ha single superphosphate ([8.8% phosphorus (P), 11% sulfur (S)] was applied in November–December and May–June (total 200 kg single superphosphate/year) and 218 kg/ha urea [46% nitrogen (N)] applied to the +N plots in November at the Bingara and Manilla sites. The Trangie site was fertilised with 200 kg single superphosphate in autumn each year.

The Manilla experiment was sprayed with MCPA (750 g/L a.i.) in early March 2013 (at 1.2 L/ha) and mid-June (0.5 L/ha) to control broadleaf weeds including *Portulaca oleracea* (pigweed), *M. polymorpha* (naturalised burr medic) and *Lamium purpureum* (dead nettle). The nil legume plots (i.e. +N and -N plots) were sprayed with 2,4-D (720 g/L a.i. at 1.2 L/ha) each winter to remove *M. polymorpha* seedlings. At the Trangie site, grass weeds consisting predominantly of *Lolium rigidum* (annual ryegrass), were controlled during the legume growing season with fluazifop-P (128 g/L a.i. at 0.82 L/ha; August 2013, March and July 2014) and with haloxyfop (520 g/L a.i. at 0.1 L/ha; July 2016). During summer when the legumes had senesced, weeds were controlled with 1–2 applications of glyphosate (at 1–2 L/ha). *Halotydeus destructor* (red legged earth mite) were controlled with 0.1 L/ha omethoate (290 g/L a.i.) in August 2013 and September 2015 at the Trangie site. No herbicide was applied at the Bingara site.

Rainfall data were recorded by automatic weather station (Trangie) or manually (Bingara and Manilla). Long-term average monthly and annual rainfall data for all sites were determined from the Bureau of Meteorology (BOM) sites.

4.3 Data collection

Comparative plant performance was assessed using three traits; ability to establish (grass seedling density); productivity (legume and grass herbage production) and persistence (legume seedling density over time and grass plant frequency).

4.3.1.1 Seedling density

Counts of seedling density (seedlings/m^2) of digit grass and the legumes were taken at establishment (2013) and after legume regeneration (2014–16) in six quadrats (0.1 x 0.5 m) in each plot. Counts were conducted 4–6 weeks after emergence of digit grass and 6–8 weeks after emergence or regeneration of the legumes at each site. The total number of legume seedling counts (i.e. repeated measure) conducted at each site are shown in Table 4.4.

4.3.1.2 Herbage production

Herbage mass (kg DM/ha) was assessed at each site at approximately six weekly intervals (more frequent during periods of active growth and less frequent during low rainfall periods) and included the measurement of total herbage mass and the proportion of three pasture components: sown grass, sown legume and other. At each assessment total herbage mass was visually estimated on a continuous 0–5 scale (0, nil; 5, high) also the proportion (%) of sown grass, sown legume and other. Twenty calibration quadrats (0.4 x 0.4 m) representing the range of herbage mass at a site (with five quadrats each selected to represent the upper and lower range of herbage mass) were also scored. These quadrats were harvested to approximately 10 mm above ground level, sorted into the three components, dried at 80^{o}C for 48 h before weighing. Herbage mass scores and percentage estimates were regressed (linear or quadratic R^2>0.80) against actual herbage mass (kg DM/ha) and percentage of each component to determine the herbage mass of the sown species. After each assessment the plots were mown with a rotary mower and the herbage removed from the plots. The exception was the final legume assessment in spring each year when mowing was delayed until the legumes had set seed and senesced. The total number of assessments conducted at each site is shown in Table 4.4.

Table 4.4. Number of sample times, factor analytic (FA) model for treatment variance and antedependence (AD) model for residual variance used to analyse data for each trait with repeated measures at each location.

Site	Number of sample times	Treatment	Residual	REML log-likelihood	%vaf
		Legume plant count			
Bingara	4	FA1	AD1	11.30	94.81
Manilla	4	FA1	AD1	-68.41	80.29
Trangie	9	FA2	AD1	-18.34	96.30
		Legume HM			
Bingara	6	FA1	AD2	116.04	99.81
Manilla	6	FA1	AD1	-208.29	96.52
Trangie	6	FA1	AD3	-359.27	89.86
		Grass HM			
Bingara	13	FA1	AD3	970.62	95.43
Manilla	6	FA2	AD2	234.20	85.48
		Grass plant frequency			
Bingara	6	FA1	AD3	727.74	99.96
Manilla	7	FA1	AD3	503.73	97.22

4.3.1.3 Grass persistence

In spring and autumn each year at the Bingara and Manilla sites, plant frequency of digit grass was assessed in a permanent quadrat (1.0 x 0.5 m) centrally located in each plot. Estimates were taken 0–10 days after the experimental area was defoliated. Quadrat locations were identified using a known distance along a tape measure strung between pegs permanently located at both ends of a row of the plots. For each plot, cells containing a portion of a live digit grass plant were recorded (presence) and the proportion (%) of occupied cells used to estimate frequency of occurrence (plant frequency, Brown 1954). The total number of assessments conducted is shown in Table 4.4.

4.3.2 Statistical analyses

Five traits were used for selection among treatments (legume cultivars) in the present study: legume seedling density, legume herbage production, grass seedling density, grass herbage production and grass persistence. While a multivariate analysis would generally be considered the appropriate approach for selection based on multiple genetically correlated traits, this study was complicated by the fact that traits were measured at multiple sites and each trait was assessed multiple times at every site. An ideal approach would involve a single analysis of all traits, sampling times and sites, however, such an analysis is still an area of current research and is beyond the scope of this paper. Therefore, we chose to analyse each trait by site combination separately.

For each trait, except grass establishment plant count, each assessment within a site was considered an 'environment`, such that each subset of data corresponding to a trait by site combination was a multi-environment trial (MET) data-set. The factor analytic (FA) approach of Smith *et al.* (2001) was then used to analyse individual MET subsets. The FA approach has been used widely for the analysis of grain yield data in Australian cereal breeding program and allows for the accurate prediction of cultivar means for environments using best linear unbiased prediction (BLUP) (Kelly *et al.* 2007). Additionally, FA models account for the (unknown) cultivar by environment interaction by modelling the difference between environment genetic variance in terms of a small number of common factors, where the number of factors was the model order.

In the analysis of the grass establishment trait, for which there was only one assessment at each site, a variance component analysis was used. In all models, cultivar effects were fitted as random effects given the aim of the analysis was selection and, by definition, the BLUPs obtained using random cultivar effects best predict the true cultivar effects (Smith *et al.* 2005)

It was anticipated that there may be correlation across sampling times within plots and an antedependence (AD) model for the residuals was assumed. AD models are flexible, non-stationary covariance structures for repeated measurements data with common times of measurement across plots. They are more parsimonious for non-stationary data than the completely unstructured model of the classic multivariate approach and do not assume constant variance or equal correlations between measurements equidistant in time, as with autoregressive models (Pourahmadi 1999).

All analyses were conducted using ASReml (Gilmour *et al.* 2006) in R (R Core Team, 2011). Legume plant count, legume herbage mass, grass frequency and grass plant count data were cube-root transformed and grass herbage mass data were log-transformed to more closely resemble a Gaussian distribution.

An appropriate FA order for cultivar by environment interaction and AD order for residuals was determined for each analysis by comparing sequences of models using the residual maximum likelihood (REML) (Patterson and Thompson, 1971) and the overall percentage of genetic variance accounted (%vaf) by the FA model component (Smith *et al.* 2015). Table 4.4 presents a summary of the number of environments at each trait by site combination as well as the FA and AD order of the final model fitted to each MET data set.

The BLUPs of the overall performance for each legume treatment (Smith *et al.* 2018) for each trait by site combination were calculated, as well as the 90% confidence interval for these predictions. The overall performance was added to the BLUP of the overall mean for cultivars, averaged across environments. This value was then back-transformed as an approximation of the overall mean performance on the scale of the original data to provide a value for each legume treatment that was biologically meaningful. This value and the BLUPs of the overall performance for each legume treatment with the 90% confidence interval are presented for each trait. For some treatments, the resulting overall mean performance was negative, typically for varieties with a high proportion of zero or very small observations and may be interpreted biologically as a value of zero. For some traits, the order of treatments changed when ranking by overall mean performance compared to ranking by the BLUPs of overall performance. This is due to the overall mean performance using an average of treatment effects across environments and the presence of cultivator by environment interaction. When interpreting BLUPs, the confidence intervals provided are not a formal test for comparison of treatments (i.e. significance) because treatment effects were fitted as a random effect. Instead they are a test for the true value of each treatment individually.

4.4 Results

4.4.1 Rainfall

Environmental conditions at the three sites were challenging with rainfall below average most years that the experiment was conducted. Based on long term records, Bingara site was the wettest site (long term average (LTA) annual rainfall 745 mm), but received only 80% of LTA in 2013 when the experiment was establishing and 47% in 2014 (Fig. 4.2). The site received below average rainfall for 17 consecutive months (July 2013–November 2014). Total rainfall for 2015 was average and first six months of 2016 were again below average (76% of LTA). The Manilla site had below average rainfall every year; most years about 76–78% of LTA except 2014 when only 44% of LTA rainfall was received (253 mm *cf.* 576 mm). The Trangie site had the lowest LTA rainfall (500 mm) of the three sites and received below average rainfall during 2013–2014. Annual rainfall was above average for the final 18 months of the study (858 *cf.* 760 mm).

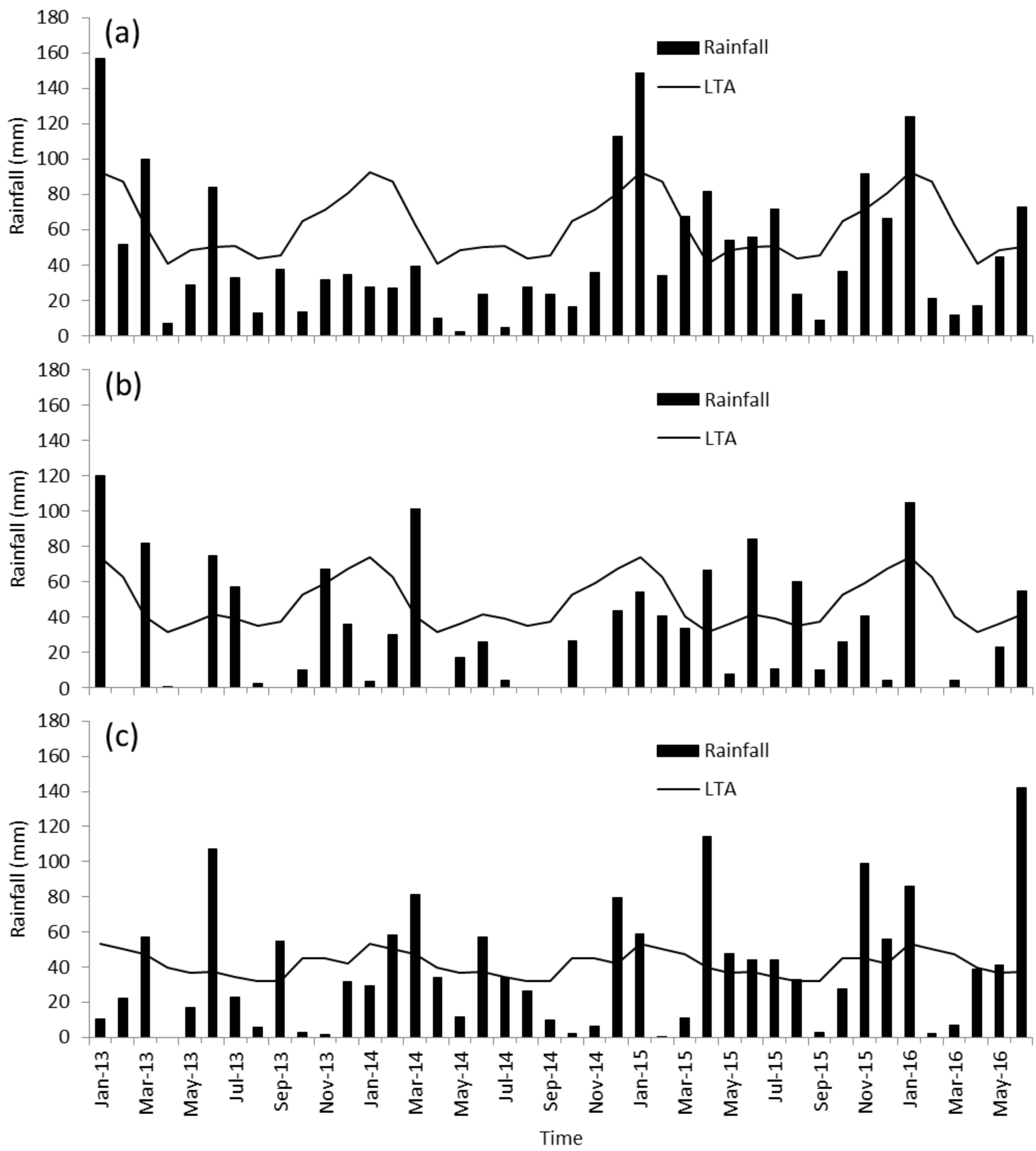

Fig. 4.2. Actual and long term average monthly rainfall (mm) at (a) Bingara, (b) Manilla and (c) Trangie, January 2013–June 2016. LTA data are from BOM site number (a) 054004 (1878–1997), (b) 55274 (1909–2013) and (c) 51049 (1922–2017).

4.4.2 Legume seedling density

Woolly pod vetch cv. Haymaker had the highest seedling density (ranked 1st) over the 4 years of the experiment at Bingara with a predicted plant density of 419 seedlings/m^2 (backtransformed predicted treatment mean, herein referred to by the units only) (Table 4.5). Legumes with similar plant densities were barrel medic cv. Jester, snail medic and woolly pod vetch cv. Capello (ranked 2nd–4th, ≥280 seedlings/m^2). Bladder clover cv. Bartolo had the lowest seedling density ($P < 0.05$), similar to six other legumes all with <5 seedlings/m^2.

At the Manilla site, the barrel medic cultivars had the highest seedling densities, with cvv. Caliph ranked 1st (676 seedlings/m^2; Table 4.5). Snail medic, two woolly pod vetch cultivars and gland clover also had above average seedling densities (BLUP > 0). Persian clover was ranked 16th with the lowest seedling density, only regenerating in small numbers, and was similar to legumes ranked 11th–15th.

At the Trangie site, legumes fell into three distinct groups; two groups with BLUPs >0. The first group with the highest seedling densities were the two barrel medics (335–412 seedlings/m^2; Table 4.5). The second group consisted of six legumes: rose clover, two woolly pod vetch cultivars, biserrula, subterranean clover cv. Campeda and snail medic (11–53 seedlings/m^2). The remaining species had BLUPs < 0 and consisted of seven legumes (ranked 9th–16th) with densities generally <10 plants/m^2.

4.4.3 Legume herbage production

At Bingara the most productive legumes were a distinct group consisting of four legumes. Woolly pod vetch cv. Haymaker was ranked 1st (average 2191 kg DM/ha/assessment) and similar to snail medic, barrel medic cv. Jester and the other woolly pod vetch (>1400 kg DM/ha). Subterranean clover cv. Dalkeith was the least productive, but similarly productive as seven other legumes (ranked 9th–15th); all with below average productivity (BLUP < 0, Table 4.6).

Legumes fell into two distinct groups based on herbage production at the Manilla site; those with >3000 kg DM/ha and those with <50 kg DM/ha; Table 4.6). The productive group consisted of five legumes with barrel medic cv. Caliph ranked 1st. The other legumes were barrel medic cv. Jester, snail medic and the two woolly pod vetch cultivars. The remaining legumes (ranked 6th–16th) had BLUPs < 0 and similar herbage production.

At the Trangie site, the most productive legumes were the two barrel medic cultivars (>5000 kg DM/ha). Biserrula was ranked 3rd followed by wooly pod vetch cv. Haymaker (4th) and snail medic (5th)(916–1686 kg DM/ha). Nine legumes had below average herbage production (≤300 kg DM/ha) and purple clover was the least productive legume (ranked 16th) at this site (Table 4.6).

4.4.4 Grass seedling density

Digit grass established consistently across all treatments at both sites; ranging 24–25 seedlings/m^2 at Bingara and 23 seedlings/m^2 at Manilla (data not shown).

4.4.5 Grass herbage production

At Bingara, digit grass herbage production was highest in woolly pod vetch cv. Haymaker plots (2302 kg DM/ha), but similar to legumes ranked 2nd–4th: barrel medic cv. Jester, woolly pod vetch cv. Capello and snail medic (>1550 kg DM/ha; Table 4.7). Digit grass with 50 kg/ha applied N (+N treatment) had the lowest herbage production (ranked 16th, 409 kg DM/ha) (Table 4.7).

At the Manilla site, digit grass in the barrel medic cv. Jester was highest (ranked 1st) with productivity similar to the woolly pod vetch cv. Haymaker plots (ranked 2nd). Other treatments that had productive digit grass were woolly pod vetch cv. Capello (3rd), barrel medic cv. Caliph (4th) and snail medic (5th).

Table 4.5. Average legume seedling density (seedlings/m^2) at the Bingara, Manilla and Trangie sites. Empirical best linear unbiased predictors (BLUPs) of overall performance are presented with lower and upper confidence intervals (90%), also sum of the overall performance and individual predicted treatment mean which has been backtransformed to provide an estimated seedling density (plants/m^2).

			Bingara					Manilla					Trangie				
ID	Treatment	Cultivar	BLUP	Lower	Upper	Rank	Seedling density (/m^2)	BLUP	Lower	Upper	Rank	Seedling density (/m^2)	BLUP	Lower	Upper	Rank	Seedling density (/m^2)
1	Biserrula	Casbah	-0.15	-0.70	0.40	7	25	0.00	-0.73	0.72	7	13	0.52	0.05	0.99	5	53
2	Snail medic	Silver	1.82	1.27	2.37	3	321	1.94	1.21	2.67	3	307	0.08	-0.39	0.56	8	26
3	Barrel medic	Caliph	0.48	-0.08	1.03	5	86	2.84	2.11	3.57	1	676	2.27	1.81	2.74	1	412
4	Barrel medic	Jester	1.90	1.35	2.45	2	416	2.24	1.51	2.97	2	458	2.05	1.58	2.51	2	335
5	Yellow serradella	Santorini	-1.03	-1.58	-0.48	12	4	-1.33	-2.06	-0.61	14	-1	-0.67	-1.14	-0.20	11	10
6	French serradella	Margurita	-0.48	-1.03	0.07	9	10	-0.97	-1.70	-0.24	13	-1	-0.91	-1.38	-0.44	16	0
7	Gland clover	Prima	0.10	-0.45	0.65	6	52	0.40	-0.33	1.13	5	144	-0.90	-1.36	-0.43	15	0
8	Rose clover	SARDI Rose	-1.04	-1.59	-0.49	13	0	-0.27	-1.00	0.46	8	1	0.59	0.12	1.06	3	14
9	Purple clover	Electra	-0.96	-1.52	-0.41	11	1	-0.94	-1.67	-0.21	12	0	-0.67	-1.13	-0.20	10	2
10	Persian clover	Nitro Plus	-1.11	-1.66	-0.56	15	-1[1]	-1.37	-2.10	-0.64	16	-1	-0.86	-1.33	-0.39	12	0
11	Bladder clover	Bartolo	-1.12	-1.67	-0.57	16	0	-0.64	-1.37	0.09	10	6	-0.66	-1.13	-0.20	9	3
12	Subterranean clover	Dalkeith	-1.11	-1.66	-0.56	14	1	-1.36	-2.09	-0.63	15	2	-0.87	-1.34	-0.40	13	17
13	Subterranean clover	Campeda	-0.36	-0.91	0.19	8	8	-0.76	-1.49	-0.04	11	1	0.09	-0.38	0.55	7	11
14	Arrowleaf clover	Cefalu	-0.82	-1.37	-0.27	10	2	-0.34	-1.07	0.39	9	10	-0.87	-1.34	-0.41	14	0
15	Woolly pod vetch	Capello	1.71	1.16	2.26	4	280	0.46	-0.27	1.19	4	51	0.28	-0.18	0.75	6	33
16	Woolly pod vetch	Haymaker	2.17	1.62	2.72	1	419	0.11	-0.62	0.84	6	29	0.53	0.06	0.99	4	52

[1]Treatments with a high proportion of zero or very small observations often resulted in a negative value for overall mean performance and may be interpreted biologically as a value of zero.

Table 4.6. Average legume herbage production (kg DM/ha) at the Bingara, Manilla and Trangie sites. Empirical best linear unbiased predictors (BLUPs) of overall performance are presented with lower and upper confidence intervals (90%), also sum of the overall performance and individual predicted treatment mean which has been backtransformed to provide an estimated herbage production (kg DM/ha).

			Bingara					Manilla					Trangie				
ID	Treatment	Cultivar	BLUP	Lower	Upper	Rank	Herbage mass (kg DM/ha)	BLUP	Lower	Upper	Rank	Herbage mass (kg DM/ha)	BLUP	Lower	Upper	Rank	Herbage mass (kg DM/ha)
1	Biserrula	Casbah	-0.89	-1.86	0.09	8	19	-2.34	-3.98	-0.70	9	-8	2.39	0.41	4.37	3	1686
2	Snail medic	Silver	3.49	2.51	4.46	2	1624	5.02	3.39	6.66	3	4200	1.19	-0.79	3.17	5	916
3	Barrel medic	Caliph	0.56	-0.41	1.53	5	195	6.24	4.60	7.88	1	5777	5.30	3.32	7.28	2	5137
4	Barrel medic	Jester	3.34	2.37	4.32	3	1521	6.03	4.40	7.67	2	5670	5.62	3.64	7.61	1	5418
5	Yellow serradella	Santorini	-1.83	-2.81	-0.86	14	1	-2.65	-4.28	-1.01	12	-10	-1.02	-3.00	0.96	10	241
6	French serradella	Margurita	-1.21	-2.18	-0.24	9	3	-2.83	-4.47	-1.19	15	-10	-2.07	-4.05	-0.09	12	4
7	Gland clover	Prima	0.32	-0.65	1.30	6	146	-1.57	-3.21	0.06	6	20	-2.49	-4.48	-0.51	13	-6
8	Rose clover	SARDI Rose	-1.59	-2.56	-0.62	11	-8[1]	-2.47	-4.10	-0.83	10	-10	-0.65	-2.63	1.34	9	14
9	Purple clover	Electra	-1.58	-2.55	-0.61	10	-6	-2.61	-4.25	-0.98	11	-10	-3.37	-5.35	-1.39	16	-10
10	Persian clover	Nitro Plus	-1.81	-2.78	-0.84	13	-9	-2.84	-4.48	-1.20	16	-10	-3.10	-5.08	-1.12	15	-10
11	Bladder clover	Bartolo	-1.87	-2.84	-0.89	15	-9	-2.07	-3.71	-0.43	8	-6	-0.11	-2.09	1.87	8	311
12	Subterranean clover	Dalkeith	-1.88	-2.85	-0.90	16	-8	-2.78	-4.42	-1.14	14	-10	-2.75	-4.73	-0.76	14	42
13	Subterranean clover	Campeda	-0.77	-1.74	0.21	7	1	-2.74	-4.38	-1.10	13	-10	0.76	-1.22	2.74	6	245
14	Arrowleaf clover	Cefalu	-1.71	-2.68	-0.74	12	-7	-1.62	-3.26	0.01	7	11	-1.91	-3.89	0.07	11	9
15	Woolly pod vetch	Capello	3.29	2.32	4.27	4	1447	4.66	3.02	6.30	4	3404	0.44	-1.54	2.42	7	409
16	Woolly pod vetch	Haymaker	4.12	3.14	5.09	1	2191	4.57	2.94	6.21	5	3078	1.76	-0.22	3.74	4	996

[1]Treatments with a high proportion of zero or very small observations often resulted in a negative value for overall mean performance and may be interpreted biologically as a value of zero.

Table 4.7. Average grass herbage production (kg DM/ha) at the Bingara and Manilla sites. Empirical best linear unbiased predictors (BLUPs) of overall performance are presented with lower and upper confidence intervals (90%), also sum of the overall performance and individual predicted treatment mean which has been backtransformed to provide an estimated herbage production (kg DM/ha).

			Bingara					Manilla				
ID	Treatment	Cultivar	BLUP	Lower	Upper	Rank	Herbage mass (kg DM/ha)	BLUP	Lower	Upper	Rank	Herbage mass (kg DM/ha)
1	Biserrula	Casbah	-0.02	-0.15	0.11	9	877	-0.02	-0.08	0.05	7	494
2	Snail medic	Silver	0.26	0.13	0.39	4	1563	0.03	-0.04	0.10	5	605
3	Barrel medic	Caliph	0.09	-0.04	0.22	6	1093	0.09	0.02	0.15	4	633
4	Barrel medic	Jester	0.28	0.15	0.42	2	1640	0.18	0.12	0.25	1	625
5	Yellow serradella	Santorini	-0.10	-0.24	0.03	13	776	-0.10	-0.17	-0.03	18	431
6	French serradella	Margurita	-0.06	-0.19	0.07	10	812	-0.02	-0.09	0.05	9	486
7	Gland clover	Prima	0.08	-0.05	0.21	7	1081	-0.04	-0.11	0.03	11	439
8	Rose clover	SARDI Rose	-0.20	-0.33	-0.07	16	596	-0.07	-0.14	0.00	16	407
9	Purple clover	Electra	0.09	-0.04	0.23	5	1111	-0.03	-0.10	0.04	10	508
10	Persian clover	Nitro Plus	-0.17	-0.30	-0.04	15	646	-0.02	-0.09	0.05	8	477
11	Bladder clover	Bartolo	-0.08	-0.21	0.05	12	784	-0.04	-0.11	0.02	15	461
12	Subterranean clover	Dalkeith	-0.24	-0.37	-0.11	17	581	-0.04	-0.11	0.03	13	426
13	Subterranean clover	Campeda	-0.07	-0.20	0.06	11	780	-0.04	-0.11	0.03	12	408
14	Arrowleaf clover	Cefalu	-0.17	-0.30	-0.04	14	666	-0.04	-0.11	0.03	14	444
15	Woolly pod vetch	Capello	0.26	0.13	0.39	3	1561	0.11	0.04	0.18	3	611
16	Woolly pod vetch	Haymaker	0.46	0.33	0.59	1	2302	0.15	0.09	0.22	2	635
17	-N		0.01	-0.12	0.14	8	954	-0.01	-0.08	0.06	6	652
18	+N		-0.41	-0.54	-0.28	18	409	-0.09	-0.16	-0.02	17	390

4.4.6 Grass persistence

At Bingara, plant frequency of digit grass was highest in woolly pod vetch cv. Haymaker plots (90%), and similar to barrel medic cv. Jester (2nd). Plant frequency of digit grass in the barrel medic cv. Jester plots was similar to treatments ranked 1st–5th which included the -N treatment plots (3rd), woolly pod vetch cv. Capello (4th) and snail medic (5th)(>70%). Digit grass in the subterranean clover cv. Dalkeith plots had the lowest plant frequency (45%), but was similar to digit grass in treatments ranked 11th–15th (Table 4.8).

Plant frequency of digit grass at the Manilla site ranged from 20 (purple clover)–30% (yellow serradella cv. Santorini)(Table 4.8).

4.5 Discussion

This study was conducted during a series of seasons that were less than optimal for legume growth and production, but ideal to illustrate the persistence of some of these pasture species. The results from this study have identified: (1) superior legumes at each site, (2) similarities in the best performing legumes between sites despite their different environments, and (3) the importance of good legume establishment and performance in the first year and regeneration in subsequent years and the challenge for their persistence if this is not achieved is evident.

4.5.1 Best performing legumes

The best performing legumes at each site were similar for each trait showing that good establishment and regeneration is related to high herbage production which can positively affect grass productivity and persistence. At Bingara, the highest rainfall site, woolly pod vetch (both cultivars), barrel medic cv. Jester and snail medic cv. Silver were the best performing legumes. At Manilla the intermediate rainfall site, the medics (both barrel cultivars and snail medic cv. Silver) were the superior legumes with woolly pod vetch also being productive and persistent. At Trangie, the lowest rainfall site, barrel medic (both cultivars) was the superior legume followed by woolly pod vetch cv. Haymaker and biserrula cv. Casbah.

The consistent high performance of barrel medic and woolly pod vetch (in particular cvv. Jester and Haymaker respectively) across the three sites shows their broad environmental adaptive range. The ranking of these legumes changed north to south with woolly pod vetch being the most productive at the northern (highest rainfall) site, declining in rank as rainfall deceased. In contrast barrel medic was the superior legume at Trangie, the lowest rainfall and most western site, declining in rank as rainfall increased. The results from this study have some similarities to other studies conducted in northern NSW. At a higher rainfall site, similar to Bingara, woolly pod vetch was the most productive and persistent temperate annual legume in a native pasture (Archer 1981), while in environments similar to Manilla, barrel medic (Tow 1975) and woolly pod vetch (Lodge 1991b) were reported as productive and persistent. Barrel medic has had a long history of use within Central Western NSW (e.g. Michalk and Beale 1976) and may be considered a benchmark legume in this area and this study has shown that it continues to be well adapted, productive and persistent, but woolly pod vetch and biserrula may also be adapted in this area and warrant further investigation. Research in southern and central NSW showed that both French serradella and biserrula were more 2.5–3 times more productive than subterranean clover with the ability to set sufficient seed for regeneration the following season, even in lower rainfall years (Hackney *et al.* 2012a,c) .

Table 4.8. Average plant frequency (%) at the Bingara and Manilla sites. Empirical best linear unbiased predictors (BLUPs) of overall performance are presented with lower and upper confidence intervals (90%), also sum of the overall performance and individual predicted treatment mean which has been backtransformed to provide an estimated plant frequency (%).

			Bingara					Manilla				
ID	Treatment	Cultivar	BLUP	Lower	Upper	Rank	Frequency (%)	BLUP	Lower	Upper	Rank	Frequency (%)
1	Biserrula	Casbah	-0.10	-0.22	0.02	12	49.6	-0.03	-0.13	0.06	13	23.6
2	Snail medic	Silver	0.13	0.01	0.25	5	70.2	-0.07	-0.17	0.02	16	21.4
3	Barrel medic	Caliph	-0.07	-0.19	0.05	11	51.9	-0.01	-0.11	0.08	11	24.2
4	Barrel medic	Jester	0.25	0.13	0.37	2	83.1	0.03	-0.06	0.13	7	27.0
5	Yellow serradella	Santorini	-0.07	-0.19	0.05	10	54.7	0.09	0.00	0.19	1	27.2
6	French serradella	Margurita	-0.14	-0.26	-0.02	17	46.1	0.01	-0.08	0.11	9	25.8
7	Gland clover	Prima	0.05	-0.07	0.17	6	62.6	0.07	-0.03	0.16	3	28.9
8	Rose clover	SARDI Rose	-0.13	-0.26	-0.01	16	44.8	-0.04	-0.14	0.05	14	25.2
9	Purple clover	Electra	-0.02	-0.14	0.10	8	56.2	-0.11	-0.21	-0.02	18	19.7
10	Persian clover	Nitro Plus	-0.04	-0.16	0.08	9	54.8	0.05	-0.04	0.15	4	27.8
11	Bladder clover	Bartolo	-0.13	-0.25	-0.01	15	47.1	0.09	0.00	0.18	2	30.1
12	Subterranean clover	Dalkeith	-0.18	-0.30	-0.06	18	44.9	0.04	-0.05	0.14	5	25.2
13	Subterranean clover	Campeda	-0.01	-0.13	0.11	7	54.7	-0.01	-0.11	0.08	10	27.3
14	Arrowleaf clover	Cefalu	-0.10	-0.22	0.02	13	49.3	-0.05	-0.15	0.04	15	22.4
15	Woolly pod vetch	Capello	0.16	0.04	0.28	4	73.8	-0.02	-0.11	0.08	12	24.4
16	Woolly pod vetch	Haymaker	0.31	0.19	0.43	1	90.4	-0.10	-0.19	0.00	17	20.4
17	-N		0.19	0.07	0.31	3	76.5	0.04	-0.06	0.13	6	27.1
18	+N		-0.10	-0.22	0.02	14	49.2	0.02	-0.07	0.12	8	26.4

Considering the similarity in results across the three sites, and that the potential of both barrel medic and woolly pod vetch was recognised in the 1970–1980s (e.g. Archer 1981), it is surprising that neither are currently widely sown. This may be due to a number of factors such as the dominance of mixed farming enterprises across the study area may result in woolly pod vetch being viewed as a weed in cropping systems (Dann 1976) and ripening seed pods may be toxic to livestock (Enneking 1995). Medics and woolly pod vetch have also been reported to be difficult to maintain in grazed pastures (e.g. Lodge *et al.* 1991) and may be related to grazing management. Our experiments were not grazed, however, the superiority of these legumes is clear, and while grazing management is still important it may not be as much an issue because over the last three decades there has been a shift in livestock dominance from sheep to cattle (Lodge 2011). Cattle generally do not graze pastures as short or consume temperate legumes pod from the ground which may suit their inclusion in mixed pastures.

Other legumes which were identified as having potential were biserrula and gland clover. Both species have been evaluated in central and southern NSW, but not in areas with low rainfall like Trangie or summer rainfall like Bingara and Manilla.

4.5.2 Persistence

We consider there to be two key components to persistence of an annual temperate legume: successful establishment in autumn and large seed set in spring. Establishment failure occurs (Lodge 1991b) and in this environment, rainfall in some years can be insufficiently low and/or untimely that legumes fail to either establish or set seed. This risk is higher in a sown tropical grass pasture as the grass pasture has typically utilised all or the majority of stored soil water by autumn (Murphy *et al.* 2018), leaving little for the regenerating legume. Establishment failure due to insufficient autumn rainfall to achieve large seedling establishment and poor rainfall in spring to achieve high seed production in the first year, were the likely reasons for the demise of many legumes in our study, especially at the two northern sites.

Hagon (1974) suggested that successful legume regeneration required high seed production together with sufficient hard seed levels and a seed softening pattern that prevent germination during summer, but provided sufficient soft seed for good regeneration in autumn were necessary. In areas characterised by summer rainfall, legumes with low levels of hardseededness are prone to germination following summer rainfall (Hagon 1974) and are unlikely to survive unless they are able to establish quickly and develop strong root systems (Archer *et al.* 1985). At the two northern sites in our study, woolly pod vetch and subterranean clover seed tended to germinate on late summer rainfall and while many vetch seedlings survived, most subterranean clover seedlings died unless there was sufficient follow-up rainfall. The difference in survival may be associated with their relative growth habits and ability to develop a deep root system. For example, subterranean cover establishment is also restricted by shading form the perennial pasture (e.g. Boschma *et al.* 2018a) while woolly pod vetch has a twining habit which allows it to climb the grass as it growths. This difference was not observed at the Trangie site as both legumes tended to regenerate from early autumn (i.e. from March).

Early studies reported poor regeneration of medics in the second year (Hagon 1974; Archer 1981) with seed recovered from the soil being almost 100% hard seed (Archer 1981) leading to the conclusion that their hard seed levels were too high for the summer dominant rainfall zone (Hagon 1974; Archer 1981). In another study, the proportion of hard seeds of annual medics declined 70–80% over a 4 year period, in contrast to several subterranean clovers (cv. Seaton Park, Woogenellup, Clare), woolly pod vetch (cv. Namoi) or rose clover (cv. Hykon) having no residual hard seeds. Interestingly, even when the legumes were allowed to set seed every year, seed numbers of subterranean clover and barrel medic (cv. Sephi) declined prompting suggestion that additional strategies may be required long term (Lodge *et al.* 1990).

There has been conjecture about the role of hardseededness in areas which receive summer rainfall. This confliction is possibly reflective of the cultivars available, seasonal environments and knowledge at the time the research was conducted. However, there is general agreement that pasture management is important for long-term persistence of a species (e.g. Hagon 1974; Archer 1981). Different cultivars were used in previous studies, and it is not known whether the level of hardseededness of old and new varieties has changed with time, whether the changes in our environment (rainfall distribution, changing temperatures and increasing rainfall variability; e.g. Cullen *et al.* 2009; Boschma *et al.* 2010b) have been sufficient that seed softening occurs more quickly, or whether current climate changes mean that hardseededness is more important now than previously. It may also be that the current understanding of pasture ecology has progressed such that high hard seed levels are currently considered advantageous making species like medics more favourable.

Hardseed levels of barrel medic cultivars used in earlier studies (Hagon 1974; Archer 1981; Lodge *et al.* 1990) were similar (e.g. cvv. Sephi and Jemalog, 80–90% hard seed) or lower (e.g. cvv. Paraggio and Borung, 65–80%) than cvv. Caliph and Jester (80–95% hardseed) used in our study (Nair *et al.* 2007). Other characteristics of the barrel medic cultivars used in this experiment which increase their adaptive zone include a greater tolerance of aphids compared to earlier cultivars (cvv. Jester and Caliph), better suited to red clay loams (cv. Jester) and early-mid maturity (cv. Caliph). Cultivar Haymaker was selected from cv. Namoi for early and uniform flowering and has similar high levels of hardseed (80%). Cultivar Capello was bred from an induced mutation of cv. Namoi and selected for low levels of hard seed (1%) (Anon 1998). A current study to determine hardseed breakdown patterns of a range of temperate legumes in the summer dominant rainfall zone found cv. Haymaker to have low hardseed levels (18%) about 8 months after senescence, compared to barrel medic cv. Caliph (98%) and snail medic cv. Silver (92%), subterranean clover cvv. Clare and Dalkeith (65–77%), and five other legumes (SP Boschma, unpublished data).

Lodge *et al.* (1990) suggested that a high level of hard seededness may not be as important if large quantities of seed are produced, but concluded that legumes with relatively high hard seed levels but slow seed softening patterns (such as medics) require seed production in most years, while legumes with lower levels of hard seed and/or faster seed softening patterns need to set seed annually. They also suggested that for some species, annual seed set may not be sufficient to maintain a large viable seed bank and additional strategies such as grazing and resowing may be required. We suggest that long term persistence of temperate legumes is dependent on using adapted species and cultivars, maintaining a seed bank through the production of large quantities of seed with high hard seed levels. In addition, high seedling vigour and deep rootedness will also allow seedlings to establish quickly when prevailing seasonal conditions are suitable developing deep roots quickly.

4.5.3 Poor nodulation affecting legume performance?

Poor performance of some legumes in this study may also be linked to a decline in rhizobia numbers over the course of the study (Waters *et al.* 2015). Poor nodulation of legume pastures has been reported as a significant issue in southern and central NSW with a recent survey finding over 90% of pastures ineffectively nodulated (Hackney *et al.* 2017a,b). Acidity and fertility status, also high summer temperatures and low rainfall may contribute to poor nodulation or rhizobia persistence (Slattery *et al.* 2001; Hackney *et al.* 2017a,b). A number of legumes, including biserrula and serradella, have highly specific rhizobia strain requirements (Hackney *et al.* 2012a,c), and while many of the *Medicago* and *Trifolium* species may nodulate with background rhizobia strains these can be either ineffective or less efficient than the commercial strains resulting in less N fixed for use by the perennial pasture (Hackney *et al.* 2017a). All legumes in these experiments were inoculated with recommended commercial rhizobia strains at sowing, and nodules were found on plants at the Trangie site in the establishment year, however, they were not assessed in subsequent years or at either of the northern sites.

4.5.4 Effect of legume on grass productivity

Pasture availability has been reported to increase with addition of legumes (Archer 1981), and this was evident in our study also. At Bingara, plots containing productive legumes also had higher grass productivity and plant frequency, indicating larger plant crowns. This increase was likely due to greater soil N availability from the legumes (Peoples and Baldock 2001) and was also noted at Manilla, although the effects were smaller. Research has shown that tropical grasses are highly responsive to N, improving resilience and ability of the grass pasture to respond when it does rain, however, rainfall is required for the potential to be expressed (Boschma *et al.* 2014a, 2016). Both the Bingara and Manilla sites experienced below average rainfall throughout the study, Manilla receiving less than Bingara each year, insufficient rainfall to express the true effect of N fixed by the temperate legumes.

4.5.5 Future opportunities

This study has identified a number of areas of future work:

1. Alternative temperate legume sowing times. Ideally all components of a pasture (e.g. grass and legume) are sown within a relatively short time period when soil water and nutrient resources are available (Boschma *et al.* 2018b). Further research is required to determine if it is better to establish temperate legumes, before, at the same time or after the grass the grass has been sown.
2. Establishing legumes into established pastures. This study established legumes into newly established grass pastures when soil water and nutrient resources are known to be better for legume establishment (Boschma *et al.* 2018b), however, there are large areas of established tropical pastures requiring legumes, and strategies to establish, or reestablish legumes into these pastures warrants investigation.
3. Development of a hardseeded woolly pod vetch. Both cultivars used in this study were derived from cv. Namoi and both previous and current research suggests that higher levels of hardseededness may be advantageous in grazing systems.
4. Optimum sowing time of tropical grasses in central NSW. Tropical pastures are sown in the low-medium rainfall Central West of NSW, but failure to establish the digit grass suggests that further work is required to better understand the best sowing time and strategies to improve establishment in low rainfall environments with an even or winter dominant rainfall pattern.

4.6 Conclusion

This study has shown that woolly pod vetch and barrel medics are the superior temperate annual companion legumes for sown tropical grass pastures in Northern Inland NSW and showed their potential in Central West NSW. In higher rainfall environments woolly pod vetch was the highest ranking legume while barrel medic was the superior legume in lower rainfall environments.

5 Companion legumes for tropical perennial grass pastures in inland New South Wales. 2. Tropical perennial legumes

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5.1 Introduction

In Northern Inland NSW the area sown to tropical perennial grasses has increased over the last 15 years, and it has been estimated that over 450,000 ha have been sown and seed sales (2011–15) indicate that at least 25,000 ha continue to be sown annually. Tropical perennial grasses are highly responsive to fertiliser, in particular nitrogen (Boschma *et al.* 2014a, 2016). Nonetheless, the cost of inorganic nitrogen and its annual/biannual application is prohibitive to some producers, plus variable rainfall makes application somewhat risky due to volatilisation if insufficient rainfall is received (e.g. Schwenke 2014). Productive and persistent companion legumes are a cost effective, sustainable alternative means of providing nitrogen in tropical pastures (e.g. Peck *et al.* 2012b), additionally, in a mixed pasture, legumes can be important for animal production through their own high protein content.

Northern Inland NSW has a summer dominant rainfall distribution (60% falling October–March), but evaporation is high during summer making winter rainfall more effective providing the potential for either temperate or tropical legumes to be used as companion legumes in tropical grass pastures. Rainfall capture efficiency (change in profile stored soil water divided by total rainfall over a period of time) of a tropical pasture during winter (55–64%, Murphy *et al.* 2010a) can be almost double that for a summer fallow (22–25%, Dalgliesh and Foale 1998) in this environment. Soil water accumulated over winter could be utilised by a companion temperate legume, or stored for use by the tropical pasture in spring. While temperate legumes utilise this soil water during the winter period (Murphy *et al*. 2018), research in northern NSW has shown that soil profiles do not tend to refill to fallow conditions after the pasture is about 2 years old, except during years with above average cool season rainfall (Murphy *et al*. 2018). The growth patterns of tropical legumes are similar to tropical perennial grasses; responsive to spring-summer rain, but susceptible to frost over winter, as a result tropical grass-tropical legume pastures are productive for 6–8 months of the year with no growth in winter. As there is no growth over this period, stored soil water levels can increase and be available for when growth recommences in spring; initial pasture growth would not be dependent on receiving spring rainfall. The disadvantage to this system is that there is no green feed available over winter, however residual dry feed could be utilised with supplementation (Harris *et al.* 2014).

Information on the productivity and persistence of tropical legume species for Northern Inland NSW is limited compared with that of temperate legumes. This group of legumes have not been widely tested with evaluation of species being mostly opportunistic and, with a few exceptions (e.g. Moylan and Crocker 2000; Boschma *et al.* 2012), largely unpublished. However, they are an important component of pastures in Southern Queensland (e.g. Peck *et al.* 2012a) where climate is similar, suggesting that they may also have potential in Northern Inland NSW. Evaluation of single cultivars of several species in Northern Inland NSW (2009–12; Boschma and Harris, unpublished) highlighted that tropical herbaceous legume species such as desmanthus (*Desmanthus virgatus*) may have potential and warrant a more detailed investigation.

The primary objective of this study was to evaluate the production and persistence of a range of herbaceous tropical perennial legume species and cultivars grown in Queensland and two temperate perennial legumes in mixtures with a tropical perennial grass (digit grass (*Digiteria eriantha*) cv. Premier) to identify those suitable for northern NSW. The agronomic traits used to determine suitability were establishment plant density, legume and grass herbage production and legume and grass persistence over four years. The aims of the study were to compare species and identify those adapted to specific locations and those more broadly adapted to North-West Slopes of NSW.

5.2 Methodology

5.2.1 Sites

This study consisted of two experiments located at sites near Bingara and Manilla on the North-West Slopes of northern NSW. These sites represent areas where tropical perennial grasses are currently grown but with different annual average rainfall and altitude. A third experiment was sown at Trangie in Central West NSW, but was abandoned after two failed establishment attempts. No further details of the experiment at Trangie are provided.

Individual site details are outlined in Table 5.1 and their location in shown in Chapter 4 (Fig. 4.1). For two years prior to sowing the experiment, both sites were sown to forage oat (*Avena sativa*) during winter and left as a fallow over the summer period with summer weeds controlled with non-residual herbicides.

Table 5.1. Characteristics of the experimental sites including location, elevation, soil classification (Isbell 1996), soil pH ($CaCl_2$, 0–0.1 m, pH_{Ca}) and pre-experimental management.

Characteristic	Bingara	Manilla
Location	29°42’39” S, 150°27’07” E 27 km north-west of Bingara	30°42’11” S, 150°30’10” 18 km west of Manilla
Elevation (m)	297	412
AAR (mm)	745	576
Soil classification	brown Chromosol soil	brown Chromosol soil
Soil pH_{Ca}	5.0	6.1
Pre-sowing treatment	In December 2012, area was cultivated with a rotary-hoe and levelled with pasture harrows	In November–December 2012, area was irrigated (30 mm) to encourage summer weed germination and sprayed with 1.5 L/ha glyphosate

Soil cores (0–10 cm, 10–20 cm) were collected in November 2012 and analysed for pH, organic carbon, nitrate nitrogen (N), sulphate sulfur (S, extracted in 1 M KCl at 40°C), phosphorus (P, Colwell), potassium, calcium, magnesium, sodium, chloride, copper, zinc, manganese, iron and electric conductivity. The full analysis results are presented in Table 4.2.

Rainfall data were recorded in manual rain gauges located either at or near each experimental site. Long-term average monthly and annual rainfall data for both sites were determined from the Bureau of Meteorology (BOM) sites located near the experimental sites.

5.2.2 Species, sowing and experimental design

This study consisted of 14 cultivars of tropical legumes, and four cultivars of two temperate perennial legumes, all sown in plots with digit grass cv. Premier giving a total of 18 treatments (Table 5.2). Each experiment was a randomised complete design with three replicates (total 54 plots). The grass and legumes were sown at about 5 mm depth in alternate rows with a disk seeder (total 6 rows per plot, each row 0.25 m apart) into plots 1.5 wide x 6.0 m long. The Manilla site was sown in December 2012 and the Bingara experiment in January 2013. Digit grass was sown at 1 kg/ha viable seed and the legumes at commercially recommended rates and inoculated with the recommended strain of Rhizobia the day prior to sowing (Table 5.2). The Manilla site was also irrigated with 55 mm water (6–14 December) to assist emergence.

Table 5.2. Legume species and cultivar sown with digit grass at Bingara and Manilla.

ID	Common name	Botanical name	Cultivar/Line	Coated seed	Sowing rate (kg/ha)	Inoculant group
1	Lucerne	*Medicago sativa*	Pegasis	No	1	AL
2	Lucerne	*M. sativa*	Q31	No	1	AL
3	Lucerne	*M. sativa*	Venus	No	1	AL
4	Lotus	*Lotus corniculatus*	Phoenix	No	1	SU343
5	Desmanthus	*Desmanthus virgatus*	Marc	No	2	CB3126
6	Desmanthus	*D. leptophyllus*	JCU 1	No	2	CB3126
7	Desmanthus	*D. virgatus*	JCU 2	No	2	CB3126
8	Desmanthus	*D. virgatus*	JCU 3	No	2	CB3126
9	Desmanthus	*D. bicornutus*	JCU 4	No	2	CB3126
10	Desmanthus	*D. virgatus*	JCU 5	No	2	CB3126
11	Fine stem stylo	*S. guianensis* var. *intermedia*	-	Yes	4	CB82
12	Common stylo	*S. guianensis* var. *guianensis*	Beefmaker	Yes	4	CB82
13	Common stylo	*S. guianensis* var. *guianensis*	Beefbuilder	Yes	4	CB82
14	Caatinga stylo	*Stylosanthes seabrana*	Unica	No	1	CB3481
15	Round-leaf cassia	*Chamaecrista rotundifolia*	Wynn	Yes	2	M
16	Burgundy bean	*Macroptilium bracteatum*	B1	Yes	4	CB1717
17	Burgundy bean	*M. bracteatum*	AT10[1]	Yes	4	CB1717
18	Burgundy bean	*M. bracteatum*	AT12[1]	Yes	4	CB1717

[1]Experimental line

No fertilisers were applied the year of sowing. Each year from October 2013, 200 kg/ha single superphosphate (8.8% P, 11% S) was applied during spring at both sites. At the Manilla site 2.0–2.5 L/ha paraquat and diquat (135 g/L and 115 g/L a.i respectively) were applied in July 2014 and 2015 to control winter-growing grass and broadleaf weeds. No herbicides were applied at the Bingara site.

5.2.3 Data collection

5.2.3.1 Establishment and regeneration

Seedling densities (seedlings/m^2) of digit grass and legume were taken 4–6 weeks after emergence in six quadrats (0.10 x 0.50 m) in each plot, avoiding the outer rows of each plot. Regeneration was noted on a number of occasions throughout the conduct of the experiment and assessed three times at Bingara and once at Manilla using the same technique.

5.2.3.2 Herbage production

Herbage mass was assessed approximately every 6 weeks (less frequent during periods of low rainfall) during the period October–May from the second year, also early spring (August–September) to quantify winter-early spring production. Plots were subdivided into three equal strata. For each strata, total herbage mass was visually estimated on a continuous 0–5 scale (0, nil; 5, high) also the percentage of digit grass and sown legumes (dry weight herbage mass). At each sampling time 20 calibration quadrats (0.40 x 0.40 m) representing the range of herbage mass were also scored. These quadrats were harvested (about 10 mm above ground level), sorted into three components (digit grass, sown legume and other species), and dried at 80°C for 48 h before weighing. Herbage mass scores and percentage estimates were regressed (linear or quadratic R^2>0.80) against actual herbage mass (kg DM/ha) and percentage of each component to determine the herbage mass of the sown species. After each assessment the plots were mown with a rotary mower and the herbage removed from the plots. The exception was the final legume assessment in spring each year when the plots were left unmown until the legumes had set seed. Herbage mass was assessed a total of 9 and 14 times (2013–16) at Bingara and Manilla respectively.

5.2.3.3 Plant persistence

In spring and autumn each year, plant frequency of the grasses and legumes were assessed in a permanent quadrat (1.0 x 0.5 m) centrally located in each plot. Estimates were taken 0–10 days after defoliation by recording the number of cells containing a portion of a live digit grass or legume plant (presence) and frequency of occurrence estimated from the proportion (%) of occupied cells (plant frequency, Brown 1954). At Bingara, plant frequency was assessed a total of six and seven times for the grass and legumes respectively (November 2013–July 2017). Grass and legume plant frequency was assessed eight times (April 2013–April 2017) at Manilla.

5.2.4 Statistical analyses

5.2.4.1 Establishment and regeneration

Legume and grass establishment seedling densities (seedlings/m^2) at Bingara and Manilla were analysed by ANOVA with legume cultivar as the explanatory factor and replicate as a block term. Plots of the residuals showed no transformation of the data was necessary. Legume regeneration seedling counts (seedlings/m^2) were analysed using the same technique, but only those legumes that recorded regeneration were included in the analysis. Plots of the residuals showed that the Bingara data did not require transformation while the Manilla data required square root transformation.

5.2.4.2 Herbage production

Legume and grass herbage mass was assessed a total of 9 and 14 times at Bingara and Manilla respectively. To estimate herbage production across all assessments a meta-analysis of series of trials was performed using GENSTAT (Release 19.1, VSN International 2018). Each assessment being a trial, legume cultivar the explanatory factor and replicate within trial as a block term. Linear row or column effects were fitted for each assessment if necessary. Data were cube root transformed to improve the distribution of the residuals. At Manilla, two grass assessments were omitted (September 2014 and August 2015) from the analysis as all treatments had nil herbage production. Also at Manilla, five legume treatments failed to persist giving all zero values for herbage mass from year 2 (fine stem stylo, two common stylo cultivars, Caatinga stylo and round-leaf cassia). These treatments were omitted from the analysis.

5.2.4.3 Plant persistence

Legume and grass plant frequency (%) were analysed using the same meta-analysis technique as herbage production. The data were square root transformed to improve the distribution of the residuals. The five treatments omitted from the analysis of herbage production at Manilla were also omitted from this analysis of both legume and grass plant frequency at Manilla as values dropped to zero after the second assessment and did not recover.

5.3 Results

5.3.1 Rainfall

Environmental conditions experienced at the sites during the conduct of this study were challenging and have been described in more detail in Chapter 4. Rainfall was below average three of the four years at Bingara, which included a period of 17 consecutive months (July 2013–November 2014) with below average monthly rainfall (monthly rainfall data are presented in Fig. 4.2). At the Manilla site, annual rainfall was below average all years (Table 5.3), and especially low in 2014 when rainfall was 44% of the long term average.

Table 5.3. Long term annual average (LTA, mm), annual rainfall (mm) and proportion (%) of LTA received each calendar year at the Bingara and Manilla sites.

Year	Bingara		Manilla	
	Rainfall (mm)	Proportion of LTA (%)	Actual (mm)	Proportion of LTA (%)
LTA	745	-	567	-
2013	594	80	451	78
2014	352	47	253	44
2015	743	100	440	76
2016	643	86	451	78

5.3.2 Establishment and regeneration

Legume establishment plant counts at the Bingara site were highly variable; highest for common stylo cv. Beefmaker (119 seedlings/m^2) and lowest for desmanthus cv. JCU 1 (10 seedlings/m^2; P < 0.05). There were no differences in the seedling density of digit grass (56–88 seedlings/m^2; P > 0.05).

Both legume and digit grass establishment densities were much lower at the Manilla site. Lucerne cv. Venus had the highest seedling density (18 seedlings/m^2; P < 0.05) and was similar to eight other cultivars. Fine stem stylo had the lowest density (1 plant/m^2; P < 0.05) and similar to eight other legumes (Table 5.4). Digit grass ranged from 11 (in mixture with lucerne cv. Venus) to 28 seedlings/m^2 (mixture with burgundy bean (AT10); P > 0.05).

Legume regeneration was observed on three occasions at Bingara and one occasion at Manilla. At Bingara nine legumes had seedling recruitment in January 2015 (1–5 seedlings/m^2) and 12 legumes in March 2015 (1–13 seedlings/m^2) and January 2016 (1–16 seedlings/m^2; Table 5.4). Nine legumes were consistent on the three occasions: six desmanthus cultivars and three burgundy bean cultivar/lines. The additional three entries at the latter assessments were Caatinga stylo and lucerne cvv. Pegasis and Q31 with ≤2 seedlings/m^2. The legumes with the greatest recruitment were ranked the same on each occasion: 1st, desmanthus cv. Marc; 2nd, burgundy bean (AT12) and 3rd desmanthus cv. JCU 2 (Table 5.4).

Seven legumes recruited seedlings in February 2014 at the Manilla site. Burgundy bean cv. B1 and desmanthus cv. JCU2 had the highest recruitment (85–162 seedlings/m^2, back transformed; Table 5.4), followed by the other burgundy bean lines and desmanthus cvv. JCU3, Marc and JCU 5 (3–23 seedlings/m^2; Table 5.4).

5.3.3 Herbage production

At Bingara, herbage production of lucerne cvv. Pegasis and Venus was highest (1011 and 791 kg DM/ha/assessment respectively, backtransformed; P < 0.05) and is shown in Fig. 5.1a. Lucerne cv. Q31 was ranked 3rd with herbage production similar to lucerne cv. Venus and desmanthus cv. Marc. Burgundy bean lines AT10 and AT12 were ranked 5th and 6th. Fine stem stylo cv. Beefbuilder had the lowest herbage production (P < 0.05) and were similar to the other fine stylos, round-leaf cassia and lotus (< 10 kg DM/ha/assessment; P > 0.05; Fig. 5.1a).

At Manilla, the three cultivars of lucerne were the most productive (P < 0.05), herbage mass ranging 1508–1955 kg DM/ha/assessment (Fig. 5.1c). Desmanthus cvv. Marc and JCU2 were ranked 4th and

5th and had above average herbage production of the legumes tested. Burgundy bean line AT12 was the least productive (14 kg DM/ha/assessment; $P < 0.05$), with the three burgundy bean entries ranked lowest (Fig. 5.1c).

Table 5.4. Legume and digit grass establishment and legume regeneration seedling densities (seedlings/m^2) at the Bingara and Manilla sites. Square-root transformed (in parentheses) and backtransformed means have been provided where appropriate. Least significant differences (LSD; P = 0.05) among treatment means are also provided where appropriate.

		Establishment				Legume regeneration				
		Bingara		Manilla		Bingara			Manilla	
ID	Legume treatment	Legume	Grass	Legume	Grass	Jan15	Mar15	Jan16	Feb14	
1	Lucerne cv. Pegasis	66	87	13	16	-[1]	1	1	-	-
2	Lucerne cv. Q31	61	82	12	13	-	1	1	-	-
3	Lucerne cv. Venus	76	61	18	11	-	-	-	-	-
4	Lotus cv. Phoenix	48	73	8	19	-	-	-	-	-
5	Desmanthus cv. Marc	74	88	15	23	5	13	16	(3.3)	11
6	Desmanthus cv. JCU 1	10	68	3	17	1	2	5	-	-
7	Desmanthus cv. JCU 2	47	56	9	16	2	8	10	(9.2)	85
8	Desmanthus cv. JCU 3	22	86	6	13	1	4	6	(4.4)	19
9	Desmanthus cv. JCU 4	19	70	10	13	1	2	4	-	-
10	Desmanthus cv. JCU 5	37	69	9	14	1	2	3	(1.8)	3
11	Fine stem stylo	20	67	1	18	-	-	-	-	-
12	Common stylo cv. Beefmaker	119	76	7	19	-	-	-	-	-
13	Common stylo cv. Beefbuilder	100	64	10	15	-	-	-	-	-
14	Caatinga stylo cv. Unica	32	63	6	21	-	2	2	-	-
15	Round-leaf cassia cv. Wynn	30	74	5	17	-	-	-	-	-
16	Burgundy bean cv. B1	52	70	12	13	1.7	3	5	(12.7)	162
17	Burgundy bean (AT10)	63	67	14	28	1.7	5	7	(4.8)	23
18	Burgundy bean (AT12)	91	76	12	23	2.7	9	11	(4.0)	16
	Average	54	72	9	17	2	4	6	(5.8)	
	LSD (P = 0.05)	22.2	NS[2]	8.8	NS	1.5	5.1	5.1	(3.78)	

[1]No regeneration [2]NS = not significant

Digit grass production in the mixtures at the Bingara site ranged from 967 (lucerne cv. Venus) to 1379 kg DM/ha/assessment (lucerne cv. Q31) and are presented in Fig. 5.1b. Four legumes had similar production to lucerne cv. Q31: Caatinga stylo, lucerne cv. Pegasis, fine stem stylo cv. Beefmaker and burgundy bean cv. B1, while digit grass in mixtures with desmanthus cvv. JCU2, JCU4 and JCU5 had low productivity similar to lucerne cv. Venus.

At Manilla, digit grass production was highest in mixtures with burgundy bean cv. B1 (1091 kg DM/ha/assessment) and similar to the other two burgundy bean lines and desmanthus cvv. JCU 1, JCU 2, JCU 3 and JCU 5 (Fig. 5.1c). Grass production was lowest in mixtures with the three lucerne cultivars (average 637 kg DM/ha/assessment; $P < 0.05$).

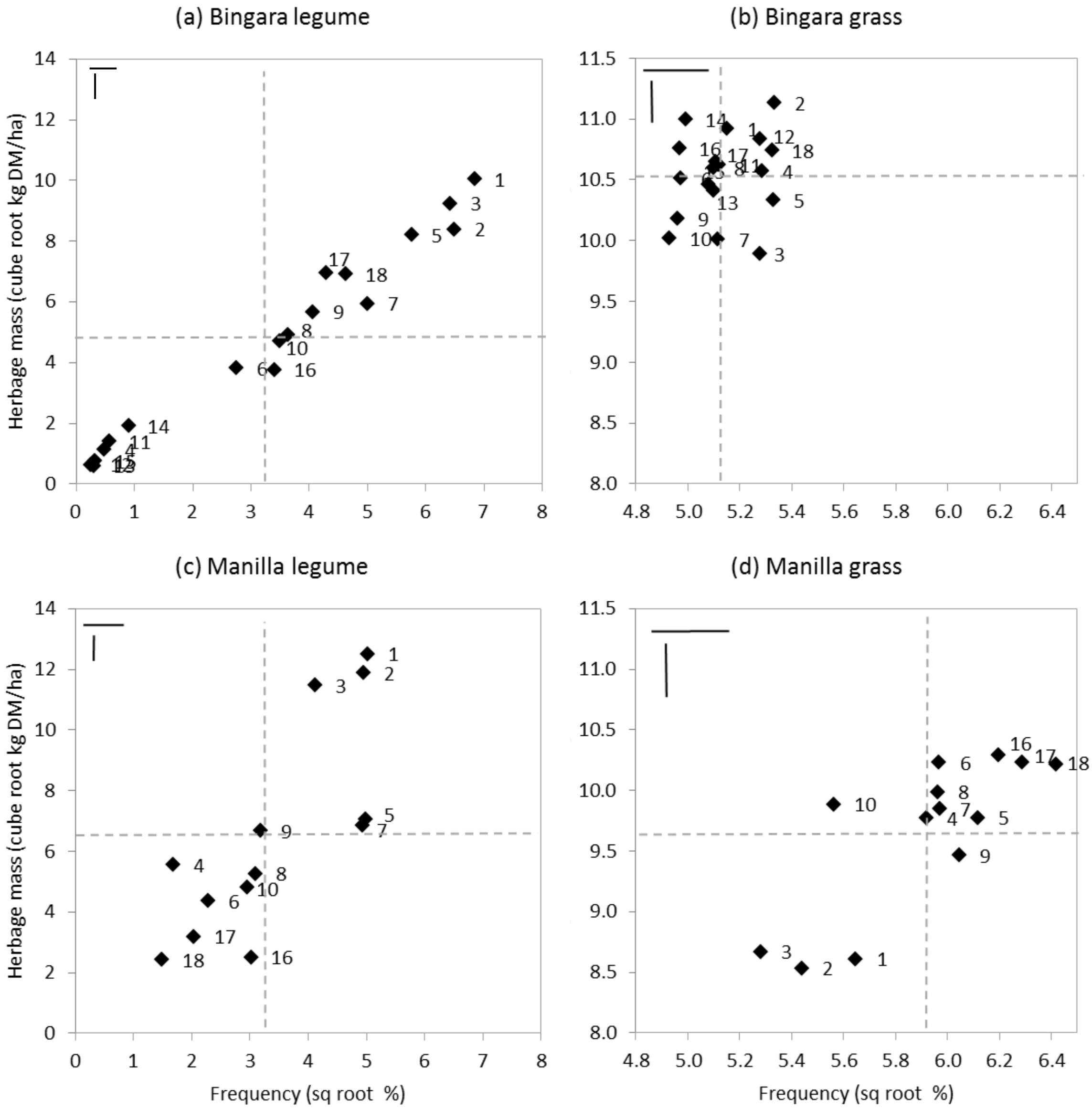

Fig. 5.1. Average predicted legume (a,c) and digit grass (b,d) herbage production (cube root kg DM/ha) and plant frequency (square root %) for 18 legume-grass treatments at (a,b) Bingara and (c,d) Manilla. Numbers on each figure relate to legume treatment number provided in Table 5.1. Horizontal and vertical lines on each figure represent LSD (P = 0.05) for plant frequency and herbage mass respectively.

5.3.4 Persistence

Plant frequency at Bingara showed that lucerne cv. Pegasis was the most persistent legume in a mixture with digit grass (47% backtransformed, $P < 0.05$, Fig. 5.1a) followed by the other two lucerne cultivars (41–42%, $P < 0.05$). Desmanthus cvv. Marc and JCU2 were ranked 4th and 5th ($P <$ 0.05). The legumes with the lowest plant frequency in mixtures were fine stem cvv. Beefbuilder and Beefmaker, round-leaf cassia and lotus (<1%, $P < 0.05$, Fig. 5.1a).

At the Manilla site, lucerne cvv. Pegasis and Q31 and desmanthus cvv. Marc and JCU2 had the highest plant frequencies 24–25%, $P < 0.05$, Fig. 5.1c). Burgundy bean cvv. AT10 and AT12 and lotus had the lowest plant frequencies (<5%, $P < 0.05$).

Digit grass plant frequency at Bingara ranged from 24% (backtransformed, desmanthus cv JCU5) to 28% (lucerne cv. Q31) and while the range was significant ($P < 0.05$), 11 legume entries had plant frequency values similar to both desmanthus cv. JCU5 and lucerne cv. Q31 (Fig. 5.1b).

Digit grass plant frequency at Manilla was highest in mixtures with the three burgundy bean entries and desmanthus cv. Marc (37–41%, $P < 0.05$, Fig. 5.1d) and lowest for lucerne cvv. Venus and Q31 and desmanthus cv. JCU5 (28–30%, $P < 0.05$).

5.3.5 Comparative herbage production and persistence

At Bingara, plots of average predicted legume herbage production and plant frequency showed that the legume treatments were divided into three groups: lucerne with high production and plant frequency (top right quadrant), stylos and lotus with low production and frequency (bottom left quadrant) and other species which were intermediate (Fig. 5.1a). Other legumes that had above average herbage production and plant frequency were desmanthus cvv. Marc, JCU2, JCU3 and JCU4, also burgundy bean lines AT10 and A12.

The same plots for Manilla also showed lucerne in the upper right quadrant (Fig. 5.1c), burgundy bean entries in the lower right quadrant and desmanthus intermediate (Note that the four stylo entries and round-leaf cassia failed to persist beyond year 2 and were removed from the analysis and are therefore not presented). In addition to the lucerne cultivars, desmanthus cvv. Marc and JCU2 also showed above average production and persistence and were positioned in the upper right quadrant.

Plots of digit grass production and frequency for the legume treatments did not show any grouping by legume type at Bingara (Fig. 5.1b), while at the Manilla site, there were clear groupings but the inverse to the legume relationship, that is, burgundy bean entries were in the upper right quadrant (indicating above average digit grass production and plant frequency) while lucerne was in the lower left quadrant (below average digit grass production and plant frequency). Desmanthus entries were again intermediate, but the majority of cultivars (Marc, JCU 1, JCU2, JCU3) were located in the upper right quadrant.

5.4 Discussion

This study has shown that:

1. Lucerne is the most productive companion legume in mixtures with digit grass, followed by desmanthus.
2. At Manilla, the drier of the two sites in this study, the relative competitiveness of individual legumes in mixtures was evident; lucerne was highly competitive against digit grass while there appeared to be little to no competitive effect of desmanthus and burgundy bean.
3. Stylos had poor persistence.

In perennial pastures, good establishment of both the grass and legume components is essential as this initial plant density sets the scene for the future productivity; poor establishment will result in poor performance unless the species are able to thicken from seed set. This was the case at the Manilla site of this study, the poor establishment (<10 plants/m^2) of nine legumes including the stylos and round leaf cassia, resulted in poor persistence and production throughout the experiment, especially with the ensuing three years of below average rainfall.

5.4.1 Lucerne was the superior legume

Lucerne is the most productive and persistent temperate perennial legume in Northern Inland NSW (Boschma *et al.* 2011), but is not as well utilised by graziers as its potential indicates due to its high bloat risk and poor ground cover (Boschma *et al.* 2011). Its inclusion as a companion legume may overcome both risks, but reducing competitiveness of lucerne in a mixture is pivotal to increasing productivity and persistence of the mixture.

In this study, lucerne negatively impacted the productivity of digit grass during periods of stress. The competitive effect of lucerne on digit grass was clearly evident at the Manilla site with three lucerne mixtures located in the upper right quadrat for legume production and plant frequency and lower right quadrant for digit grass production and plant frequency. Competition between the grass and lucerne, or any legumes was not evident at the Bingara site. This may be due to higher altitude and rainfall (despite dry conditions) at Bingara.

Further work is required to see whether the competition between the two species can be reduced by adjusting the configuration of the two species in the mixture. For example, the experiments were sown in alternate single grass-legume rows, however, further banding of plants of a species and separation from the other species (e.g. alternating bands of three rows of lucerne with three rows of grass) may increase intraspecific competition and reduce interspecific competition (Harper 1977) resulting in a more productive, persistent pasture. The cultivars used in our study ranged in winter activity rating from highly winter active (rating 9, cv. Pegasis) to semi winter dormant (rating 3, cv. Q31). Winter-dormant lucerne cultivars typically have slower rates of regrowth in both summer and spring than highly winter-active lucerne cultivars (Boschma and Williams 2008) which may affect their relative competiveness with digit grass, however, this was not evident in our study and further separation of the species may be required to allow differences to be expressed (see Chapter 12).

Lucerne competitiveness could potentially also be manipulated by reducing lucerne plant density. In a replacement series experiment using seedling lucerne and a range of seedling tropical grasses, lucerne tended to produce similar quantities of herbage at mixture plant population proportions ranging 25–100% (Boschma *et al.* 2010a). The difference appeared due to the proportion of lucerne stem, but plant density and proportion may warrant investigation.

5.4.2 Desmanthus

Desmanthus has not been widely evaluated in Northern Inland NSW, but these studies, and those that have been reported (e.g. Moylan and Crocker 2000; Boschma *et al.* 2012) have consistently shown the potential of desmanthus in this environment. While they are not as productive as lucerne, the best entries were still quite productive and persistent, they set seed and readily recruited seedlings in mixed pasture following a substantial (e.g. >20 mm) summer rainfall event. Desmanthus also had the advantage of complimentary grass productivity, that is, desmanthus did not appear to restrict productivity of the grass due to strong competition. All are important characteristics for a long term or permanent pasture. An additional benefit of desmanthus is the presence of condensed tannins (Adjei *et al.* 1993) making it a non-bloating legume (Cook *et al.* 2005). Desmanthus is reported to have high levels of hard seed (Lawrence *et al.* 2012; Cook *et al.* 2005), and current seed softening studies being conducted at Tamworth support this and also suggest that there are differences between cultivars (Chapter 13).

This study used current Australian commercial cultivars of desmanthus: Marc and Progardes (composite blend of five individual cultivars; JCU 1–5; www.progardes.com.au). Both cultivars were selected for central and northern Queensland where frosts are commonly fewer and less severe. Cultivar Marc is also recognised as having low productivity (Pengelly and Conway 2000; Cook 2005), but a broad range of germplasm is held in the Australian Pastures Genebank. These accessions have been collected across the globe from environments and latitudes similar to Southern Queensland and northern NSW, including Central and South America and Mexico (Pengelly and Liu 2001). This germplasm offers the potential to select and/or develop lines with improved productivity and frost tolerance which would be better suited to this environment. Desmanthus was a genera recognised as having potential for pastures across the summer dominant rainfall zone from northern NSW to northern Queensland and a priority for development (Bell *et al.* 2016).

When a cultivar is sold as a composite, such as Progardes (composite of JCU 1–5), it is important to evaluate the individual cultivar entries separately to better understand the relative productivity and persistence of each in an environment. In northern NSW, JCU 1, 3 and 5 were the least productive and persistent and could potentially be excluded from blends sold in the region. Interestingly, all cultivars in the cv. Progardes composite were collected from discontinued evaluations sites in northern Australia (Latitudes 17–15°S, Gardiner 2016). Their persistence in northern NSW shows the incredible adaptation of *Desmanthus*.

5.4.3 Burgundy bean

Burgundy bean is considered a drought tolerant species, with greater cool temperature tolerance than many tropical legumes. Interestingly burgundy bean has been reported as effective in a grass pasture for 3–4 years, failing to persist beyond that due to its high palatability (Cook *et al.* 2005). In our study, burgundy bean failed to persist in a mixture, which supports anecdotal and unpublished findings of others in Southern Queensland and Northern NSW that burgundy bean is most persistent in pure swards, but has potential as a short term ley in subtropical grain systems (Bell *et al.* 2012).

Burgundy bean established well when sown with a tropical grass, and actually competed strongly with the grass during the establishment year. At the higher rainfall Bingara site, plants survived winter and plant populations were supplemented by seedling recruitment, however, at the Manilla site, burgundy bean had an annual habit and under the management imposed was not able to maintain a sufficiently large seed bank or recruit sufficient number of seedlings annually to persist beyond two years. Seedling regeneration was observed during this study (and several regeneration periods assessed), although recruitment seedling densities were low (<13 plants/m^2) and their subsequent survival low. Burgundy bean has rapid hard seed breakdown with about 90% hard seed broken down within 2 years (Lawrence *et al.* 2012). These findings suggest that long-term persistence of burgundy bean in a grass pasture would require higher rainfall to ensure greater seedling survival (although competition from the grass would also increase) and grazing management to ensure annual seed set so that the seed back could be maintained.

5.4.4 Stylos and round leaf cassia

Fine stem stylo, and particularly caatinga stylo are persistent legumes in grass pastures in Southern Queensland, so it is somewhat surprising that they were not persistent in our study. Previous studies in Northern Inland NSW have also found that the legume and grass established well, but failed to persist (Boschma and Harris, unpublished). There are a number of potential issues which include:

1. Some stylo species have specific rhizobia requirements (e.g. caatinga stylo). Nodulation was assessed 13 weeks after sowing at Manilla and no nodules were found on fine stem stylo and few nodules found on caatinga and common stylo indicating nil or poor nodulation. In addition, some plants of common stylo were pale, stunted and unthrifty next to plants which were green and healthy, suggesting problems with inoculation or rhizobia survival which has been reported (e.g. McInnes and Date 2005). All stylo entries failed to persist the first winter at Manilla. Interestingly, at Bingara visual scores of seedling health conducted in the establishment year showed common stylos were healthy with a bright green colour while

fine stem and caatinga stylos were pale and is likely a reflection of satisfactory and poor nodulation respectively.

2. The two common stylos and fine stem stylo are both *S. guianensis* but different subspecies: var. *guianensis* and *intermedia* respectively are suited to very different environments. Var. *intermedia* is tolerant of frost and heavy grazing and lower rainfall (700–900 mm) environments, while var. *guianensis* is intolerant of frost and heavy grazing and persists in higher rainfall environments (>1000 mm) (Cook *et al.* 2005). At Bingara, the higher rainfall site in this study the two cultivars of var. *guianensis* established well (≥100 seedlings/m^2), but failed to persist past the first winter of the experiment. In contrast, the single entry of var. *intermedia* had a much lower initial plant density (20 seedlings/m^2) but persisted until 2015.

Common stylo is not suited to Northern Inland NSW however fine stem stylo and caatinga stylo warrant further investigation.

Round-leaf cassia was included in this study to test the boundaries of the species as it is considered a higher rainfall species, but is reported as having an annual habit, regenerating each spring from seed in lower rainfall environments (>600 mm) (Cook *et al.* 2005). In higher rainfall environments (900–1500 mm) round-leaf cassia tends to be a short lived perennial suited to free draining soils with acid to neutral pH and low-medium fertility (Cook *et al.* 2005). Round-leaf cassia failed to persist at both sites in this experiment and is not a suitable companion legume for tropical perennial grasses for Northern Inland NSW.

5.4.5 Areas of further research

Two areas of further research include establishment of legumes into established pastures and understanding keys to successful tropical grass establishment in Central Western NSW.

This study has focused on establishing legumes and grasses at the same time, that is, newly sown pastures, however the majority of tropical pastures in this region and beyond have a minor or non-existent legume component and are not fertilised. Developing techniques to establish legumes into established pastures – sown and native pastures warrants investigation.

Failure to establish the site at Trangie was disappointing, but highlights the challenge of establishing tropical species in this low aseasonal rainfall environment. Further studies are required to understand the optimum sowing time of individual tropical grass and legume species for establishment and persistence of these establishing seedlings. For example, while November–December may be an optimum time for emergence in northern NSW, in Central Western NSW summer temperatures are higher and rainfall lower, so the optimum sowing time for emergence and seedling survival may be October–November.

5.5 Conclusions

In Northern Inland NSW lucerne was the most productive companion legume in mixtures with digit grass, followed by desmanthus. At the lower rainfall site (Manilla), lucerne was highly competitive against digit grass, resulting in less herbage production than digit in mixes with desmanthus. This competitiveness was not evident at the higher rainfall site (Bingara). Desmanthus did not appear to be competitive against digit grass at either site. Burgundy bean is not an effective companion legume and all stylos that were tested failed to persist.

6 Companion legumes for tropical perennial grass pastures in inland New South Wales. 3. Leucaena

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6.1 Introduction

Northern Inland New South Wales (NSW) is a sub-humid summer rainfall zone (Tweedie and Robinson 1963) with approximately 60% of rainfall falling between October and March each year commonly in high intensity thunderstorms. Pasture in the region are limited by low temperature in winter and high temperature and soil moisture stress over summer (Harris and Culvenor 2004) as the average period of frost occurrence is 145 days with approximately 50 frosts received per year (Hobbs and Jackson 1977), while during summer, rainfall tends to be relatively ineffective as high summer temperatures lead to high evapotranspiration (Murphy *et al.* 2004b).

In the Central West of NSW rainfall is relatively evenly distributed throughout the year with the highest falls occurring on the eastern areas of the slopes and at higher elevations. Summer rainfall is slightly higher in the northern part of the region, however, over the summer months evaporation exceeds rainfall (Central West Catchment Authority 2008). Average maximum daytime temperatures exceed 20°C for 6–8 months of the year and exceed 30°C in most areas for 2–3 months of the year. Winter temperatures are cool throughout the region with frost potential for five months of the year. These climatic conditions generally result in pasture production being limited by moisture during the summer months; this effect is greater at lower altitudes where rainfall tends to be lower and temperatures higher. In winter, rainfall typically exceeds evaporation and pasture growth is limited by temperature (Central West Catchment Authority 2008).

In both Northern Inland and Central West NSW sown grass pastures have traditionally been based on temperate species (e.g. Archer 1989, Lodge and Orchard 2000; Harris and Culvenor 2004), however, the persistence of these species under summer moisture stress has generally been poor (Harris and Culvenor 2004; Boschma *et al.* 2009), such that they have now largely been replaced by tropical grasses in many areas, particularly in Northern Inland mixed farming zone of NSW (Harris *et al.* 2014). To maintain the productivity of tropical pastures, nutrients, in particular nitrogen are required (Boschma *et al.* 2014a). This nitrogen could be applied as inorganic sources or by addition of a companion legume.

There is need to expand the range of legume options available as a companion to the tropical perennial grass based pastures and studies investigating the potential of a range of temperate and tropical herbaceous legumes were described in Chapters 4 and 5. Another legume that showed potential in preliminary studies conducted at Tamworth NSW is leucaena (*Leucaena leucocephala* ssp. *glabrata*) (SP Boschma, unpublished data). Leucaena-grass based pasture systems are widely utilised in Central Queensland (>150,000 ha) (Shelton and Dalzell 2007) and a similar system may have potential to the grazing industry in Northern Inland and Central West NSW.

Leucaena is a perennial shrub or tree legume native to neutral and alkaline soils of Southern Mexico and Central America and is now naturalised throughout the tropics between 30^{0}N and 30^{0}S latitudes, on less acid soils and at elevations up to 1500 m above sea level (Hill 1971). It is widely grown throughout Central Queensland in hedgerows with a companion grass and is reported as being one of the most productive, profitable and sustainable grass-legume pasture systems in subtropical and tropical northern Australia (Shelton and Dalzell 2007). However, its susceptibility to cooler temperatures is thought to limit its value in more frost prone regions (Lambert 2009) and potential as a weed is an issue in both NSW and Western Australia (Walton 2003a, b). There are a small number of commercial sowings of leucaena in NSW near the Queensland border, but no records on or south of the North-West Slopes of NSW. Leucaena established in January 2009 in a study at Tamworth (Boschma and Harris 2009) continues to be productive with no plant losses (SP Boschma, pers. comm.) indicating that it may have more potential in these environments than previously considered.

Once established, leucaena is known to have superior persistence (>30 years) under regular grazing (Jones and Bunch 1995, 2000) and as a result of its deep-rootedness is able to tolerate prolonged dry periods (Dalzell *et al.* 2006). Leucaena-grass based pastures can provide forage with high nutritive value for livestock with one research study reporting cattle live weight gains of 0.70–1.70 kg/head/day in leucaena-grass pastures. This growth is higher than, grazing buffel grass (*Cenchrus ciliaris*) alone (0.47–1.30 kg/head/day) (Esdale and Middleton 1997; Shelton and Dalzell 2007). Leucaena-grass pastures can also support higher stocking rates than grass pasture alone (Shelton and Dalzell 2007). Leucaena-grass pastures also have a number of environmental benefits including; high water use efficiency (Dalzell *et al.* 2006), enhanced soil fertility through nitrogen fixation (>75 kg N/ha/year; Shelton and Dalzell 2007), and increased ground cover (promoted by grass growth) leading to reduced erosion and better competition against weeds (Shelton and Dalzell 2007).

Leucaena is a tropical species and requires warm temperatures (25–30°C) for optimum growth. It has poor cold tolerance, and production can be significantly reduced or non-existent during cool winter months in subtropical areas (Cooksley *et al.* 1988). In environments which receive a significant frost period, leaves and stems will die when exposed to heavy frosts (below 3°C). These frosts will not kill the plant (Cook *et al.* 2005).

Experiments evaluating the potential role of leucaena as a companion legume for tropical grass based pastures in Northern Inland and Central West NSW were conducted 2013–2017. The main objective of this study was to evaluate four cultivars and an experimental line of leucaena in a mix with a tropical perennial grass (digit grass (*Digitaria eriantha*) cv. Premier) at two sites (Bingara and Manilla) in the possible geographic range for this tropical legume in Northern Inland NSW and a third site (Trangie) to test adaptation. The agronomic traits used to determine suitability were establishment, persistence and herbage production of both the leucaena and tropical grass over four years.

6.2 Methodology

6.2.1 Sites

Two experimental sites were located near Bingara and Manilla in Northern Inland NSW and one at Trangie in Central West NSW. A summary of the site locations, soil type and condition, and pre-sowing weed management are provided in Table 6.1, a more detailed description of the sites can be found in Chapter 4.

Table 6.1 Latitude, longitude, annual average rainfall (AAR, mm), elevation (m), soil type, soil chemical properties [pH (pH_{Ca}, $CaCl_2$), phosphorus (P, Colwell, mg/kg) and sulfur (S, KCl_{40}, mg/kg)] and pre-sowing management for the experimental sites in Northern Inland NSW and Central West NSW.

Site	Latitude Longitude	AAR (mm)	Elevation (m)	Soil Type	Soil chemical properties (0–0.1 m)			Management pre-sowing
					pH_{Ca}	P	S	
Bingara	29°42’39” S, 150°27’07 E	743	297	Brown Chromosol	5.0	50	3.2	Two winter oat crops, with a fallow period over summer. Summer weeds controlled with glyphosate (450 g/L a.i., 1.5L/ha). December 2012 area prepared with rotary hoe and pasture harrows.
Manilla	30°42’11” S, 150°30’10” E	576	412	Brown Chromosol	6.1	36	4.2	Two winter oat crops, with a fallow period over summer. Summer weeds controlled with glyphosate (1.5L/ha). In Nov–Dec area irrigated with 50 mm to encourage germination. Summer weeds sprayed with 1.5 L/ha glyphosate.
Trangie	31°59’45” S, 147°56’18” E	493	214	Brown Chromosol	5.1	10	3.0	Approximately twelve months prior to sowing the native pasture (dominated by *Enteropogan acicularis* (curly windmill grass) was sprayed twice with glyphosate (1 L/ha) and 500 ml/ha 2,4-D amine (625 g/L a.i). The experimental area was cultivated with offset disks sprayed with glyphosate (1 L/ha and deep ripped prior to sowing.

6.2.2 Species, sowing and experimental designs

Four commercial cultivars of leucaena (Wondergraze, Cunningham, Tarramba and Peru) and two experimental lines developed by the University of Queensland for tolerance to psyllids (*Heteropsylla cubana*) (S&D#20 and S&D#36) were sourced from seed companies and University of Queensland respectively. Seedlings of each line were established in glasshouses in December 2012 (Bingara and Manilla) and August 2013 (Trangie). Germination of S&D#20 was poor and was removed from the experiment. Prior to transplanting, the seedlings were inoculated with commercial rhizobia (CB3126); the inoculant was suspended in clean water and evenly distributed across the seedling trays. In January 2013, the 150–200 mm tall (about seven weeks old) seedlings were transplanted into the field at Bingara and Manilla. Seedlings were transplanted into the field at Trangie in early November 2013 (approximately 150 mm high).

The three experiments were designed as randomised complete blocks with three replicates. Each plot consisted of 16 plants of a cultivar/line of leucaena transplanted 0.5 m apart in twin rows (1 m apart). Each row and plot was 4 m in length and each replicate was 20 m long (5 cultivars/line x 4 m each) with an additional 1 m row of leucaena (i.e. two plants) at each end of both twin rows as a buffer. The alley between individual replicates was 6 m. At Bingara, the leucaena seedlings were transplanted into furrows 0.1 m deep, while at Manilla and Trangie the seedlings were planted into rip lines (0.3–0.6 m deep). After transplanting the plants were watered either by hand held hose (Bingara and Trangie) or by an inverted soaker hose and watered three times over an eight day period (Manilla).

Digit grass cv. Premier was sown in the 6 m alleys between leucaena twin rows at 1 kg/ha (viable seed) at Bingara and Manilla in December and November 2013 respectively and at Trangie in October 2013. It failed to establish at all sites. At the Manilla site, forage oats was sown into the area in May 2014, sprayed with glyphosate (1.5 L/ha) in September 2014 to allow soil water to accumulate and digit grass was resown in November 2014. Subsequent attempts to establish digit grass at Bingara failed and the experiments at Bingara and Trangie continued without a sown grass in the alleys.

6.2.3 Site Management

At the Bingara site, grass weeds along the twin leucaena rows were controlled with haloxyfop (520 g/L a.i.) at 100 ml/ha in August 2014 and 2015. The alley between leucaena rows was maintained in weed free fallow with three applications of glyphosate (1.5 L/ha) during the period April–November 2013. After 2015, grass and broadleaf weeds were controlled in alleys with glyphosate (1.5 L/ha) and 2,4-D ester (680 g/L a.i. at 1.3 L/ha) on three occasions.

At the Manilla site grass weeds along the twin leucaena rows were controlled with fluazifop-P (128 g/L a.i. at 0.5 L/ha) in February 2012. Imazethapyr (240 g/L a.i. at 70–100 g/ha) was applied in July and December 2013, and July and October 2015 to provide residual weed control. The alley between leucaena rows was maintained in weed free fallow with five applications of glyphosate (1.5 L/ha) during the period April–November 2013. Broadleaf weeds in digit grass were controlled with 2,4-D (720 g/L a.i. at 1.7 L/ha) in June 2015.

At the Trangie site grass and broadleaf weeds along the twin leucaena rows and alleys between the leucaena rows were controlled by glyphosate (1.0–1.8L/ha) six times in 2014–15. At this site the leucaena plants were sprayed with 400 ml/ha of fenitrothion (1000 g/L a.i.) in March 2015 to control locusts.

At all sites single superphosphate (8.8% P, 11% S) was applied at 200 kg/ha in spring–early summer each year from 2013. In September each year, as leucaena plants were recommencing growth, the dead frosted stems were cut to a height of 0.3 m and the woody material removed from the experiment. Leucaena pods were removed and destroyed to eliminate the risk of recruitment throughout the experiment.

6.2.4 Data collection

Rainfall data were recorded either manually (Bingara and Manilla) or by automatic weather station (Trangie). Long-term average monthly and annual rainfall data for all sites were determined from Bureau of Meteorology (BOM) sites.

6.2.4.1 Leucaena establishment and persistence

Plant numbers were recorded 2–3 months after transplanting in the paddock to determine establishment success. Persistence of individual leucaena plants was assessed in spring and autumn each year by recording their presence and health. Plants were categorised as present and healthy, present but in poor condition (notes were recorded on the nature of the poor condition and photos taken), or absent/dead.

6.2.4.2 Leucaena herbage production

Leucaena herbage mass was assessed from spring to autumn each growing season whenever the tallest leucaena plants reached approximately 1.8 m in height. At each assessment the number of stems was recorded for eight plants. The middle four plants in each row were used except when the plants were not representative of those in the plot (e.g. significantly smaller or dead) then plants from those remaining in the plot were assessed. A representative stem on each assessed plant was selected, cut at the point where the stem diameter was about 10 mm and bagged. All leaves from the remainder of this stem were also removed to the base of the plant and placed in the same bag. This harvested stem and leaf material represented the edible proportion of the plant and was dried in a dehydrator for 48 h at 80°C then weighed to calculate herbage dry weight (g DM/plant and kg DM/ha. After each assessment all leucaena plants were cut back to a height of 0.5 m and material removed from the plots.

6.2.4.3 Grass establishment

Counts of seedling density (seedlings/m^2) of digit grass were taken 4–6 weeks after sowing in 6 quadrats (0.10 x 0.50 m) in each plot; three from the alley on each side of the leucaena rows.

6.2.4.4 Grass herbage production

The grass sown between the rows was assessed at the same time as the leucaena using the same visual assessment described in Chapter 4. In summary, for each plot, the alley on either side of the twin rows was sub-divided into two equal strata. For each strata, total herbage mass was visually estimated (continuous 0–5 scale, where 0 = nil and 5 = highest herbage mass) also the percentage of digit grass (dry weight herbage mass) was assessed. Fifteen calibration quadrats (0.40 x 0.40 m) representing the range of herbage mass at a site were also scored, harvested to approximately 10 mm above ground level and sorted into digit grass and other species. The samples were dried at 80°C for 48 h and weighed. Herbage mass scores and percentage estimates were regressed (linear or quadratic R^2>0.80) against actual herbage mass (kg DM/ha) and percentage of digit grass to determine herbage mass of the sown grass. After each assessment the plots were mown with a rotary mower and the herbage removed from the plots.

6.2.4.5 Grass persistence

In spring and autumn each year plant frequency of the digit grass was assessed in a two permanent quadrats (1.0 x 0.5 m) located in the alley on either side of the leucaena twin rows. Estimates were taken 0–10 days after the experimental area was defoliated. For each plot, cells containing a portion of a live digit grass plant were recorded (presence) and the proportion (%) of occupied cells used to estimate frequency of occurrence (plant frequency, Brown 1954).

6.2.4.6 Leucaena flowering

Growth stage and maturity were recorded for individual plants during the growing season. Plants were categorised as vegetative, buds present, flowers present and pods present. At Bingara, flowering was recorded eight times (approximately every two weeks) over the first growing season (2013–14) and then at the start and finish of the growing seasons in the following years. At the Manilla site, individual plant flowering assessments were conducted during the 2013–14 (5 assessments), 2014–15 (1 assessment) and 2015–16 growing season (3 assessments); assessments generally coinciding with a herbage mass assessment. At Trangie, flowering was assessed approximately monthly during the 2014–15 growing season.

6.2.5 Statistical analyses

6.2.5.1 Variance components analysis

Three traits were analysed; leucaena herbage mass, grass herbage mass and grass frequency. Selection of best performing varieties among genetically correlated traits involved a multivariate analysis. The study had the additional complications, however, that traits were measured at multiple sites and all traits had repeated measures on each plot within a site. An ideal approach would have involved a single analysis of all traits, sampling times and sites. Such an analysis is still an area of current research and is beyond the scope of this paper. Therefore, data for each combination of trait and site were analysed individually using a variance component analysis.

For each trait, each assessment within a site was considered an 'environment`, such that each subset of data corresponding to a trait by site combination was a multi-environment trial data-set. A linear mixed model was fitted to the data for each trait by site combination using the software ASReml (Gilmour *et al.* 2006) in R (R Core Team 2017) and estimates of variance parameters were obtained using residual maximum likelihood (REML) estimation (Patterson and Thompson 1971), using the average information REML algorithm (Gilmour *et al.* 2006).

Leucaena herbage mass, grass herbage mass and grass frequency data were first cube-root transformed to more closely resemble a Gaussian distribution. Non-genetic effects associated with the experimental design of the trials were crossed with the longitudinal factor for sampling times (Brien and Demetrio 2009) and fitted as random effects. In terms of the genetic effects, the random component of the model included a main effect for legume varieties and an interaction term between sampling times and varieties and assumed a simple variance component structure for these effects. This is commensurate with a so-called compound symmetric variance structure which assumes equal variety variance at each sampling time and equal covariance between pairs of sampling times. The statistical significance of genetic terms in the model was assessed using the residual maximum likelihood ratio test (REMLRT) to compare the likelihood of the full model against the model excluding the effect under examination. The resulting test statistic was then compared to the reference distribution of a mixture of chi-squared variates (Stram and Lee 1994).

Effects related to varieties were fitted as random effects given the aim of the analysis was selection and, by definition, the best linear unbiased predictors (BLUPs) obtained using random effects best predict the true variety effects (Smith *et al.* 2005). A simplified residual model was used which assumed a unit variance for the residuals rather than assuming correlation among sampling times within a plot, as may be done with larger repeated measures data.

The BLUPs of the overall performance for each cultivar (Smith and Cullis 2018) for each trait were calculated for each site, as well as the 90% confidence interval for these predictions. The overall performance was added to the BLUP of the overall mean for cultivars, averaged across environments. This value was then back-transformed as an approximation of the overall mean performance on the scale of the original data to provide a value for each legume treatment that was biologically meaningful. This value and the BLUPs of the overall performance for each legume treatment with the 90% confidence interval are presented for each trait. When interpreting BLUPs, the confidence intervals provided are not a formal test for comparison of treatments (i.e. significance) because treatment effects were fitted as a random effect. Instead they are a test for the true value of each treatment individually.

6.2.5.2 Leucaena persistence and flowering

There was very little change in persistence during the trial and little variation between many of the species, so no statistical analyses were conducted. Notable exceptions are described in the results section.

For the flowering data, variation between replicates for a species was much larger than variation between species with no consistent trend over time. Notable aspects of the flowering data are described in the results section.

6.2.5.3 Grass establishment

Seedling densities (seedlings/m^2) were analysed by ANOVA with leucaena cultivar/line as the explanatory factor and replicate as a block term. Plots of the residuals showed no transformation of the data was necessary.

6.3 Results

6.3.1 Rainfall

During the establishment period (January–April 2013), rainfall at the Bingara and Manilla sites was above average and average respectively (Table 6.2). In contrast, the Trangie site received below long term average (LTA) rainfall during the establishment period. Growing season rainfall at the Bingara site was well below average in 2013–14 (39% of the LTA), but above average for each subsequent year. At Manilla growing season rainfall was below average (62–83% of LTA) each year that the experiment was conducted, while at Trangie growing season rainfall was above average each year. The non-growing season (May–October) each year was below the LTA at all sites for all years except 2016, when rainfall ranged 130% (Manilla) to 226% of the LTA (Trangie). Monthly rainfall are provided in Chapter 4 (Fig. 4.2).

Table 6.2. Rainfall (mm) received during each leucaena growing season (November–April) and non-growing season (May–October) from January 2013 to April 2017, at the Bingara, Manilla and Trangie sites. Long term average (LTA) rainfall data are from Bureau of Meteorology sites Bingara (054004 (1878–1997)), Manilla (55274 (1909–2013)) and Trangie (51049 (1922–2017)).

		Bingara	Manilla	Trangie
Growing season 2013	Jan–Apr13	316	203	90
Non-growing season 2013	*May–Oct13*	*211*	*145*	*212*
Growing season 2013–14	Nov13–Apr14	172	238	237
Non-growing season 2014	*May–Oct14*	*99*	*75*	*142*
Growing season 2014–15	Nov14–Apr15	482	239	271
Non-growing season 2015	*May–Oct15*	*252*	*200*	*200*
Growing season 2015–16	Nov15–Apr16	333	155	289
Non-growing season 2016	*May–Oct16*	*411*	*314*	*492*
Growing season 2016–17	Nov16–Apr17	362	150	235
Growing season LTA	Nov–Apr	436	336	278
Non-growing season LTA	May–Oct	305	242	217

6.3.2 Leucaena establishment and persistence

Plants established successfully at both the Bingara and Manilla sites with 98–100% survival 2.5–3.0 months after transplanting. At Trangie, plant survival was less than the other sites; cvv. Peru and

Tarramba had the highest survival rate (68%) while the experimental line had the lowest survival rate (44%). It was noted that some plants had been grazed by hares or kangaroos at all three sites, with damage most evident, but generally not fatal at Trangie.

At the Bingara site plant numbers for all cultivars declined in the first 12 months of the experiment (by 12–25%), but after the first summer (2013–14) plant numbers remained relatively stable. At the end of the experiment cvv. Tarramba, Cunningham, Wondergraze and Peru had similar plant survival (85–81%) and S&D#36 was the least persistent at 71% (Fig. 6.1).

Despite the dry conditions at the Manilla site cvv. Tarramba, Wondergraze and Peru did not decline at all over the course of the experiment. The experimental line S&D#36 declined by 2% over the first winter (2013), but remained steady for the remainder of the experiment. Cultivar Cunningham declined by 2% over the first winter (2013) of the experiment and declined a further 2% over the winter of 2015, but still retained high persistence (96%) at the end of the experiment (Fig. 6.1).

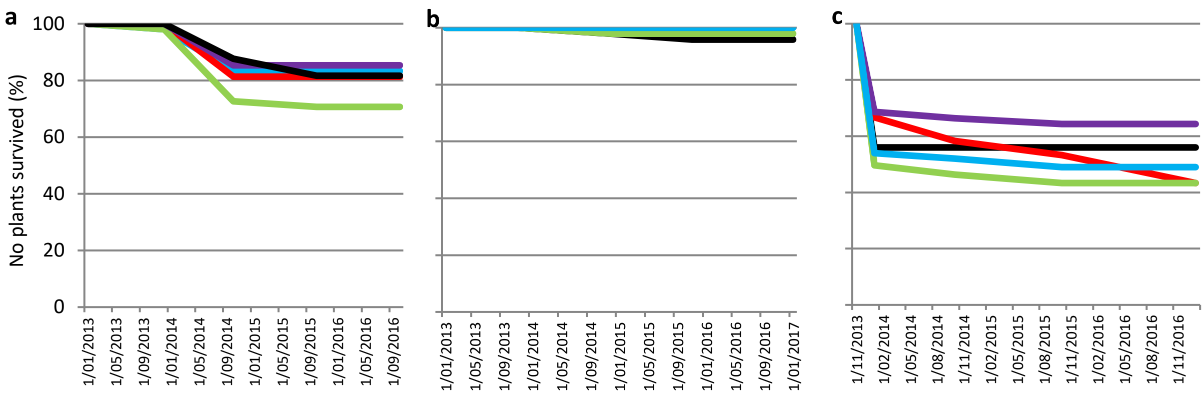

Fig. 6. 1 Leucaena plant survival (%) of five leucaena cultivars; Cunningham (black line), Peru (red line), S&D#36 (green line), Tarramba (purple line) and Wondergraze (light blue line) at (a) Bingara, (b) Manilla and (c) Trangie from 2013–17.

At the Trangie site where there were lower plant numbers due to poorer establishment, survival of all treatments was lower than the Bingara and Manilla site (43–64%). However, after the establishment year cv. Cunningham did not decline over the course of the experiment. The experimental line S&D#36 and cvv. Tarramba and Wondergraze declined by a similar amount (4–6%) over the course of the experiment. Cultivar Peru was the least persistent cultivar at Trangie, declining 23% over the course of the experiment (Fig. 6.1). At the end of the experiment cv. Tarramba had the highest persistence (64%), while experimental line S&D#36 and cv. Peru had the lowest persistence (43%, Fig. 6.1).

6.3.3 Leucaena herbage production

At Bingara cv. Wondergraze was ranked 1st over the four years of the experiment with an average herbage mass of 2394 kg DM/ha per assessment (backtransformed predicted mean herein referred to by units only) which was similar to cv. Cunningham (2059 kg DM/ha per assessment). The remaining treatments, cv. Tarramba, S&D#36 and cv. Peru were ranked 3rd–5th respectively (Table 6.3) and had below average productivity (BLUP<0, Table 6.3).

At Manilla cv. Cunningham had an average herbage mass of 1904 kg DM/ha per assessment and was ranked 1st, followed by cv. Wondergraze (1704 kg DM/ha per assessment); both had above average productivity (BLUP>0). The experimental line S&D#36 was ranked 5th (1303 kg DM/ha per assessment; Table 6.3).

The average herbage mass per assessment at Trangie ranged from 708 kg DM/ha for experimental line S&D#36 to 1053 kg DM/ha for cv. Wondergraze (data not analysed, Table 6.3)

6.3.4 Leucaena flowering

In 2013–14, cvv. Peru, Wondergraze and the experimental line S&D#36 commenced flowering approximately 114 days after the first signs of regrowth (30/09/2013), but cvv. Cunningham and Tarramba did not commence flowering until approximately 156 days after first signs of regrowth. The experimental line S&D#36 was the most prolific flowering cultivar and cv. Tarramba the least (Table 6.4).

In the growing seasons of 2014–15 and 2015–16, flowering for each cultivar followed a similar pattern to 2013–14 with flowering commencing approximately 110 days after first signs of regrowth in spring for cvv. Peru, Wondergraze and the experimental line S&D#36, and at approximately 150 days for cvv. Cunningham and Tarramba. The experimental line S&D#36 was again the most prolific flowering line in these years and cv. Tarramba the least (Table 6.4).

At the Manilla site, cv. Wondergraze was the first cultivar to commence flowering (November 2013) and was flowering at each assessment during the 2013–14 growing season (4–44%). The other cultivars were flowering in January 2014, with the exception of cv. Tarramba which only flowered (2%) in February 2014. During the 2014–15 growing season, the cultivars responded similarly and flowering ranged from 44% (cv. Wondergraze) to 0% (cv. Tarramba) in February 2015. During the 2015–16 growing season, all cultivars were flowering at each assessment with the exception of Tarramba which only flowered (6%) in December 2015 (Table 6.5).

Flowering data for the 2014–15 growing season at Trangie are presented in Table 6.6. At this site cv. Peru and experimental line S&D#36 commenced flowering earlier than at the Bingara site (about 88 days after first signs of regrowth in mid-September), but all cultivars were flowering by about 110 days after growth recommenced. Cultivar Peru was the most prolific flowering cultivar, but was similar to cvv. Tarramba and Wondergraze. The experimental line S&D#36 was the least prolific flowering line, but was similar to cv. Cunningham.

6.3.5 Grass establishment, herbage production and persistence

Establishment of digit grass at the Manilla site at the second attempt of sowing was poor and ranged from 4 plants/m^2 in the alley adjacent to cv. Tarramba to 0.5 plants/m^2 adjacent to cv. Wondergraze ($P > 0.05$).

Digit grass herbage mass varied, although the range was small. Average grass herbage mass in the S&D#36 treatment was ranked 1st (530 kg DM/ha per assessment) and was similar to cv. Tarramba (456 kg DM/ha per assessment); both with BLUPs>0 (Table 6.7). Cultivars Wondergraze and Peru had the lowest grass herbage mass (386 kg DM/ha per assessment; Table 6.7).

Digit grass plant frequency in the S&D#36 treatment was ranked 1st with an average grass frequency of 25%; similar to cvv. Tarramba and Cunnigham (22–23%) and above average (BLUP>0, Table 6.7), Peru and Wondergraze were ranked 4th and 5th respectively (Table 6.7).

Table 6.3. E-BLUPs of the treatment effects, treatment means and their confidence intervals (CI), also back-transformed means (scaled mean kg DM/ha per assessment) for leucaena herbage mass at the Bingara and Manilla sites. Herbage mass (kg DM/ha/assessment) for the Trangie site are also presented (data not analysed).

	Bingara					Manilla					Trangie
Treatment	E-BLUP	Mean	CI lower	CI Upper	Herbage mass (kg DM/ha)	E-BLUP	Mean	CI lower	CI Upper	Herbage mass (kg DM/ha)	Herbage mass (kg DM/ha)
Wondergraze	0.91	13.38	12.34	14.42	2394	0.37	11.94	11.20	12.69	1704	1053
Cunningham	0.25	12.72	11.68	13.76	2059	0.82	12.40	11.65	13.14	1904	943
Tarramba	-0.16	12.31	11.27	13.35	1866	-0.06	11.51	10.77	12.26	1526	863
Peru	-0.66	11.81	10.76	12.85	1646	-0.48	11.09	10.35	11.84	1365	794
S&D#36	-0.34	12.13	11.09	13.17	1784	-0.65	10.92	10.17	11.64	1302	708

Table 6.4. Percentage (%) of plants with flowers and/or pods at the Bingara site during the 2013–14, 2014–15 and 2015–16 growing seasons.

	2013-14								2014-15		2015-6	
Treatment	22/01/2014	05/02/2014	19/02/2014	05/03/2014	19/03/2014	02/04/2014	16/04/2014	30/04/2014	06/01/2015	06/05/2015	12/01/2016	20/04/2016
Cunningham	0	0	0	8	7	27	29	70	0	42	5	47
Peru	5	5	5	27	33	34	39	56	11	42	8	48
S&D#36	17	23	26	33	33	40	44	49	18	47	26	51
Tarramba	0	0	0	3	0	5	10	32	0	17	7	20
Wondergraze	5	7	7	33	24	27	37	70	3	46	6	50

Table 6.5. Percentage (%) of plants with flowers and/or pods at the Manilla site during the 2013–14, 2014–15 and 2015–16 growing seasons.

	2013–14					2014–15	2015–16		
Treatment	20/11/2013	16/01/2014	25/02/2014	8/04/2014	14/05/2014	11/02/2015	8/12/2015	2/02/2016	12/05/2016
Cunningham	0	10	10	2	0	2	8	2	26
Peru	0	13	10	2	2	8	23	4	19
S&D#36	0	15	23	8	6	15	17	26	17
Tarramba	0	0	2	0	0	0	6	0	0
Wondergraze	8	40	44	6	4	44	67	8	46

Table 6.6. Percentage (%) of plants with flowers and/or pods at the Trangie site during the 2014–15 growing season.

Treatment	22/11/2014	16/12/2014	06/01/2015	23/03/2015
Cunningham	0	3	0	13
Peru	8	16	18	35
S&D#36	19	7	7	9
Tarramba	0	3	0	27
Wondergraze	0	17	5	26

Table 6.7. E-BLUPs of the treatment effects, treatment means and confidence intervals (CI), also back-transformed means for digit grass herbage mass (kg DM/ha) and plant frequency (%) at the Manilla site.

Treatment	Herbage mass					Plant frequency				
	E-BLUP	Mean	CI lower	CI Upper	Back-transformed (kg DM/ha)	E-BLUP	Mean	CI lower	CI Upper	Back-transformed (%)
S&D#36	0.53	8.09	7.50	8.69	530	0.12	2.91	2.72	3.10	24.6
Tarramba	0.13	7.70	7.10	8.29	456	0.04	2.83	2.63	3.02	22.6
Cunningham	-0.10	7.47	6.87	8.06	416	0.01	2.79	2.60	2.98	21.8
Wondergraze	-0.28	7.28	6.69	7.88	386	-0.09	2.70	2.51	2.89	19.6
Peru	-0.28	7.28	6.69	7.88	386	-0.08	2.71	2.52	2.90	19.8

6.4 Discussion

This study showed that leucaena can establish successfully with high seedling survival in Northern Inland NSW, although mortality can be higher in the Central West; once established, leucaena is persistent; productivity varied with site but cvv. Wondergraze and Cunningham were consistently the most productive and S&D#36 the least; and grass establishment was poor.

At the Bingara and Manilla sites leucaena established successfully and was productive over the experimental period (best cultivar producing approximately 7 and 5.3 t DM/ha per growing season respectively). This confirms the previous (Boschma and Harris 2009) research conducted at Tamworth that signposted the potential of leucaena for Northern Inland NSW. At Trangie, where, leucaena had previously not been evaluated, establishment was less successful, but the majority plants which did establish were persistent for the four years of the experiment and were relatively productive (best cultivar produced approximately 3.3 t DM/ha per growing season). The poorer production at the Trangie site was most likely a factor of the lower number of established plants, with most losses occurring in the establishment year. Poor establishment was possibly due to the small plant size at transplanting, low overnight temperatures at the time of transplanting in spring and below average rainfall during the growing season. This highlights the importance of good establishment for subsequent production.

Once established leucaena is highly persistent with stands of >30 years in Queensland (Radrizzani *et al.* 2010). In this study persistence at the Bingara and Manilla sites was high with >70% of plants surviving throughout the experiment; few losses occurring after the first 12 months. Similarly, there were few losses at Trangie after the establishment period (with the exception of cv. Peru). Leucaena is reported to have poor cold tolerance (Cooksley *et al.* 1988) which could impact on plant persistence in frost prone regions such as Northern Inland and Central West NSW. In this study, plant growth ceased over winter at all sites, but it did not appear to have a large impact on plant survival, therefore demonstrating that leucaena is able to survive in these colder environments and be productive, despite the shorter growing season.

Cultivars Wondergraze and Cunningham were the most productive cultivars; ranked 1st and 2nd at all three sites. Cultivar Cunningham was bred by CSIRO and released in Australia in 1976. It has good basal branching giving it a 'bushy' habit (Cook *et al.* 2005; Dalzell *et al.* 2006). Cultivar Wondergraze was bred in Hawaii, released in Australia in 2010 and has higher basal branching than cv. Tarramba. Both cvv. Wondergraze and Tarramba are reported as being higher yielding than cvv. Cunningham and Peru (Cook *et al.* 2005; Shelton *et al.* 2017).

It was a challenge to establish a leucaena-grass pasture, especially when trying to establish the species in separate years as evidenced by the failure of digit grass at two sites in this study. Best-practice recommendations for establishing leucaena-grass pastures developed in Queensland were followed in this study, that is, establish leucaena hedgerows in first summer and the grass the following summer (Dalzell *et al.* 2006). This is to allow the leucaena to establish well as it is a weak seedling and slow to establish (Lambert 2013), before applying competition from the grass. This method was unsuccessful at all sites in this study, resulting in resowing which was only successful at one site. This practice is unlikely to be commercially viable in these environments for the following reasons:

1. Producer concerns that the pasture is out of production for at least two years while the pasture is establishing.
2. Established leucaena is highly competitive against seedling grasses. Established leucaena plants will have a developed root system (Radrizzani *et al.* 2010) drying the soil profile in alley making grass harder to establish. This can be exacerbated if rainfall is low in the second summer when grass is sown. If grass does not established in the second year, grass would need to be resown extending establishment to three years. This is time consuming and expensive; that is, additional costs of weed control, fertiliser and seed, also lost productivity and grazing opportunity while the pasture is establishing.
3. Failure to establish a grass in the alley may result in poor ground cover, weed invasion, increased potential for erosion and reduced livestock production.

An alternative technique to establish a leucaena-grass pasture is to sow both species in the same year leaving a 2–3 m buffer on either side of each leucaena hedgerow to minimise competition from the grass during the first year. A similar strategy, using 1 m buffers was found to have merit in southern inland Queensland (Lambert 2013), but this would need to be tested in Northern Inland and Central West NSW environments. In this study the alleys were 6 m and extensive cracks were present across the full width of the alley suggesting that the distance between the twin rows could be increased to 8–10 m on soils similar to the three sites.

It is important to achieve a leucaena-grass pasture to optimise livestock production. Studies have shown that grazing leucaena-grass based pastures can support higher stocking rates (Shelton and Dalzell 2007) and result in higher cattle weight gains than leucaena or grass alone. Esdale and Middleton (1997) reported live weight gains of 0.7–1.70 kg/head/day in leucaena-grass pastures. This growth is comparable to, or higher than, grazing on buffel grass (*Cenchrus ciliaris*) alone (0.47–1.30 kg/head/day) and to grain-fed lot feeding (1.41 kg/day) (Shelton and Dalzell 2007). Mullen *et al.* (2005) showed that leucaena can increase beef cattle liveweight gains by >70% compared with pure grass pastures, achieving gains of 275–300 kg/head/year.

The genus *Leucaena* has a short juvenile phase and can commence flowering 3–4 months after growth commences although generally plants do not set seed until the second year (Walton 2003b). Kaminski *et al.* (2000) reported that the vegetative period (number of days from leaf regrowth to first flower bud) of ssp. *glabrata* was 56–170 days. This was confirmed at the Bingara site (where detailed flowering data was collected). Flowering of all cultivars and the experimental line commenced 88–156 days after first regrowth. This work only provided a snapshot on the phenology of leucaena in the Northern Inland and Central West environments. A better understanding of plant phenology will also assist with the development of grazing guidelines to maximise production and forage quality by timing grazing to occur around the time of first flower (approximately every 6–8 weeks depending on cultivar) (Garcia *et al.* 1996). In addition, a more detailed understanding of seed set and seed bank persistence is required to inform management guidelines for the grazing industry if leucaena does become weedy.

During this study, flowers and pods were removed before pods could ripen to reduce the potential for seed spread. Leucaena has weed potential (Walton 2003a, b) due to its ability to produce seed year round (in the tropics), build a substantial seed bank, re-sprout after cutting or burning, tolerate drought and to produce thickets (Hughes and Jones 1998). This is a biosecurity concern to a number of state government agencies in NSW, also Western Australia (WA). In Queensland, leucaena management is recommended from a Code of Practice developed by the Leucaena Network (Leucaena Network 2010). The Leucaena Network was established in 2000 by industry and supported by technical expertise from Queensland Department of Primary Industries (currently called Department of Agriculture and Fisheries) and University of Queensland to counter the anti-leucaena movement from environmental groups. Their charter is to "promote the responsible development of leucaena in sustainable and productive grazing and agroforestry systems to build stronger rural communities". The most effective means of overcoming weed potential is development of a seedless or sterile leucaena. In 2017 a project commenced with WA Department of Primary Industries and Regional Development, University of Queensland and Meat and Livestock Australia Donor Company. The weediness of leucaena means that the NSW Department of Primary Industries will not be recommending leucaena in the near future, but would welcome the opportunity to evaluate sterile lines when they become available.

Another management issue that requires further investigation in NSW before leucaena can be recommended is the long-term productivity in older leucaena-grass pastures. In Queensland, older leucaena pastures (>10 years old) are reported to be maintaining grass and animal productivity, however, leucaena productivity is declining on lower fertility soils as a result of little or no fertiliser application (Radrizzani *et al.* 2010). The sites established in Northern Inland and Central West NSW for this study warrant further monitoring to evaluate the long-term productivity of leucaena in these environments.

If/when leucaena is recommended to producers in NSW a technology and training package will be required for producers and advisors outlining best management practice for establishment, management and animal husbandry. For example, leucaena contains the toxic non-protein amino acid called mimosine which can impact livestock productivity from reduced liveweight gains (Jones *et al.* 1976; Jones and Jones 1984; Quirk *et al.* 1988) to ulcers and liver and kidney tissue damage (Dalzell *et al.* 2006). Mimosime is degraded in the rumen by the bacterium *Synergistes jonesii* (Allison *et al.* 1992) and livestock need to be inoculated with the rumen bacterium and allowed to graze leucaena regularly to maintain the bacterium (Dalzell *et al.* 2012). A best management package will improve producer confidence to incorporate leucaena into their grazing systems, as evidenced, by the parallel development of NSW DPI guidelines for establishment and management of tropical perennial grasses and the increase in area sown over the past 15 years, now estimated to be over 450,000 ha.

6.5 Conclusion

This study has confirmed the persistence and productive potential of leucaena as a summer growing companion legume for tropical perennial grasses in the Northern Inland and to a lesser degree in Central West NSW. It has, however, highlighted challenges in establishing a productive and persistent perennial tropical grass base. This requires further research to develop the agronomy around the establishment of the grass and leucaena in the same year.

The weed potential of leucaena means that NSW DPI will not be recommending leucaena in the near future, but the authors recommend that leucaena continues to be monitored at these sites to understand long-term persistence, production and agronomy on this species in sub-tropical and temperate regions. This information will assist in the development of a best management practice (establishment, management and animal husbandry) for producers and advisors if/when leucaena is recommended in NSW.

7 Temperatures for seedling emergence of tropical perennial grasses

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7.1 Introduction

Sown tropical perennial grasses are able to utilise summer rainfall; a number being productive and persistent in grazed pasture systems in summer dominant rainfall areas prone to frost in Northern Inland NSW and Southern Queensland (e.g. Lloyd *et al.* 2007a,b; McCormick *et al.* 1998; Boschma *et al.* 2009, 2014a, 2016). The persistence of species such as *Digitaria eriantha* (digit grass), *Chloris gayana* (Rhodes grass), *Megathyrsus maximus* (panic grasses) and *Panicum coloratum* (Bambatsi panic) in this region, shows their broad adaptation and has resulted in their widespread use across Northern Inland NSW, a region traditionally considered marginal for these species. Pasture establishment is costly (Scott *et al.* 2000) and reports of failed establishment over recent years, in part due to insufficient rainfall, emphasise the risks associated with establishing these grasses in this environment. While there is good understanding of many factors which influence establishment; including the size of grass weed seed banks (Lodge *et al.* 2010a), sowing depth (Lodge and Harden 2009) and seed quality (McCormick *et al.* 2009), there is limited knowledge of the temperature requirements for successful establishment and seedling vigour at emergence.

Plant species can be grouped into two categories based on their optimum temperature for germination: 10–20°C and 25–35°C broadly representing temperate and tropical species respectively (Mott and Groves 1981). However, it is recognised that variation occurs among grass species within each group; both for the optimum temperature for germination and range of temperatures at which germination is near optimal. For example, the optimum temperature range for germination of *Chloris gayana* was found to be 20–30°C (Watt and Whalley 1982), while *Eragrostis leptostachya* had a similar, but narrower range of 20–25°C (Lodge and Whalley 1981) and *Heteropogon contortus* a narrow, but higher temperature range of 30–35°C (Tothill 1977). In contrast, *Dichanthium sericeum* and *Chloris truncata* both have a broad optimum temperature range of 15–35°C (Lodge and Whalley 1981).

Recommendations for Southern Queensland are to either sow tropical grasses from mid-August to early-November or mid-January to mid-March, thereby avoiding the extreme summer heat (e.g.

Lloyd *et al.* 2007b) while in Northern Inland NSW sowing from mid-late November until January is recommended (Lodge and Harden 2009; Harris *et al.* 2014). This coincides with the period when soil temperatures are high and rainfall more likely. However, despite a summer dominant rainfall distribution (60–65% of annual average rainfall falling October–March), warm season rainfall in this region is highly variable typically falling in high intensity storms (Hobbs and Jackson 1977). Together these provide a highly challenging environment for tropical pasture establishment. A better understanding of the optimum temperature and range could help guide industry recommendations of the best time to sow particular species in current areas and indicate areas where these species may be adopted in the future.

A growth cabinet study was conducted to determine the range and optimum temperature for emergence of seven tropical perennial grasses commonly sown in northern NSW and Southern Queensland. Optimum temperatures for germination have been determined for *Panicum coloratum* var. *makarikariense (*Watt and Whalley 1982*), Megathyrsus maximus* (Nichols *et al.* 2012b), *Digitaria eriantha* (Nichols *et al.* 2012b) and *C. gayana* (Watt and Whalley 1982, Nichols *et al.* 2012b), however, optimum temperature for emergence has not been determined for these species, nor *Bothriochloa insculpta* or *B. bladhii* ssp*. glabra*.

7.2 Methodology

The experiment was conducted at the University of New England, February–May 2013 using growth cabinets (Thermoline Scientific Model P.G. Cab Temp and Humidity, 415V, 32 amp 3 phase power 50 Hz).

The treatments were seven constant temperatures and seven tropical grasses. The temperature treatments ranged from 10 to 40°C in 5°C increments. The grasses were: *B. bladhii ssp. glabra* (forest bluegrass) cv. Swann, *B. insculpta* (creeping bluegrass) cv. Bisset, *C. gayana* (Rhodes grass) cv. Katambora, *D. eriantha* (digit grass) cv. Premier, *P. coloratum* var. *makarikariense* (Bambatsi panic) cv. Bambatsi, and *M. maximus* (panic) cvv. Gatton and Megamax 059. Seeds of each grass were obtained from commercial suppliers, except the seed of Megamax 059 which was obtained from a plant breeder. For each seed lot, viability of each seed lot was tested by placing 25 florets/seeds of each seed lot into four petri dishes each, lined with filter paper and watered with distilled water. The petri dishes were placed in a germination cabinet (alternating 30/20°C) for 10 days. Germination results are shown in Table 1. Each seed lot was well mixed prior to subsampling to ensure the sample was representative.

The base of shallow plastic trays (340 x 280 x 50 mm) were lined with paper towel to prevent soil loss and filled with a sieved soil mix consisting of 2 parts sand and 1 part peat. Fifty seed units (details provided in Table 7.1), herein referred to as seeds, of each grass were counted from a representative subsample of the seed lot, and sown at 10 mm depth in single species rows, 250–260 mm long and 40 mm apart, across the short axis of the tray and pressed lightly to ensure good seed-soil contact. Each tray therefore had seven rows, one for each of the seven grass entries.

The growth cabinets were set at a constant temperature with 16 h light and 8 h dark and allowed to come to equilibrium at the designated temperature over a 5 hr period before 4 trays were placed inside.

On the commencement day of each temperature treatment (day 0), 4 trays were soaked in tubs of water for 60 min to allow complete wetting. Once water ponding on the soil surface was observed, the trays were placed side by side into the growth cabinets. The trays were checked daily for soil moisture and placed in tubs of water for 15 minutes every second day to ensure they remained moist. Trays were returned to the same position within the growth cabinet after watering. Water was stored in a separate container in the growth cabinet to minimise temperature changes in the soil during watering. During conduct of the 40°C temperature, trays were placed into larger containers containing water that was maintained at a depth of 5–10 mm. The temperature within each cabinet was monitored with a minimum-maximum thermometer and fluctuated by a maximum of 2°C.

Seedling emergence, recorded as zero upon commencement of each temperature treatment in the growth cabinet (day 0), was recorded on day 4, 6, 8, 11 and 14 with emergence defined as the appearance of the first leaf through the soil surface.

Table 7.1. Details of species global occurrence, also origin, seed unit and pre-experimental germination of seven tropical grass cultivars used in the study

Grass species and common name	Global occurrence			Study details		
	Latitude (°)	Altitude (m asl)	Temperature (°C)	Cultivar and origin	Seed unit	Germination
Panicum coloratum var. *makarikariense* (Bambatsi panic)	20	1000	22	Bambatsi – Combination of cvv. Burnett, Pollock and Bambatsi found in Botswana, South Africa and Zimbabwe respectively (Bernard 1972; Cook *et al.* 2005)	Coated	64
Bothriochloa insculpta (creeping bluegrass)	0–37	0–2000	17–27	Bisset – Combination of 2 accessions from, Kenya and, Tanzania (Cook *et al.* 2005)	Coated	37
Digitaria eriantha (digit grass)	14–34	0–2250	16–24	Premier – unknown, but thought to be South Africa (Anon 1987)	Bare floret	45
Megathyrsus maximus (panic grass)	0–>30	0–>2000	Varies with genotype	Gatton – Zimbabwe (Cook *et al.* 2005) Megamax 059 – South Africa (CA Harris, pers. comm.)	Coated	71
Chloris gayana (Rhodes grass)	0–34.5 (optimum growing temperatures range 20–37°C, extremes of 5 and 50°C)	0–>1000	16.5–>26	Katambora – thought to be Zambia (Cook *et al.* 2005)	Bare floret	64
Bothriochloa bladhii ssp. *glabra* (forest bluegrass)	0–32	0–>2500	14	Swann – South America (Cook *et al.* 2005)	Coated	62

7.2.1 Statistical analyses

Total emergence for Swann forest bluegrass and Bisset creeping bluegrass was higher at 40°C than 35°C (Fig. 7.1); possibly a result of a difference in soil moisture regime due to the trays being placed in larger containers with water. Since the trays were treated differently and emergence of two species was obviously affected, data for 40°C were excluded from the analysis. All seedlings in two trays died during the 35°C treatment. The cause was not moisture deficit and remains unknown. Data for these two trays were excluded from statistical analyses.

For each temperature there were 4 replicates of each species (i.e. 4 trays), and each temperature was conducted once meaning there is no true replication of the temperature treatments. This meant that inferential tests such as t-tests would not meet the assumption of independence of the replicates for the temperature treatments (Morrison and Morris 2000) and alternative statistic methods were required. A smoothed response surface for cumulative seedling emergence of each grass was developed for each species over the range of temperatures (10–35°C) and days (0 to 14) of the study. The response surface was estimated using the thin plate spline function from the 'fields' package available in the statistical software program R. Values of the seedling emergence response surface for each grass at day 14 were used to estimate:

1. Temperature for maximum emergence,
2. Optimum temperature range determined as temperatures for which predicted emergence was within two standard errors of the maximum emergence and
3. Temperature when 50% of maximum emergence occurred.

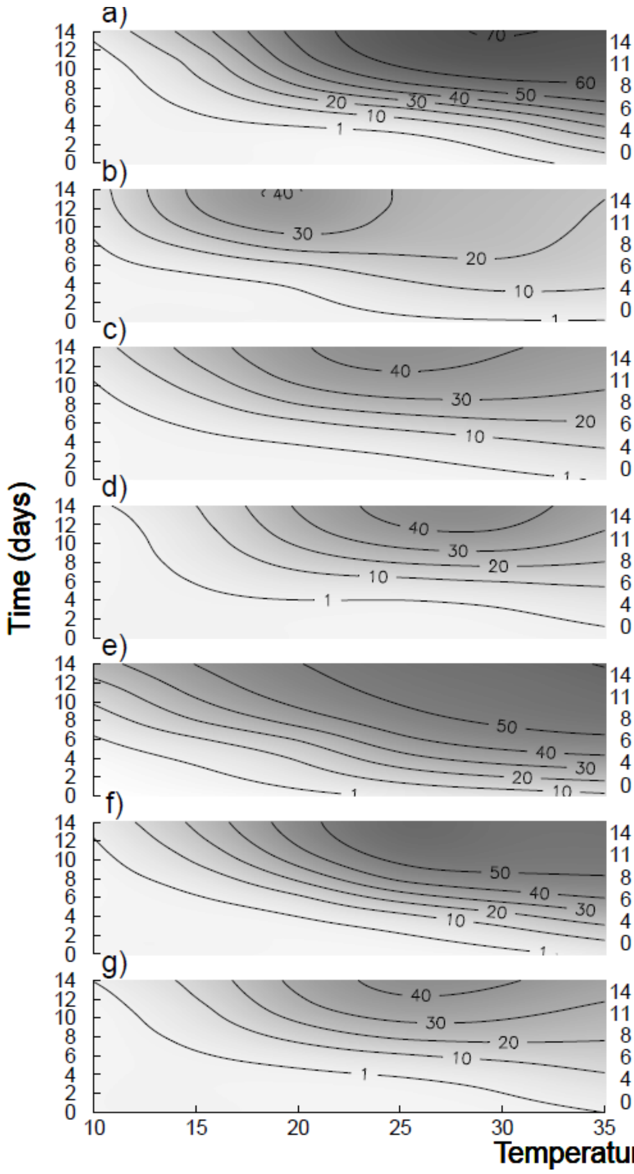
7.3 Results

Smooth response surface plots for cumulative seedling emergence (%) of each grass are shown in Fig. 7.1. Maximum emergence of the grasses varied from 40% for Bisset creeping bluegrass to 70% for Bambatsi panic (Table 7.2). The emergence of most grasses was within 6-percent units of their germination in a petri dish with water; the exceptions were Swann forest bluegrass and Gatton panic which were 15 and 26-percent units lower respectively (Table 7.1).

Table 7.2. Predicted temperatures (°C) for maximum emergence (%), optimum range and 50% of maximum emergence of each tropical grass used in the study.

	Maximum emergence		Optimum temperature range (°C)	Temperature for 50% maximum emergence (°C)
	Temperature (°C)	Emergence (%)		
Bambatsi panic	30	70.3	24–35[1]	17
Bisset creeping bluegrass	19	40.4	16–22	13
Premier digit grass	25	43.0	21–32	14
Gatton panic	28	45.2	24–32	18
Megamax 059 panic	26	58.9	23–35[1]	17
Katambora Rhodes grass	35[1]	60.4	24–35[1]	12
Swann forest bluegrass	27	43.0	23–31	17

[1]35°C was the highest temperature tested



a)

Time (days)	10	15	20	25	30	35	40
14	0	24.5	60.5	65.5	70.5	62	58
11	0	1	54.5	63	67	61	55.5
8	0	0	36.5	55	61	58	55.5
6	0	0	7.5	11	38	53	37
4	0	0	0	0	0.5	43	18
0	0	0	0	0	0	0	0

b)

Time (days)	10	15	20	25	30	35	40
14	0	38	45	23	26.5	19	30.5
11	0	30	41.5	22.5	25	18	30
8	0	10.5	31.5	20.5	23	15	28.5
6	0	0	3	19	20.5	15	21.5
4	0	0	0	7.5	13.5	12	19
0	0	0	0	0	0	0	0

c)

Time (days)	10	15	20	25	30	35	40
14	0	28	44	42	39.5	33	31.5
11	0	10.5	42.5	40	36.5	28	30.5
8	0	0	30.5	31	31.5	28	29.5
6	0	0	0	10.5	19.5	20	19.5
4	0	0	0	0.5	4	15	9.5
0	0	0	0	0	0	0	0

d)

Time (days)	10	15	20	25	30	35	40
14	0	1	38.5	44	47.5	26	18.5
11	0	0	28.5	39.5	44	21	17.5
8	0	0	15	22.5	26.5	20	16.5
6	0	0	8	1	6.5	14	14
4	0	0	0	0	0.5	6	0
0	0	0	0	0	0	0	0

e)

Time (days)	10	15	20	25	30	35	40
14	27	45.5	48	54	53.5	58	61
11	0	40	46	53	50.5	55	61
8	0	32	44.5	51.5	49.5	55	60.5
6	0	0	0.5	48	46	55	57.5
4	0	0	0	35.5	35.5	52	54
0	0	0	0	0	0	0	0

f)

Time (days)	10	15	20	25	30	35	40
14	0	21	49	64.5	50	54	49
11	0	10.5	41	60.5	49.5	53	48
8	0	0	21.5	52	47.5	50	48
6	0	0	0.5	27.5	39	49	36.5
4	0	0	0	0.5	8	29	6.5
0	0	0	0	0	0	0	0

g)

Time (days)	10	15	20	25	30	35	40
14	0	7	39	44.5	43	29	44
11	0	1	31.5	39.5	31	24	41.5
8	0	0	13.5	27.5	27	20	35
6	0	0	0.5	4	10.5	18	18
4	0	0	0	0	0.5	14	5.5
0	0	0	0	0	0	0	0

Fig. 7.1. Surface plots of smoothed cumulative seedling emergence (%) at temperatures ranging 10–35°C over a 14 day period and average cumulative seedling emergence (%) at temperatures ranging 10–40°C of (a) Bambatsi panic, (b) Bisset creeping bluegrass, (c) Premier digit grass, (d) Gatton panic, (e) Katambora Rhodes grass, (f) Megamax 059 panic and (g) Swann forest bluegrass.

The temperature range tested in this study encompassed the optimum for all grasses except Katambora Rhodes grass, which had predicted optimum emergence at 35°C, also Bambatsi panic and Megamax 059 panic which had 35°C as the upper limit of the optimum temperature range. Katambora was also the only grass that had seedling emergence at 10°C; seedlings emerged in the final 3 days of assessment (i.e.no seedlings had emerged by day 11).

Optimal temperatures for emergence ranged from 19°C for Bisset creeping bluegrass to 35°C for Katambora Rhodes grass (Table 7.2). Bisset creeping bluegrass also had the narrowest range of optimal temperatures of 6°C from 16–22°C. Premier digit grass and Megamax 059 panic had similar optimal temperatures (25 and 26°C respectively), but different ranges; Premier digit grass had optimal temperatures of 21–32°C varying 11°C, while Megamax 059 panic had higher optimal temperatures (23–35°C) with similar range (12°C, Table 7.1). Gatton panic had a slightly higher optimum temperature (28°C) and narrower temperature range (24–31°C) spanning only 7°C compared with Megamax 059 panic. Bambatsi panic had optimum seedling emergence at 30°C and a wide temperature range that was limited by the temperatures tested, spanning 11°C (24–35°C). The optimum temperature range for emergence of Katambora Rhodes grass included 35°C, the highest temperature tested. The optimum temperature for emergence of Swann forest bluegrass was 27°C with a range of 23–31°C.

The predicted temperature at which 50% of maximum emergence occurred ranged from 12°C for Katambora Rhodes grass to 18°C for Gatton panic. Fifty-percent of maximum emergence occurred at low temperatures for Bisset creeping bluegrass and Premier digit grass (13 and 14°C respectively) while Bambatsi panic, Megamax 059 panic and Swann forest bluegrass had 50% maximum emergence at 17°C, similar to Gatton panic (18°C).

In general, the number of days to initial emergence declined as temperature increased; even at temperatures above the optimum for emergence. For example, 10% emergence of Megamax 059 panic seedlings occurred in almost half the time at 35°C compared to the optimum temperature (26°C; Fig. 7.1).

7.4 Discussion

Germination and seedling emergence in the field is affected by a multitude of micro-environmental variables (McDonald 2002a) and the resultant establishment success, or failure, is further influenced by many interrelated seed and seedbed biological and physical properties (Hopkinson 1993).This study shows that tropical grass species which have good adaptation to frost-prone, summer dominant rainfall regions vary in their capacity to emerge from soil at low temperatures and have different optimum temperatures and ranges for emergence.

Optimum temperature range for emergence of the grasses tested fell into three groups: low (<20°C), intermediate (20–30°C), and high (>30°C) optimum temperature for emergence.

7.4.1 Low optimum temperature

Bisset creeping bluegrass had the lowest optimum temperature range of the grasses tested and the only grass to have optimum <20°C; 17–22°C (range of 5°-units). These are the first reported data for this species. Cultivar Bisset is a combination of two accessions originating from higher altitudes in Nairobi, Kenya and Namanga, Tanzania, (Table 7.1; Cook *et al.* 2005).

7.4.2 Intermediate optimum temperature

Premier digit grass, Swann forest bluegrass and panic grass had optimum temperatures which were intermediate. The optimum temperature range for emergence of Premier digit grass determined in our study (21–32°C) was wider than the optimum for germination reported by Nichols *et al.* (2012b; 20–25°C), although the lower temperatures of both ranges were similar. Origin of cv. Premier is unknown, but thought to be Katombora, Zambia (Cook *et al.* 2005) (Table 7.1; Cook *et al.* 2005).

These are the first reported data for optimum temperatures for emergence of forest bluegrass. Swann forest bluegrass had an optimum temperature range of 23–31°C, similar to Premier digit grass and Gatton panic. It originated from an accession collected in the Guyana Highlands (4ºN), South America. *B. bladhii* ssp. *glabra* is a widely adapted species (Table 7.1; Cook *et al.* 2005).

The reported optimum temperature for germination of *M. maximus* at constant temperatures was 25–30°C (cv. Gatton; Nichols *et al.* 2012b) which was similar to cv. Gatton (24–32°C) in our study and a little narrower than cv. Megamax 059 (optimum temperature range 23–35°C). The variation between the two cultivars in our study was not large and could be related to their centre of origin or simply seed quality. Variation in germination responses for some crop species has been directed to their centre of origin, for example, grain legumes (Covell *et al.* 1986) and sunflower (Mwale *et al.* 1994). Cultivar Gatton originated from Zimbabwe (Cook *et al.* 2005) while seed of cv. Megamax 059 was collected from South Africa (CA Harris, pers. comm.).

7.4.3 High optimum temperature

Bambatsi panic and Katambora Rhodes grass had the highest optimum temperatures for emergence of the grasses tested. Optimum temperatures for germination have previously been reported for both of these species; those reported for Bambatsi panic were similar to those determined in our study (25–35°C; Watt and Whalley 1982) while Katambora Rhodes grass were different. Cultivar Bambatsi is a variable, but persistent and resilient cultivar and the only cultivar currently available in Australia (Cook *et al.* 2005; Table 7.1). Bambatsi panic had the widest optimum temperature range (13°-units) of the grasses tested in this study.

The optimum temperature for germination of *C. gayana* has been reported as 20–30°C (Watt and Whalley 1982) and 25–30°C (Nichols *et al.* 2012b); both lower than the optimum determined for emergence in our study. Similar to our study, Watt and Whalley (1982) reported germination at temperatures 10–40°C (nil at 5 and 45°C), with maximum occurring at 25°C, while in our study maximum emergence at 35°C the highest temperature assessed. The ability of Rhodes grass to emerge at a wide range of temperatures highlights the broad adaptation of the species. Rhodes is reported as having a wide geographical distribution and environmental tolerance (Cook *et al.* 2005).

Despite the recognised wide adaptation of Rhodes grass, the differences between the two previously reported temperatures for germination and our emergence data are somewhat surprising, but highlight the difference methodology can make on results. Nichols *et al.* (2012b) placed seeds in a water agar germination medium to provide a constant water potential. Watt and Whalley (1982) placed seed on germination pads which were watered regularly to ensure they remained moist. In contrast, our study conducted in soil, more closely resembled the environment from which seed emerges. Water potential was likely to have varied in our study and the study conducted by Watt and Whalley (1982), however, our study, conducted in soil was the most realistic of the three methods.

Emergence of seedlings of most of the grasses from soil at 25°C was lower than germination in a petri dish (alternating 30/20°C temperature with an average of 25°C) and experience is that emergence in the field is commonly less than germination in a petri dish. Seedling emergence in the field is affected by a range of soil biological, physical and chemical factors (e.g. pH, organic matter content, moisture content), also environmental conditions (e.g. temperature, humidity, light intensity), predation and attack by pests and diseases, and competition from other species (grasses, legumes and weeds) (e.g. Hegarty 1973; Watt and Whalley 1982; Hardegree and van Vactor 2000). There are many potential sources of variation in germination response to temperature; between cultivars of a species and between seed lots due to physiological age, vigour and viability of the seed (McDonald 2002a). In our study, seed of cv. Gatton used in our study had higher germination in a petri dish than emergence from soil (Table 7.1), however, total emergence (Table 7.2) was similar to cv. Megamax 059 clearly highlighting differences in methodology and variation that can occur between cultivars and/or seed lots. The seed lot of cv. Gatton used in our study was supplied by a seed company (harvest date unknown). While it was stored correctly for long term storage during the time we had it, it was at least 12 months older than the cv. Megamax 059 seed lot which was 18 months old at the time of this study. A single seed lot was used in this study due to limited resources, however, comparing multiple seed lots in studies such as this has merit as it will ensure that the results are robust and allow variation between seed lots to be estimated.

The temperature when 50% of maximum emergence occurred could be used to develop guidelines for minimum temperatures for sowing. At these temperatures a good level of seedling emergence and seedling vigour can be achieved. This information is potentially useful for determining 'earliest' or 'latest' sowing times in spring and autumn respectively. Of the grasses tested in this study the temperature to achieve 50% of maximum emergence varied 6°C (i.e. 12–18°C; Table 7.2). If used as a guide for spring sowing, soil and air temperatures would be rising toward the optimum range for emergence and increasing seedling vigour. Katambora could be sown the earliest, when average temperatures are 12°C and increasing. This again fits with its published wide adaptation (e.g. Cook *et al.* 2005). Bisset creeping bluegrass and Premier digit grass could also be sown early; when average temperatures are increasing and reach a consistent 13–14°C. In contrast, sowing the three panic grasses and Swann forest bluegrass would ideally be delayed until temperatures reach 16°C, with Bambatsi panic having the highest temperature, requiring 18°C. These temperatures are similar (±2°C) to those proposed by McDonald and Bowman (2002) who used the same method to provide recommendations for sowing temperature from results of the study conducted by Watt and Whalley (1982).

An alternative time to sow tropical grasses is potentially from late summer until early autumn since the emergence of several of these grasses has been reported as higher in autumn than spring (Lodge and Harden 2009). Maximum emergence of summer growing native grasses from seed, presumably on the soil surface, occurred in summer, however, recruitment was most successful from mid-summer to early autumn (Lodge 1981). A study using exotic tropical grass species, greater emergence occurred in autumn than spring and surface sowing was found to adversely affect seedling emergence compared to sowing at 10–25 mm depth (Lodge and Harden 2009). Although temperatures at the soil surface and 10 mm depth varied less than 1°C, the difference in emergence was suggested to be due to rapid drying of the soil surface resulting in seed not remaining moist for sufficient periods to germinate.

The maturity of plants at the onset of frosts is also an important consideration for sowing time as it affects the ability of some tropical grass species to overwinter and their production in subsequent years. Premier digit grass and Katambora Rhodes grass sown in March overwintered similarly to stands established in spring-summer (November and January), however, Bambatsi panic, Floren bluegrass (*Dichanthium aristatum* cv. Floren) and Swann forest bluegrass were adversely affected by sowing in March (Lodge *et al.* 2010a). These findings are better understood in light of our findings; Premier digit grass and Katambora Rhodes grass both had lower temperatures for 50% emergence and optimum range while Bambatsi panic and Swann forest bluegrass had higher temperature ranges for optimum emergence and 50% emergence. As soil and air temperatures are declining from late-summer to autumn, optimum temperatures would not have been maintained throughout establishment resulting in weaker seedlings less able to survive winter ensuing ongoing production losses for 2 or more years (Lodge *et al.* 2010a).

There is increasing interest in tropical grass pastures in more southern latitudes where rainfall has an even or winter dominant distribution and tropical grasses are currently not sown. The data from this study will be important to assist determining sowing time in spring and/or late-summer and autumn. For example, the opportunity to sow some tropical grass species earlier in spring as temperatures are rising while the probability of receiving adequate rainfall for establishment is still high could be advantageous.

Findings from this study provide insight into the potential competitive ability of the grass species and could be used the develop strategies to manipulate the competitiveness of the sown grasses with weeds species and companion species in mixes. For example, our findings help explain the competitiveness of Rhodes grass in mixes with other tropical grass species (Lodge *et al.* 2009) and lucerne in mixes with tropical grasses (Boschma *et al.* 2010a). They could also be useful to understand the optimal sowing time for establishment of tropical grasses in mixes with lucerne (*Medicago sativa*), a commonly sown temperate perennial legume in this region.

McDonald (2002a) reported that the differences between alternating and constant temperatures tend to be minor and of no biological importance when they were within the optimum range, but when daily fluctuations resulted in time spent at suboptimal temperatures (i.e. above or below optimum) differences were significant with total germination and the rate of germination being less than at a constant temperature with the same mean. Alternating temperatures can affect germination response compared to constant temperatures with three possible effects: stimulated germination of seed that would be dormant at a constant temperature; increased or decreased total germination; and increased or decreased rate of germination (e.g. Murdoch *et al.* 1989; McDonald 2002a), however, these differences are greatest at temperatures above and below the optimum (e.g. Garcia-Huidobro *et al.* 1982; Roundy and Biedenbender 1996; McDonald 2002a).

Ideally, this type of study includes temperatures that extend beyond the range that the grasses germinate. Previous germination studies involving tropical species have included temperatures ranging from 5°C (Lodge and Whalley 1981; Watt and Whalley 1982) to 42–45°C (e.g. Lodge and Whalley 1981; Watt and Whalley 1982). In our study an inability to cool the growth cabinets to temperatures below 10°C restricted our minimum temperature to 10°C. However, emergence of Katambora Rhodes grass at 10°C only occurred during the final 3 days indicating that the lower limit was close and 5°C would most likely have resulted in nil emergence, as was reported by Watt and Whalley (1982). Germination of a number of sown and Australian native and naturalised grasses have been reported at 40°C; the sown tropical grasses including Bambatsi panic, Rhodes grass, *Setaria incrassata* (purple pigeon grass) and *Cenchrus ciliaris* (buffel grass) (Watt and Whalley 1982). However, none of the grasses tested germinated at 45°C (Watt and Whalley 1982). Extending the range to include at least 45°C would be beneficial for germination and especially soil emergence studies involving tropical species.

7.5 Conclusion

In this study the optimum temperature for emergence of seven tropical grasses with known adaptation to frost-prone regions was determined. The species could be divided into three groups based on the temperature for optimum emergence: low (<20°C), intermediate (20–30°C), and high (>30°C). Temperature for 50% emergence ranged from 12–14°C for Katambora Rhodes grass, Bisset creeping blue and Premier digit to 17–18°C for the panic grasses, Swann forest bluegrass and Bambatsi panic. The findings of this study will be useful to guide development of sowing time recommendations of tropical grass pastures in current and new areas considering sowing tropical grass pastures and may also be useful to develop strategies based on sowing times to reduce competition with weed species and/or companion species.

8 Evaluating the establishment and production of tropical perennial grasses in Central West NSW

Y. Alemseged and W. J. Smith

8.1 Introduction

Central West NSW has a low-medium annual rainfall (400–600 mm) with a near even distribution across all seasons. During summer, temperatures are high and frosts are common during winter. Establishing tropical perennial grasses can be challenging in this environment and this has been experienced firsthand throughout the course of this project. In this lower rainfall region, as temperatures increase and become suitable for good emergence and growth, rainfall becomes less reliable and less effective due to higher evaporation rates resulting in prolonged periods of soil water deficit at the soil surface. A better understanding of seedling emergence and survival during the summer period will provide optimum times and management for sowing tropical pastures.

Nitrogen (N) is commonly limited in soils in the Central West of NSW which in turn limits plant growth (e.g. Bowman *et al.* 2005). Therefore to maintain high production, pastures need to either be fertilised with a N-based fertiliser or a reliable legume established. There is considerable data from fertiliser experiments and published studies that indicate that N application can significantly increase dry matter yield of tropical grasses (e.g. Lawrence *et al.* 2015; Boschma *et al.* 2014a).

During the conduct of this project, repeated failed attempts to establish tropical grasses (for example Chapter 4–6) have highlight gaps in our basic knowledge of tropical grass establishment and production in Central West NSW: what is the optimum sowing time for emergence and seedling establishment? Are tropical grasses responsive to N fertiliser? Can the tropical legume *Desmanthus virgatus* (desmanthus) be established in tropical grasses? To address these issues preliminary studies were conducted to better understand (i) the optimum sowing time for emergence of tropical grasses, (ii) seedling establishment and survival of five tropical grasses, (iii) productivity response to N fertiliser, and (iv) establishment of desmanthus in established grasses in Central West NSW.

8.2 Methodology

The studies were conducted at the Trangie Agricultural Research Centre located 6 km north-west of Trangie (31°59’45” S, 147°56’18” E, elevation 214 m above sea level) on a red Chromosol soil with pH_{Ca} 5.1 (0–0.1 m).

Rainfall and temperature were collected from an automatic weather station and weekly rainfall and average minimum and maximum temperatures during the experimental period are presented in Fig. 8.1.

8.2.1 Optimum sowing time for seedling emergence

Seedling emergence of five tropical grasses was determined at six sowing times. Each sowing time was a completely randomised block design with three replicates. The five tropical grasses were *Panicum coloratum* (Bambatsi panic) cv. Bambatsi; *Cenchrus ciliaris* (buffel grass) cv. Gayndah; *Chloris gayana* (Rhodes grass) cv. Katambora; *Digitaria eriantha* (digit grass) cv. Premier; and *Megathyrsus maximum* (panic grass) cv. Gatton. The six sowing times were October (17 October 2016) December (5 December 2016), February (17 February 2017), March (31 March 2017), May (15 May 2017) and June (26 June 2017). Soil (0–50 mm) from the experimental site was collected and heat treated (80^{0}C for 48 hrs) to sterilise and kill weed seeds. At each sowing time, three plastic trays

(350 x 300 x 50 mm deep) with drainage holes in the bottom lined with unbleached paper towel were filled with the treated soil and 50 seeds of each grass species sown in rows across the tray (i.e. 300 mm) at the depth of 10 mm (Plate 8.1). Trays were kept moist by watering 1–2 times a day each weekday for the time the trays were in the field (i.e. 21 days).

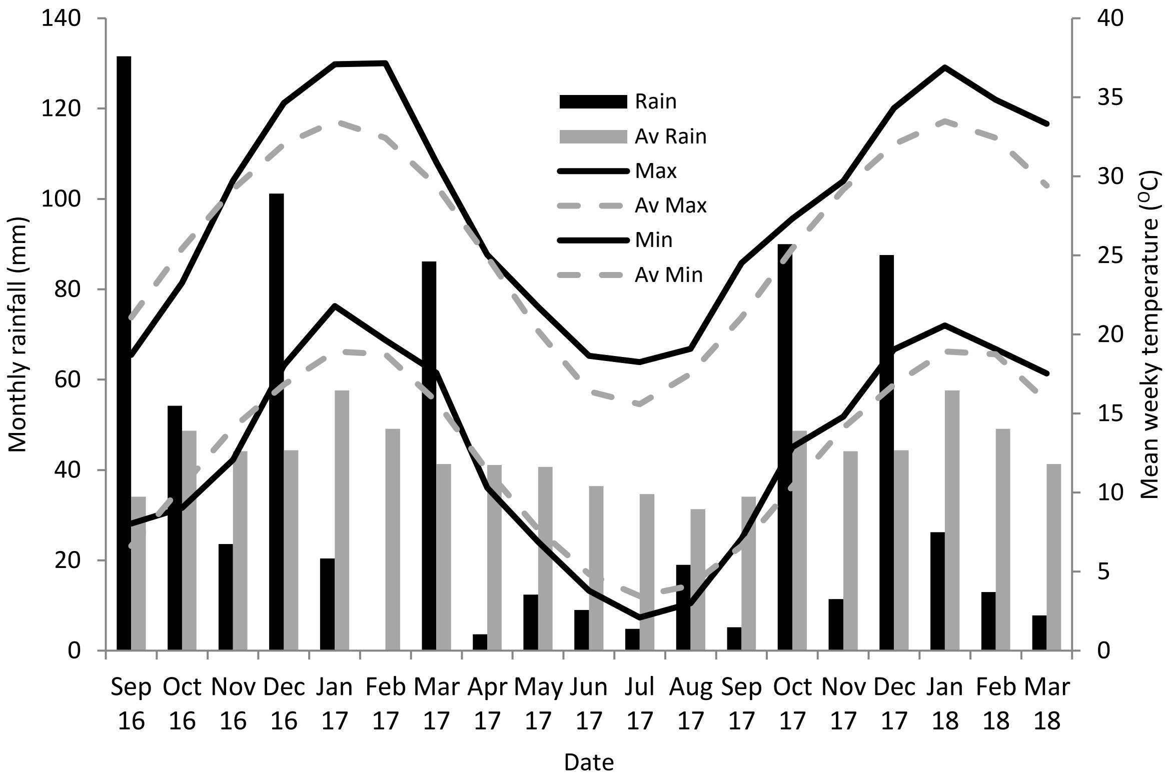


Fig. 8.1. Monthly rainfall (mm) and weekly mean maximum and mean minimum temperatures (^{0}C) during the experimental period (September 2016–March 2018) (black shade) and the long-term mean monthly rainfall and weekly mean maximum and mean minimum temperatures (light shade) of the site (Bureau of Meteorology Site 51049, 1922–2017).

All seeds except digit grass were coated and their pre-experimental germination percentages were determined under glasshouse conditions with temperature ranging from 27 to 29^{0}C (Table 8.1). The number of emerged seedlings were counted every second day for 21 days when the trays were removed.

Total number of seedling emergence data were analysed using linear mixed models of the form:

Count ~ Species + random(Rep)

Where Count = number of seedlings emerged; Species = grass species. The model was fitted separately for each sowing time, except June 2017 when no seedlings emerged. When species was significant (*P* = 0.05), least significant differences among treatment means were calculated.


Plate 8.1. Field emergence trial site.

8.2.2 Seedling establishment and survival

A series of experiments were conducted to evaluate the optimum sowing time for establishment and survival of five tropical grasses. Experiments were conducted in spring (October 2016), early-summer (mid-December 2016) and late-summer (late-February 2017) under irrigated and non-irrigated conditions. The sowing rates of each species are shown in Table 8.2. For each experiment, seeds were mixed with di-ammonium phosphate (18% N, 46% P_2O_5, 0% K_2O) fertiliser at the rate of 125 kg/ha and sown by hand broadcast onto a prepared seedbed in plots (4 x 4 m) arranged in a randomised complete block design with three replicates. Plots were lightly raked to cover the seeds to maximum of 10 mm and covered with sugar cane straw spread at the rate of 750 kg/ha over the plots to simulate stubble residue (Plate 8.2). Irrigation was provided using soaker hoses (Plate 8.3) with approximately 335 mm (over 43 day period), 250 mm (32 day period) and 110 mm (14 day period) of irrigation applied to the spring, early-summer and late-summer treatment plots respectively. Grass seedlings were counted using two 0.5 x 0.5 m quadrats eight weeks after sowing. Five plants from each plot were marked using fibre glass rods at the end of May 2017 and their survival assessed when growth recommenced in spring (September 2017) to determine winter survival.

Pasture growth was assessed using a calibrated score technique (described in Chapter 4) and botanical composition using the BOTANAL technique (Tothill *et al.* 1992). Seedlings from the late-summer sown treatment did not survive winter so there was no measurable pasture growth. Broadleaf weeds were controlled using the herbicide mixture triclopyr (300 g/L a.i.) and picloram (100 g/L a.i.) at 600 ml/ha on the 24 January 2017 for the spring sowing and the 7 February 2017 for the early-summer sowing.

Data were analysed using linear mixed models of the form:

$$Count \sim Species + random(Rep+Plot)$$

Where Count = number of plants established, Species = grass species. The model was fitted separately for each irrigation treatment (i.e. irrigated and not irrigated) and sowing time (spring, early-summer and late-summer) combination. When Species treatment was significant (P = 0.05), least significant differences among treatment means were calculated.

Plate 8.2. Experimental plots at the time of spring sowing with light cover of sugar cane straw.

Plate 8.3. Irrigating experimental plots.

8.2.3 Productivity response to nitrogen fertiliser

Two experiments were conducted to test productivity response of five established tropical grass species to N fertiliser under dryland and irrigation in 2018. Each experiment was conducted on five established grass species sown in spring 2016 in a randomised complete block design with three replicates. The grasses sown in 4 x 4 m plots were: Bambatsi panic cv. Bambatsi, buffel grass cv. Gayndah, Rhodes grass cv. Katambora, digit grass cv. Premier and panic grass cv. Gatton.

In December 2017, the experiments were mown to 50 mm height and the herbage removed. Half of each plot (i.e. 2 x 4 m) was fertilised with either nil (-) or 100 kg/ha N (+) in a strip plot design. The irrigated experiment received about 20 mm/week for 13 weeks using soaker hoses.

Herbage production and botanical composition were assessed on 13 March 2018 using the same methodology outlined above. The proportion (%) of green and dead was also estimated.

Sown grass species contributed almost 100% of the total herbage mass and was the only BOTANAL category that was statistically analysed. Sown species herbage mass was analysed using ANOVA with species, fertiliser and their interaction as explanatory factors. Rep, whole plots of fertiliser and whole plots of species were included as blocking terms. The model was fitted separately for the irrigated and non-irrigated experiments. When treatments were significant (P = 0.05), least significant differences among treatment means were calculated. Percentage green of the herbage was greater than 95% with little variation between species or between treatments and therefore the data were not analysed.

8.2.4 Establishment and production of desmanthus in established tropical grasses

Two experiments were conducted during the 2017–18 growing season to test establishment and productivity of the tropical legume desmanthus cv. Marc in five established tropical grass species under dryland and irrigated conditions.

Five grasses (Bambatsi panic cv. Bambatsi, buffel grass cv. Gayndah, Rhodes grass cv. Katambora, digit grass cv. Premier and panic grass cv. Gatton) were established into plots 4 x 4 m in a randomised complete block design with three replicates in December 2016. On 19 October 2017, the plots were mowed to 50 mm height and halved (i.e. 2 x 4 m) to form a strip plot design with subplots consisting of treatments: desmanthus and nil desmanthus. In the desmanthus plots, the soil was disturbed (using hand held hoe) and desmanthus seed broadcast at the rate of 8 kg/ha. The experiment was further mowed on 16 January 2018 to encourage desmanthus establishment. The irrigated plots were watered using soaker hoses at the average rate of 20 mm/week for 13 weeks (December 2017–March 2018). Herbage production was assessed using the same methodology outlined above on 13 March 2018. Botanical composition was assessed in the categories sown tropical grass, desmanthus, other grasses and other broadleaf weeds. The proportion of green and dead was also estimated for each category.

Grass species contributed almost 100% of the total herbage mass and was the only BOTANAL category that was statistically analysed. Sown grass species herbage mass was analysed using ANOVA with grass species, desmanthus treatment and their interaction as explanatory factors. Percent green was greater than 95% with little variation between species or between treatments and therefore the data were not analysed. Rep, whole plots of species and whole plots of desmanthus treatment were included as blocking terms. The model was fitted separately for the irrigation and non-irrigation experiments. When treatments were significant ($P = 0.05$), least significant differences among treatment means were calculated.

8.3 Results

8.3.1 Optimum sowing time for seedling emergence

The proportions of seeds which emerged at each sowing time are shown in Table 8.1. Generally emergence was highest in October and lowest in May and June for all species. Emergences of Bambatsi panic, Rhodes grass and digit grass were closely related to their glasshouse germination rates especially in the October sowing (Table 8.1). While the glasshouse germination rate of buffel grass was very low (4%), its emergence percentage was one of the highest at 42%. On the other hand panic grass had relatively high germination rate but relatively low emergence percentage. It is worth noting that Rhodes grass showed consistently high emergence percentage in all sowing times except February and June when the emergence of all the species were low. In June no seedlings had emerged after 21 days.

8.3.2 Seedling establishment and survival

The establishment plant densities of the five tropical species sown during spring, early-summer and late-summer under irrigation and dry conditions are presented in Table 8.2. The monthly rainfall during the experimental period was close to or below the long-term average (Fig. 8.1).

Table 8.1. Glasshouse germination rate (%) and field emergence percentage of the five tropical grasses. Values within a column followed by different letters are significantly different (*P* = 0.05).

Species	Germination (%)	Emergence (%)					
		October	December	February	March	May	June
Bambatsi	65	50 b	44 c	12 a	12b	2 a	0
Buffel	4	42 b	12 ab	18 a	6 ab	6 a	0
Rhodes	52	48 b	30 bc	6 a	26 c	26 b	0
Panic	37	16 a	4 a	8 a	0 a	2 a	0
Digit	15	14 a	2 a	0 a	2 a	2 a	0

Table 8.2. Established plant densities (plants/m^2) of the five tropical grasses sown in spring, early-summer and late-summer with and without irrigation. Values within a column followed by different letters are significantly different (*P* = 0.05).

Species	Sowing rate (kg/ha)	Spring sowing		Early-summer sowing		Late-summer sowing	
		Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated
Bambatsi panic	8	29 a	40 a	19 a	30 a	12 a	29 a
Buffel grass	12	5 b	14 c	6 a	14 bc	2 b	5 b
Rhodes grass	8	14 b	25 b	15 a	20 bc	7 a	13 c
Panic grass	12	28 a	29 b	25 a	22 b	14 a	18 c
Digit grass	12	11 b	22 b	11 a	11 c	11 a	15 c
Mean		*17.4*	*26.0*	*15.2*	*19.4*	*9.2*	*16.0*

When sown in spring without irrigation, seedling densities of Bambatsi panic and panic grass were significantly higher than the other grasses ($P < 0.05$). When plots were irrigated Bambatsi panic had significantly higher plant density ($P < 0.05$) than all the other species. There were no differences between Rhodes grass, panic grass and digit grass while buffel grass had the lowest establishment density ($P < 0.05$). Irrigated plots showed higher establishment density than non-irrigated plots in all species except panic grass.

Both the irrigated and non-irrigated plots were infested with the broadleaf weed common heliotrope (*Heliotropium europaeum*) but there was no grass weed emergence during spring sowing. On the other hand the main weeds during the early- and late-summer sowing were summer grass weeds such as barnyard grass (*Echinochloa* spp.) and liverseed grass (*Urochloa panicoides*).

There were no differences between grasses sown without irrigation in early-summer with plant densities ranging 6–25 plants/m^2 ($P > 0.05$). The lack of significance was due to high variability between replicates. Although plots did establish, there was no growth and after five months most were small (Plate 8.4). Under irrigation, Bambatsi panic had significantly higher establishment density than all of the other species (30 plants/m^2; $P < 0.05$), while digit grass had the lowest (11 plants/m^2). There were no significant differences between buffel grass, Rhodes grass and panic grasses or between buffel grass, Rhodes grass and digit grass. All seedlings from the spring and early-summer sowing (both irrigated and non-irrigated) that were marked after 8 weeks of sowing survived until the following spring.

Generally, late-summer sowing resulted in lower establishment plant densities than both spring and early-summer sowing. Without irrigation, buffel grass had significantly lower establishment density than all of the other species (2 plants/m^2) but there was no significant difference in establishment densities between all of the other species. Under irrigation, Bambatsi panic established at a significantly higher plant density than all species while buffel grass established at a significantly lower density (29 and 5 plants/m^2 respectively). There was no significant difference between the other three grasses. However, less than 20% plants survived the first winter and there was no measurable pasture growth by the following summer.

Plate 8.4. Early-summer sown *Panicum coloratum* (Bambatsi panic) plants five months after sowing.

Three months pasture growth (kg DM/ha) of the five species in the year after establishment is shown in Table 8.3. Overall spring sowing resulted in higher pasture growth than early-summer sowing. Pasture growth was also understandably higher under irrigation than under non-irrigation, except panic grass in spring sowing and buffel grass and Rhodes grass in early-summer sowing where the differences did not appear to be significant.

Table 8.3. Three month pasture growth (kg DM/ha) of five tropical grasses established in either spring or early-summer under irrigation and non-irrigation conditions. Values within a column followed by different letters are significantly different (P = 0.05).

Species	Spring sowing		Early-summer sowing	
	Non-irrigated	Irrigated	Non-irrigated	Irrigated
Bambatsi panic	3095 b	4969 b	2118 ab	3378 b
Buffel grass	1613 a	3159 a	2330 ab	2171 a
Digit grass	3463 b	4635 b	2075 ab	3474 b
Panic grass	2901 b	3801 ab	1498 a	2978 ab
Rhodes grass	2784 ab	4145 ab	2861 b	3417b

1.3.2. Productivity response to nitrogen fertiliser

Under dry (non-irrigated) conditions, there was no effect of N fertiliser, grass species or interaction. Herbage mass ranged from 709 (digit grass) to 1634 kg DM/ha (Bambatsi panic) (Table 8.4).
Under irrigation, Rhodes grass, panic grass and Bambatsi panic had the highest herbage mass (P < 0.05), followed by digit grass. Buffel grass had the lowest herbage mass (P < 0.05). Nitrogen had positive effect on herbage mass of all the grasses but the differences were not statistically significant (Table 8.4).

Table 8.4. Tropical grass herbage mass (kg DM/ha) averaged over fertiliser treatment in the non-irrigated and irrigated experiments. Values within an experiment not having a letter in common are significantly different (*P* = 0.05).

Species	Non-irrigated	Irrigated
Bambatsi panic	1634	6189 c
Buffel grass	1100	3552 a
Digit grass	709	5298 b
Panic grass	1612	6243 c
Rhodes grass	1241	6359 c

8.3.3 Establishment and production of desmanthus in established tropical grasses

Desmanthus cv. Marc failed to establish even when irrigation was applied and therefore it had no impact on herbage mass.

Again Bambatsi panic and panic grass were the most productive of all the grasses under non-irrigation conditions (Table 8.5) but differences were not significant. When irrigated, digit grass, Rhodes grass and Bambatsi panic were the most productive ($P < 0.05$), while buffel grass was the least productive ($P < 0.05$).

Table 8.5. Herbage mass (kg DM/ha) of five tropical grasses (main effect) under non-irrigated and irrigated conditions. Values within a column not having a letter in common are significantly different (*P* = 0.05).

Species	Non-irrigated	Irrigated
Bambatsi panic	1256	5555 bc
Buffel grass	890	3540 a
Digit grass	656	5842 c
Panic grass	1129	4499 ab
Rhodes grass	930	5691 bc

8.4 Discussion

Experiments sought to study the seed germination and emergence followed by seedling growth and survival of tropical grasses under the conditions of the Central West of NSW. The persistence of seedlings over the first winter and growth the following spring was monitored. Bambatsi panic, Rhodes grass and buffel grass had better emergence when sown in October and November than in February–June. Since soil was kept moist throughout the experimental period in the establishment experiment (i.e. 21 days), any difference would probably be due to soil temperature. The average weekly temperature during the October, November and February experimental periods were within the range described as suitable for tropical grass germination (Lodge and Harden 2009). However, there were three consecutive days with maximum temperatures over 44^{0}C just after sowing in February that may have affected germination. Lodge and Harden (2009) reported that intermittent high daily temperatures negatively affected seed emergence of tropical grasses in northern New South Wales. In this experiment Rhodes grass seedling emergence was better in the cooler autumn period than all the other species suggesting wider sowing 'window' and therefore flexibility in sowing time in the Central West of NSW. In a study to determine the optimum temperature for tropical grass seedling emergence, Egan *et al.* (2017), found that Rhodes grass to be the only species to emerge at 10^{0}C. However, Lodge *et al.* (2010) indicated poor performance of tropical grass seedlings in the following spring that emerged from an autumn sowing.

The poor establishment of digit grass in all sowing times maybe due to seed quality as the seed used was from old stock. The reason for the equally poor performance of panic grass was not clear at least during the ideal temperature regime for the species during the October and November periods.

Although germination percentages >35% are considered acceptable for tropical grasses (Moore *et al.* 2013), only three of the five species (Bambatsi panic, Rhodes and panic grass) satisfied this recommendation. On the other hand while only 4% buffel grass seed did germinate in the glasshouse, about 40% of seedlings emerged in the field studies, albeit only during the October sowing period (Table 8.1). This indicates that dormancy may have been broken down immediately under field conditions experienced when sown at this time.

As expected establishment densities of all species were higher under irrigation than under non-irrigation conditions except panic grass which had similar establishment densities under irrigation and non-irrigation in both the spring and early-summer sowing periods. The early-summer establishment densities of digit grass were also identical under irrigation and non-irrigation. However, the similarities in established densities were not reflected in herbage mass production as there were marked differences between irrigated and non-irrigated pastures of both species indicating herbage mass production is more influenced by soil moisture than by plant density. This indicates establishment of panic grass and digit grass are less affected by moisture stress than the other species.

The observed differences in establishment between irrigated and non-irrigated plots was probably due to failure to germinate and/or seedling death right after germination as a result of moisture stress. Under dry conditions, the best time to sow Bambatsi panic and panic grasses was in spring followed by early-summer. The observed rate of establishment for both species in spring of almost 30 plants/m^2 is considered more than the desirable benchmark (Moore *et al.* 2013), although research in northern NSW conducted during this project indicated that an established plant density of 4–9 plants/m^2 of digit grass is optimum for herbage production and water use efficiency (Chapter 10). The early-summer establishment density of about 20 plants/m^2 is also adequate although seedling vigour and growth were low resulting in small plants to endure winter. Even when sowed in autumn, establishment rates were between 10 and 15 plants/m^2 for both species; however very few seedlings survived the first winter. With over 10 plants/m^2, digit grass and Rhodes grass also showed acceptable establishment rate for tropical grasses without irrigation. On the other hand buffel grass showed establishment rate of well below the 10 plants/m^2 which is considered the minimum number for a good stand (Lodge and McCormick 2010).

While there was a positive relationship between emergence percentages and established densities for Bambatsi panic and to some extent for Rhodes grass (during the October sowing period) under non-irrigation conditions, no such relationships were evident for buffel grass and panic grass. Despite having relatively high emergence of 42%, buffel grass' established plant density was only 5 plants/m^2. On the other hand panic grass had only 16% emergence but the established plant density was 28 plants/m^2 at the same sowing time. This agrees with the notion put forward by Hopkinson (1993) that states "while failure to emerge guarantees failure to establish, good emergence does not guarantee establishment". However, these results should be treated with caution as there could have been seedling deaths between the times of emergence and when establishment count was undertaken.

Almost all seedlings from the spring sowing survived the extended hot dry weather that occurred during January and February (Fig. 8.1) but growth was slow. However, plants responded well to the over 80 mm rainfall that fell mid-March and growth was robust. The survival rates of established seedlings of the early-summer sown grasses were also high as no deaths were recorded. However, seedlings remained small and had lower response to rainfall than the spring sowing in the first year. Again Bambatsi panic and panic grass were the two better performing species under irrigation as they were under dry conditions.

Competition from weeds can pose a serious challenge during the early stage of seedling development by reducing moisture and nutrient availability and shading. In the current study, broadleaf and grass weeds emerged in large numbers during the spring and early-summer sowing times respectively. In spring complete weed control was achieved mainly because the weeds were broadleaf and successfully sprayed out early. On the other hand it was hard to control grass weeds that emerged during summer therefore pre-sowing grass weed control might be essential as suggested by Boschma and McCormick (2008) and Lodge *et al.* (2010). However, the extent to which weeds contributed to the lower establishment at these times is not clear.

Generally spring sowing resulted in higher pasture growth than early-summer sowing reflecting the better growing conditions that prevailed in spring. Pasture growth was higher under irrigation than under non-irrigation, possibly as a result of better establishment of irrigated treatments, in all species except for panic grass in the spring sowing and for buffel grass and Rhodes grass in the early-summer sowing where the difference did not appear to be significant.

Fertiliser did not have impact on herbage mass under dry conditions. The amount of rainfall received during the three months between fertiliser application and herbage mass assessment (16 December 2017 to 13 March 2018) was 62 mm, often in small amounts each time. There were also several days with temperatures well over 40^0C that might have also had a negative effect. Bambatsi panic and panic grass seem to handle these conditions better than the other species. Poor growth response to N during periods of low rainfall was also noted by Boschma *et al.* (2014a, 2016). In this low rainfall environment, periods without rainfall can be lengthy, so regular high inputs of N fertiliser may not be economic and persistent legumes may be a better option. These legumes may include barrel medic woolly pod vetch and biserrula (Chapter 4).

Poor establishment is the most common reason for failure of pasture legumes over-sown into existing grass pastures (e.g. Peck *et al.* 2011). Desmanthus has shown potential in co-located studies (Chapter 16), but did not establish in this experiment. Accumulation of soil water and reducing competition is important for legume establishment. In an established pasture this could possibly be achieved by removing the pasture in bands and maintaining these weed free. This approach has been tested in Southern Queensland (Peck *et al.* 2011) and requires further testing in the Central West of NSW.

Irrigation is unlikely to be a practical option for commercial enterprises in the Central West of NSW, however, including irrigation treatment in this study showed the potential of tropical grasses in 'good seasons'.

8.5 Conclusions

Based on field percent emergence as well as establishment and survival, Bambatsi panic, panic grass and Rhodes grass performed well under the climatic conditions at the time without irrigation, especially when sown in spring. Since the rainfall during the experimental period was close to or below the long-term average, these three species could prove to be viable resource for the region. However, more evaluation of their performance might be needed before firm recommendations are made.

Soil fertility is essential for grass-based pastures both for production and pasture quality. However, applying fertiliser in an environment where rainfall is not reliable may not be commercially feasible; therefore research to reliably establish legumes into existing grass pastures is needed.

9 The impact of sowing time and depth on establishment of *Desmanthus virgatus* in Northern Inland NSW

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9.1 Introduction

Sown pastures that support the grazing industries of Northern Inland New South Wales (NSW) have traditionally been based on temperate perennial and annual species, but over the past 10–15 years tropical species particularly tropical perennial grasses have replaced temperate species in much of this region (Harris *et al.* 2014). The main driver for this change has been the variable climatic conditions experienced in this region over that time e.g. dry autumn and spring periods (Lodge and McCormick 2010). Tropical perennial grass pastures have been able to respond to summer rainfall and produce large quantities of herbage in years which have been characterised by highly variable seasons (Boschma *et al.* 2009) and long dry periods with predominantly dry autumns (Lodge and McCormick 2010). This widespread adoption of tropical perennial grasses has been facilitated by the development of an agronomy management package to guide establishment, use and management (Harris *et al.* 2014). Unlike the tropical perennial grasses, tropical legumes are a relatively new suite of species for Northern Inland NSW and the factors impacting on the agronomy of these species are not well understood.

Desmanthus (*Desmanthus virgatus*) is a short, erect legume that is adapted to deep, alkaline, clay and clay loam soils (Cook *et al.* 2005; Lloyd *et al.* 2007a). Desmanthus has a number of characteristics that make it suitable as a companion legume for tropical perennial grasses including; a persistent summer growth habit, drought tolerance, rapid growth in response to rain and low bloat risk to livestock (Moylan and Crocker 2000; Pengelly and Conway 2000). In Southern Queensland, a climate similar to Northern Inland NSW, desmanthus is an important component of pastures (Peck *et al.* 2012). Evaluations by Moylan and Crocker (2000) and Boschma *et al.* (2012, Chapter 5) have highlighted the potential of desmanthus and with recommendations that it warrants more detailed investigation in this environment. While desmanthus has performed well in field experiments in Northern Inland NSW, it has yet to realise its full commercial potential on-farm. This is in part due to a lack of agronomic packages for the successful establishment and management for enhanced productivity and persistence in this region.

When introducing a new species option to a region successful establishment is vital for widespread adoption and use of that species in pasture systems. Reliable and rapid establishment achieved as cheaply as possible provides the best approach for enhanced pasture production (Gramshaw *et al.* 1993). Establishment is perceived as risky and failures are costly, which may impede the adoption of the new species.

Soil moisture and temperature are two important factors determining the success of establishment. Agronomic practices aim to improve moisture conditions for the germinating seedling by extending the time the seed is in contact with moist soil by modifying sowing time and depth. Temperature is an important factor influencing seed development, the proportion of seeds that germinate, the rate of germination and subsequent establishment (Gramshaw *et al.*1993). In studies by McDonald (2002b), the optimal range for desmanthus germination was found to be 24–28°C, with significant decreases in germination at 16°C and temperatures above 40°C. McDonald (2002a, b) concluded that total germination was not affected by alternating temperature, however, increasing amplitude of fluctuation in temperature decreased the rate of germination. At 8^{0}C amplitude, desmanthus took nearly twice as long to reach 50% germination compared to constant temperature. It is not necessarily the fluctuations in temperature that affects germination of desmanthus, but rather the time spent outside the optimum temperature range that reduces the rate of germination (McDonald 2002b).

For Northern Inland NSW the temperatures from October to March fall within the required optimal range for germination of desmanthus. These months coincide with the optimum temperature regime for sowing tropical perennial grasses and with summer rainfall that maximises the chance of receiving adequate rainfall for germination (Lodge and Harden 2009, Harris *et al.* 2014). However, the spring–autumn period in this region over is characterised by high-intensity summer thunderstorms, high temperature and high evapotranspiration (Murphy *et al.* 2004b) which make establishing a pasture challenging.

Most pasture seeds are small and have an optimum planting depth of 10–20 mm (Cook *et al.* 1987; Lodge and Harden 2009). Brandon and Jones (1998) found that the best emergence of desmanthus on a clay soil occurred at a sowing depth of 5–10 mm, however, emergence of tropical legumes can vary with soil type. Surface sowing tropical legume seed was not successful unless the seed were pushed into the surface of the soil to increase the seed-soil contact hence increase the area of seed in contact with soil moisture (Brandon and Jones 1998; Cook *et al.* 1992).

No field experiments have been conducted to determine appropriate agronomic practices required for the successful establishment of desmanthus in Northern Inland NSW. The series of field and glasshouse experiments outlined in this chapter investigate the optimum time and depth to sow desmanthus in Northern Inland NSW and how they complement sowing time and depth for tropical grasses. The hypotheses were that time of sowing (month of the year) and sowing depth would have significant impact on the seedling emergence and of desmanthus.

The experiments conducted in this study were:

1. The effect of temperature at sowing and sowing depth on the establishment of desmanthus under field conditions.
2. The effect of temperature at sowing on the establishment of desmanthus under field conditions.
3. The effect of temperature at sowing and sowing depth on the establishment of desmanthus under glasshouse conditions.

9.2 Methodology

9.2.1 Field site

The experimental site was located at Bingara (29^{0}42'39"S, 150^{0}27'07'E, elevation 297 m) (Chapter 4, Fig. 2.1) on a brown Chromosol soil (Isbell 1996) in the North West Slopes of NSW. The annual average rainfall at this site is 743 mm. At this site soil was sampled (soil cores 0–10 and 10–20 cm) and analysed for pH (in both water and $CaCl_2$), organic carbon (Walkley and Black), nitrate nitrogen (N), sulphate sulfur (S, extracted in 1 M KCl at 40°C), phosphorus (P, Colwell), potassium (K, ammonium acetate), also cations and anions and electric conductivity at commercial NATA accredited laboratories (Chapter 4, Table 4.1).

During the winter of 2011 and 2012 the area was sown to forage oat and left as a fallow over the summer period. Summer weeds were controlled with 1.5 L/ha glyphosate (450 g/L a.i.). The areas used for Experiment 1 and Experiment 2 were left fallow in subsequent years (weeds controlled with 1 .5 L/ha glyphosate (450 g/L a.i.) until 2–3 months prior to the commencement of each experiment. Rainfall data were recorded in a manual rain gauge located near the experimental site. Long-term average monthly and annual rainfall data was sourced from the Bingara Bureau of Meteorology (BOM) site (054004; 1878–1997). Maximum and minimum ambient temperature data were sourced from the BOM at Moree (053115; 1995–2018).

9.2.2 Experiment 1: Effect of temperature at sowing and sowing depth on the establishment of *desmanthus* under field conditions

The experiment was a split-plot design with the two sowing depths [surface, <5 mm deep (Sd0) and shallow, 15 mm deep (Sd15)] as the whole plot treatments. The six sowing times (October, November, December, January, February and March) were the sub-plot treatments. The experiment treatments were replicated three times. The whole plots were 9 x 6 m and the sub-plots 1.5 x 6 m each.

Desmanthus cv. Marc was sown as a monoculture. Seed that had not been scarified was sourced from Progressive Seeds and this seed was scarified by brief immersion (5–10 seconds in boiling water (Hopkinson and English 2004). A germination test was conducted and seeding rate adjusted to approximate 2 kg/ha viable seed. The seed was inoculated with the recommended strain of rhizobia (CB3126) the day of sowing.

In August 2014 the experimental area was cultivated with a rotary hoe and just prior to sowing the area was levelled with pasture harrows. The first sowing time treatment (October) was sown on 29 October 2014 and subsequent sowing time treatments were sown every 3–4 weeks thereafter. The last sowing time treatment (March) was sown on 24 March 2015. The plots were sown with a cone seeder into six rows approximately 0.25 m apart. Seeds sown into plots in the Sd0 blocks were dropped onto the soil surface and lightly raked. Seeds sown in the plots in the Sd15 blocks were sown to a depth of 15 mm and lightly raked. If required plots sown November–March were hand raked to level the soil surface prior to sowing. Plots were watered every four days for the two weeks following sowing (except for the October sown plots which were watered every second day) to eliminate moisture as the limiting factor affecting establishment, at each watering approximately 10–12 mm water per plot was applied.

No fertilisers were applied at sowing or in the establishment year, but in the following growing season (2015–16) 200 kg/ha single superphosphate (8.8% P, 11% S) was applied in early October. Grass weeds were controlled with haloxyfop (520 g/L a.i.) at 100 ml/ha in August 2014 and 2015. Broadleaf weeds were rogued by hand.

Emerging seedlings of desmanthus were counted (seedlings/m^2) approximately four weeks after sowing in three fixed quadrats (0.50 x 0.10 m), avoiding the outer rows in each plot. This count was repeated approximately 12 weeks after sowing and 10 seedlings per quadrat (or all seedlings if less than 10 present) were tagged to monitor survival in the following growing season. The survival of these tagged seedlings was assessed in October 2015. A plant was deemed to have survived if there was green leaf present. Desmanthus herbage mass was assessed two times in the 2015–16 growing season. Total herbage mass and the proportion of desmanthus were visually assessed using a continuous 0–5 scale (0, nil; 5, high). Twenty calibration quadrats (0.3 x 0.3 m) representing the range of herbage mass at a site (with five quadrats each selected to represent the upper and lower range of herbage mass) were also scored. These quadrats were harvested to approximately 10 mm above ground level, sorted into the two components (desmanthus and other), dried at 80^oC for 48 h before weighed. Herbage mass scores and percentage estimates were regressed (linear or quadratic R^2>0.80) against actual herbage mass (kg DM/ha) and percentage of the desmanthus to determine the legume herbage mass. The plots were mown with a sickle-bar mower and the herbage removed from the plots.

9.2.3 Experiment 2: Effect of temperature at sowing on the establishment of desmanthus under field conditions

As a result of unseasonal hot and dry conditions in October and November 2014 which affected the spring sowing treatments in Experiment 1 an additional experiment was sown in 2015–16.

The experiment was a randomised complete block design with six sowing times (November, December, early-January, late-January, February and March. The experiment treatments were replicated four times. The plots were 1.5 x 2 m.

Desmanthus cv. Marc was sown as a monoculture. Similar to experiment 1, seed was scarified by brief immersion in boiling water and a germination test conducted. Seeding rate was adjusted to approximate 2 kg/ha viable seed. The seed was inoculated with the recommended strain of rhizobia (CB3126) the day of sowing.

In August 2015 the experimental area was cultivated with a rotary hoe, and prior to the first sowing in November the area was levelled with pasture harrows. The first sowing time treatment (November) was sown on 12 November 2015 and subsequent sowing time treatments were approximately every four weeks (except for the two January sowings which were 2 weeks apart) with the last sowing time treatment (March) sown on 11 March 2016. The plots were sown by hand into six shallow furrows (about 0.10 m) approximately 0.25 m apart (each row comprised 40 seeds sown approximately 0.05 m apart) and lightly raked to cover the seed. If required plots sown December–March were hand raked to level the soil surface prior to sowing. Newly sown plots were watered with approximately 10 mm every 3–4 days for the two weeks following sowing to eliminate moisture as the limiting factor affecting establishment.

No fertilisers were applied the year of sowing, but in the following growing season (2016–17) 200 kg/ha single superphosphate (8.8% P, 11% S) was applied during in early October. Grass weeds were controlled with haloxyfop (520 g/L a.i.) at 100 ml/ha in August 2015 and 2016 and broadleaf weeds were rogued by hand.

Using the same methods as described in experiment 1, emerging seedlings of desmanthus were counted (seedlings/m^2) four weeks and 12 weeks after sowing; 10 seedlings were tagged and survival assessed in October 2016. Desmanthus herbage mass was assessed once in January 2017.

9.2.4 Experiment 3: Effect of temperature at sowing and sowing depth on the establishment of desmanthus under glasshouse conditions

A glasshouse experiment was conducted at the University of New England, Armidale NSW in 2015. The experiment consisted of three day-night (12 day hours, 12 night hours) temperature regimes (20–15^0C, 28–20^0C and 30–25^0C). These temperatures were selected to represent the expected range of temperatures experienced at the Bingara field site from early spring to late summer. In each temperature range six sowing depths (0, 5, 10, 15, 25 and 30 mm) were replicated four times giving an overall sample size of 72. The six sowing depths were chosen to represent a range of depths that can be achieved with conventional seeding methods.

The seed were sown in seedling trays (350 x 280 x 50 mm) that contained a seedling mix of three parts potting mix and one part sand. Trays were lined with absorbent paper to prevent soil loss and filled to the rim. Uninoculated scarified seed of desmanthus cv. Marc was sown at a density of 50 seeds per tray. The position of the 50 seeds were marked using a template board and a depth indicator used to make holes to the required depth. Individual seeds were placed into each hole and covered with adequate soil. Twenty four trays were placed in each of the glasshouse compartments at three day-night temperature regimes.

Each tray was hand watered twice a day until the soil surface was visibly saturated; preventing moisture from being a limiting factor. Trays which had surface sown seeds (0 mm) were watered by placing them in a tray of water allowing for the diffusion of water. Watering in this manner prevented seed from being moved or buried.
To ensure nutrients were not limiting, the plants were given a basal application of 800 ml per tray of liquid fertiliser (N:P:K 12.4:2.7:6.2 at 1.7 g/L) every 2 weeks.

The plants were assessed for rate of germination and total germination over a 56 day period. At the conclusion of the 8 weeks each plant was harvested. Measurements of shoot and root length were taken immediately after removing from the trays; then each plant was separated into shoots and roots by cutting them at the hypocotyl. The samples were oven-dried for 48 hours at a constant 60°C and then weighed.

9.2.5 Statistical analysis

9.2.5.1 Experiment 1 and Experiment 2

Seedling emergence (seedlings/m^2), seedling survival (%) and herbage mass (kg DM/ha) were analysed by ANOVA with sowing month and sowing depth as the explanatory factor and replicate as a block term. Plots of the residuals showed no transformation of the data was necessary. Where the main explanatory effect was significant, means were compared with Fishers's LSD t tests ($P = 0.05$).

7.2.5.2 Experiment 3

Total seedling emergence (number of seedlings per tray) and plant traits (shoot length, shoot weight, root length and root weight) at each temperature regime were analysed using ANOVA with sowing depth as the explanatory factor and replicate as a block term. Following the determination of the significant differences in the means of both the sowing depth factor, a Tukey test was completed to obtain at which level there was a significant difference ($P < 0.05$) in means in each of the factors. Data for the three day–night temperature regimes were not analysed as there was no true replication; notable data are presented in the results section.

9.3 Results

9.3.1 Rainfall and temperature at field site

At the Bingara field site over the two experimental periods (October 2014–March 2017) below average rainfall was experienced in 53% of the months (Fig. 9.1). Dry periods of particular note were the winter preceding the commencement of Experiment 1 (89 mm below average), the spring–early summer of 2014 when experiment 1 commenced (85 mm below average), October 2015 just prior to the commencement of Experiment 2 (30 mm below average) and late summer–autumn 2016 (140 mm below average).

A characteristic of the weather experienced throughout the experimental period was the above average monthly mean monthly maximum and minimum temperatures particularly in spring–early summer of each year (Fig. 9.2). In spring–summer 2014–2015 monthly mean maximum and minimum temperatures ranged from 1.9–4.7^{0}C above average and in spring 2015–2016 they ranged from 1.3–3.4^{0}C above average (Fig. 9.2).

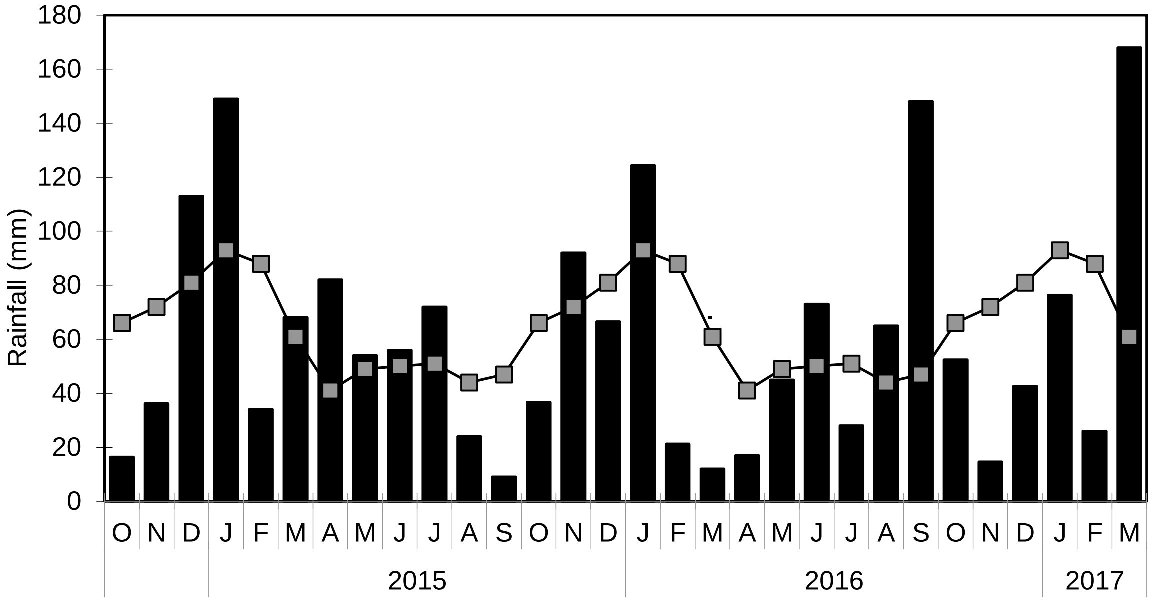

Fig. 9.1. Rainfall (mm) received during the experimental period (October 2014–March 2017) (black columns) compared to the long-term average (LTA) (grey line), at the Bingara field site. LTA rainfall data sourced from Bureau of Meteorology, at Bingara (054004; 1878–1997).

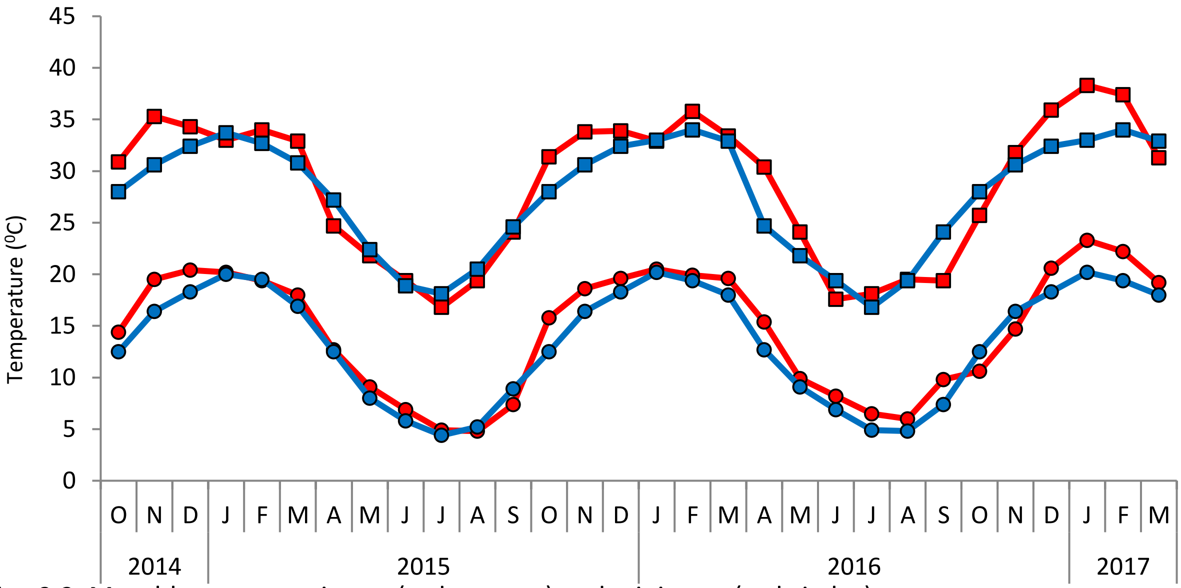

Fig. 9.2. Monthly mean maximum (red squares) and minimum (red circles) temperatures experienced over the experimental period (October 2014–March 2017) at the Bingara field site compared to long-term monthly mean maximum (blue squares) and minimum (blue circles) temperatures. Data were sourced from the Bureau of Meteorology, at Moree (053115; 1995–2018).

9.3.2 Experiment 1

No seedlings emerged from the October 2014 sowing at either sowing depth. Prior to, at, and in the four–six weeks after sowing the October treatment conditions were very hot and dry (Fig. 9.1 and 9.2) and despite hand-watering the plots more frequently than originally planned the soil was hostile for seedling emergence.

No seedlings emerged from the November sowing at Sd0. A small number of seedlings emerged in November for the November Sd15 treatment (1 and 4 seedlings/m^2 4 and 12 weeks after sowing respectively), but this was not significantly higher than the November Sd0 treatment (Fig. 9.3).

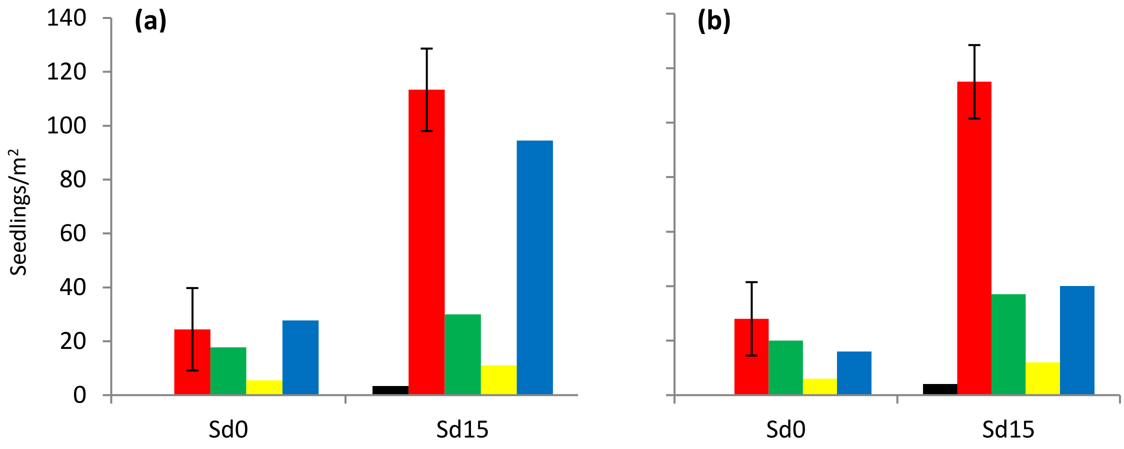

Fig. 9.3 Desmanthus seedling emergence (seedlings/m^2) (a) four weeks and (b) 12 weeks after sowing at two sowing depths; surface (0 mm, Sd0, and 15 mm Sd15) at five sowing times [months; November (black), December (red), January (green), February (yellow) and March (blue]) in 2014–2015 at Bingara. Error bars represent Fisher LSD ($P < 0.05$) comparing sowing time treatment means within sowing depth. October data not presented as there was no seedling emergence.

At 4 and 12 weeks after sowing there were significant ($P < 0.05$) effects of sowing time and depth on seedling emergence. At Sd0, seedling emergence from plots sown in February was significantly lower than from plots sown in the other months both at 4 and 12 weeks after sowing. At 4 weeks after sowing, emergence was highest for the Sd0 treatment from the March sown plots, but this was not significantly higher than the emergence from plots sown in December. At 12 weeks after sowing, emergence was highest for the Sd0 treatment from the December sown plots, which was significantly ($P < 0.05$) higher than those sown in March and January.

At Sd15, seedling emergence from plots sown in February and November was significantly lower than from plots sown in the other months both at 4 and 12 weeks after sowing. At 4 weeks after sowing seedling emergence was highest for the December sown Sd15 treatment which was significantly higher ($P < 0.05$) than the emergence from plots sown in March, which was significantly higher than emergence from the plots sown in January. At 12 weeks after sowing seedling emergence for the Sd15 treatment from the December sown plots, which was significantly ($P < 0.05$) higher than those sown in March and January which were not significantly different.

Seedling survival (hereinafter referred to as plant survival) was recorded approximately 52 weeks after the first sowing (October 2014) in October 2015. For the Sd0 treatment plant survival was highest in December sown plots (12 plants/m^2) and was significantly ($P < 0.05$) higher than the other sowing times within the Sd0 treatment. Although plant numbers were adequate at 12 weeks after sowing for the March sown plots, no plants had survived at the October 2015 measurement (Fig.

9.4). For the Sd15 treatment plant survival was highest in December sown plots (58 plants/m^2) and was significantly ($P < 0.05$) higher than the other sowing times within the Sd15 treatment. There was no significant difference in plant survival between the November and January sowing times or the February and March sowing times. Once again the number plants from the March sowing declined dramatically from that at 12 weeks to the October 2015 measurement (40 *cf.* 4 plants/m^2).

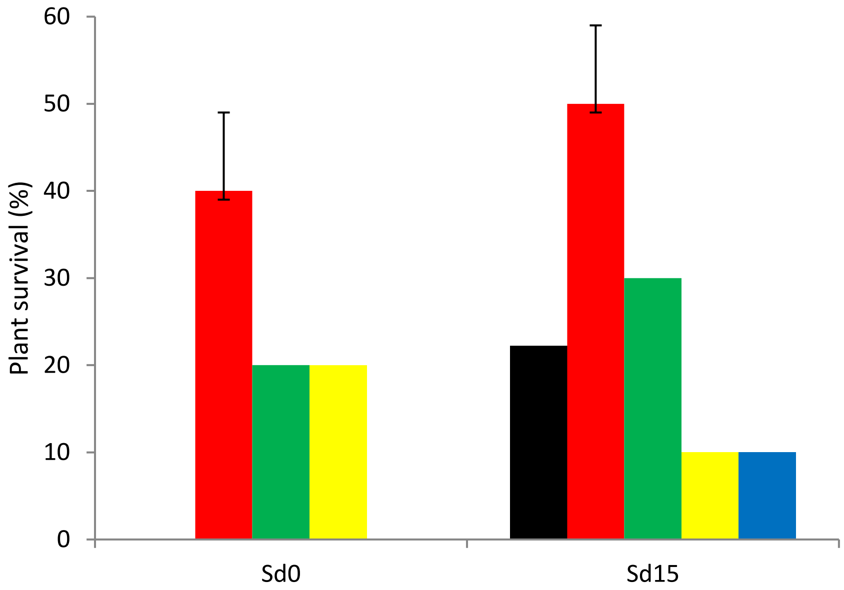

Fig. 9.4 Plant survival (%) of desmanthus recorded in October 2015 (approximately 52 weeks after the commencement of the experiment) at two sowing depths; surface (0 mm, Sd0) and 15 mm, (Sd15) at five sowing times [months; November (black), December (red), January (green), February (yellow) and March (blue)] in 2014–2015 at Bingara. Error bars represent Fisher LSD ($P < 0.05$) comparing sowing time treatment means within sowing depth. October data not presented as there was no seedling emergence.

Herbage mass assessed in December 2015 and February 2016 are presented in Table 9.1. For the Sd0 treatments there was no significant difference in herbage mass irrespective of sowing time; all treatments with ≤ 500 kg DM/ha. At the December 2015 assessment, herbage mass of desmanthus sown at 15 mm (Sd15) was significantly higher in plots sown in December (1656 kg DM/ha, $P < 0.05$), but there was no significant difference between the other treatments (<600 kg DM/ha). In February 2016 herbage mass was significantly higher in the December and January sown plots (>850 kg DM/ha, $P < 0.05$). There was no significant difference between the other sowing times.

Table 9.1 Herbage mass production (kg DM/ha) of desmanthus in December 2015 and February 2016 for five sowing times (months) at two sowing depths [Sd0 (0 mm) and Sd15 (15 mm)] at Bingara. Values within a column followed by the same letter are not significantly different ($P = 0.05$). October data are not presented as there was no emergence.

Sowing	December 2015		February 2016	
time	Sd0	Sd15	Sd0	Sd15
November 2014	180 a	148 a	175 a	136 a
December 2014	500 a	1656 b	349 a	1199 b
January 2015	199 a	595 a	104 a	856 b
February 2015	0 a	182 a	0 a	60 a
March 2015	0 a	90 a	0 a	80 a

9.3.3 Experiment 2

Four weeks after sowing, 31–46 seedlings/m^2 had emerged from the four sowing times from November to late-January, significantly more than emerged in February ($P < 0.05$). No desmanthus seedlings emerged from the March sowing (Table 9.2). At 12 weeks after sowing all seedlings in the February sown plots had died and there was no significant difference between the remaining sowing times with seedling numbers ranging from 21–37 plants/m^2 (Table 9.2).

Seedling survival (hereinafter referred to as plant survival) was recorded in October 2016, approximately 50 weeks after the first sowing (November 2015). Plant survival was highest and similar in the late- and early-January sown plots (68–75%, Table 9.2). There was no significant difference between the early-January and November sowing times, however, plant survival was significantly lower in the December sown plots ($P < 0.05$, Table 9.2).

Herbage mass was measured in January 2017 in the first growing season after the establishment year (2016–2017) and data are presented in Table 9.2. Desmanthus herbage mass was highest for plots sown in early-January ($P < 0.05$), but this was not significantly different to plots sown in late-January. There was no significant difference in herbage mass of desmanthus sown in late-January and November or November and December.

Table 9.2 Desmanthus seedling emergence (seedlings/m^2) at 4 weeks and 12 weeks after sowing, plant survival (%) recorded in October 2016 and herbage mass (kg DM/ha) in January 2017 for five sowing times (months) at Bingara. Values within the same column followed by the same letter are not significantly different ($P = 0.05$). March data not presented as there was no emergence.

Sowing time	Seedlings/m^2		Plant survival	Herbage mass
	4 weeks	12 weeks	(%)	(kg DM/ha)
November 2015	31 a	21 a	44 b	1200 bc
December 2015	47 a	37 a	20 c	675 c
Early-January 2016	36 a	35 a	68 ab	2520 a
Late-January 2016	46 a	28 a	75 a	2080 ab
February 2016	5 b	0 b	-	-

9.3.4 Experiment 3

Desmanthus emergence was first seen in trays at 30–25°C, 5 days after sowing and was followed by the 28–20°C and the 20–15°C regime, 1 and 4 days later respectively. Seeding emergence at both the 28–20^{0}C and 30–25^{0}C temperature regimes was similar and occurred at a higher rate than at the lower temperature regime (20–15°C, Fig. 9.5). After 8 weeks average seedling emergence was about double that of the lowest temperature regime (i.e. 30 *cf.* 18 seedlings/tray) (data not analysed).

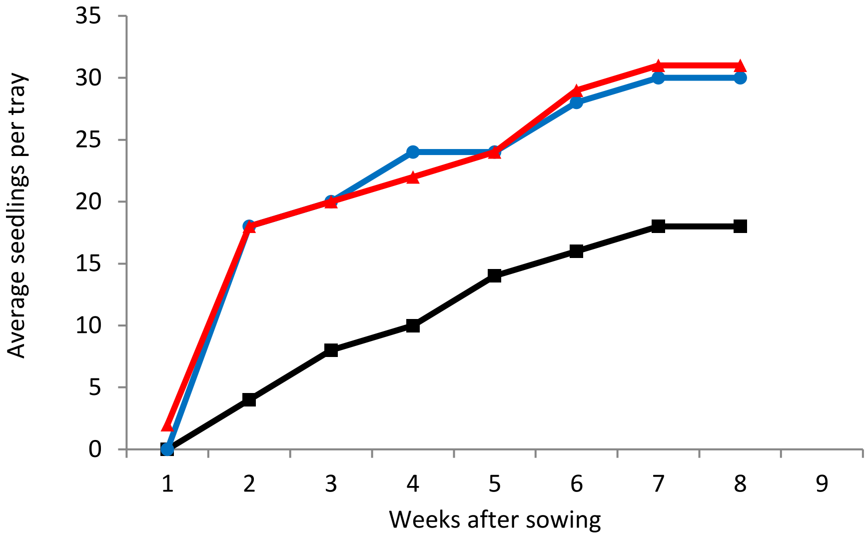

Fig. 9.5. Average total number of emerged desmanthus seedlings per tray for each day-night temperature regime; 20–15°C (black line), 28–20^{0}C (blue line) and 30–25^{0}C (red line) over 8 weeks.

Total seeding emergence after 8 weeks for the six sowing depths are presented in Fig. 9.6. Emergence at the surface (Sd0) was significantly lower than the other sowing depths, which were not significantly different.

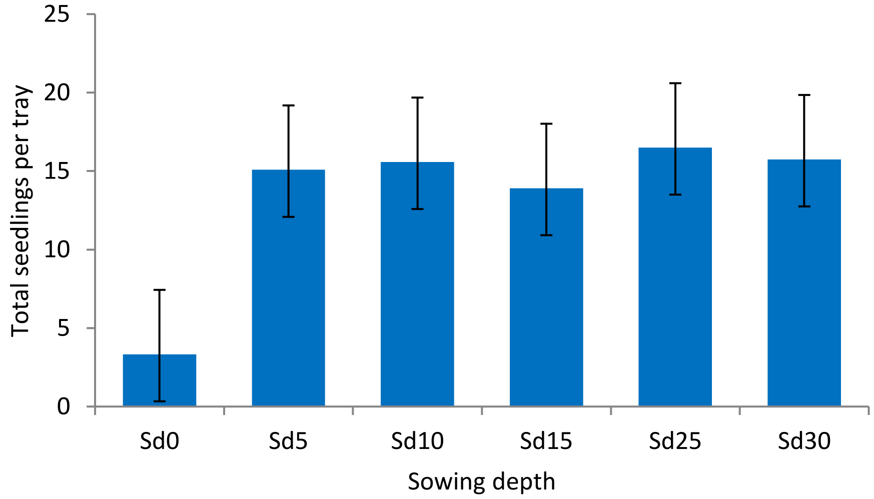

Fig. 9.6. Average total number of emerged seedlings per tray for each of the six sowing depth treatments; Sd0 (0 mm), Sd5 (5 mm), Sd10 (10 mm), Sd15 (15 mm), Sd25 (25 mm) and Sd30 (30 mm) over 8 weeks. Error bars represent Fisher LSD (P<0.05) among treatments.

Shoot length, shoot weight, root length and root weight for the three temperature regimes were not analysed but there were trends. All traits were lowest at 20–15^{0}C; shoot traits and root weight about one fifth of those at the other temperatures (Table 9.3). Shoot length and shoot weight were highest at the 30–25°C temperature regime, with the exception of root length which was highest at the 28–20^{0}C temperature regime. Root weight was the same for the two higher temperature regimes.

Table 9.3. Mean shoot length (mm), shoot weight (g), root length (mm) and root weight (g) for each of the three temperature regimes.

Day–night temperature (°C)	Shoot length (mm)	Shoot weight (g)	Root length (mm)	Root weight (g)
20–15	10.40	0.15	10.17	0.06

28–20	55.56	0.73	15.21	0.33
30–25	104.89	1.00	14.71	0.33

Shoot length, shoot weight, root length and root weight were all significantly ($P < 0.05$) lower for the surface sown treatment (Table 9.4). There were no significant differences between the remaining sowing depth treatments for all plant traits.

Table 9.4. Mean shoot length (mm), shoot weight (g), root length (mm) and root weight (g) for each of the six sowing depths. Values within the same column followed by the same letter are not significantly different at $P = 0.05$.

Sowing depth (mm)	Shoot length (mm)	Shoot weight (g)	Root length (mm)	Root weight (g)
0	17.04 a	0.10 a	3.33 a	0.04 a
5	60.87 b	0.71 b	15.08 b	0.28 b
10	60.82 b	0.66 b	15.58 b	0.24 b
15	67.76 b	0.75 b	13.92 b	0.30 b
25	64.81 b	0.73 b	16.50 b	0.29 b
30	70.38 b	0.82 b	15.75 b	0.31 b

9.4 Discussion

This study showed that time of sowing (months) and sowing depth can have a significant impact on the emergence, survival and establishment of desmanthus in both the glasshouse and under field conditions.

In Northern Inland NSW desmanthus can be sown from November to late January, but is most effective when soil moisture is non-limiting. Both the glasshouse and field studies showed that surface sowings of desmanthus will not result in a productive pasture and should be avoided.

9.4.1 Sowing time

The results from the glasshouse experiment showed that the rate of and total emergence of desmanthus was lowest for the 20–15^{0}C day–night temperature regime compared to the two higher day-night temperature regimes (28–20^{0}C, 30–25^{0}C). This was consistent with that of McDonald (2002a, b) who reported that the optimum temperature range for desmanthus was between 24–28°C, with rate of germination decreasing when temperatures were 4–8°C outside this optimum range. The lower emergence of desmanthus at the 20–15^{0}C temperature regime in this study was probably the result of the seeds spending a large portion of time each day (50%) at 15^{0}C, a constant temperature outside the optimum for germination. McDonald (2002b) also reported that there was a reduction in germination in tropical legumes when temperatures fell below 20°C.

The field experiments indicated that late spring–mid-summer (November–late January) are suitable months to sow desmanthus in Northern Inland NSW when mean daily maximum and minimum temperatures are around 28 and 20^{0}C respectively. Emergence occurred at the Bingara field site over late summer–autumn which corresponded with seedling recruitment recorded in the tropical legume evaluation experiments at Bingara and Manilla (Chapter 5). However, these experiments showed that survival of these seedlings was poor and did not result in a productive pasture. Seedlings emerging in March are exposed to warm temperatures during the day, but night temperatures are declining and seedlings would be 4–6 weeks old when the first frost in the region can potentially occur. The desmanthus seedlings emerging in February and March were likely to be too small and underdeveloped to survive frost or to flower and set seed for regeneration in the following summer. This is the case with tropical grasses where Lodge and Harden (2009) found that grass seedlings that emerged from late-summer–autumn sowings remained small, did flower and had poor survival with the onset of frost. However, cv. Marc used in these experiments has been reported to have some tolerance of frost (Graham *et al.* 1991). Shoot and root length and weight were all lower at 20–15^{0}C compared to the higher temperature regimes, supporting slower plant development in cooler temperatures. Four weeks after the March 2015 sowing, mean daily maximum and minimum temperatures were 24.7 and 12.5^{0}C respectively. Twelve weeks after sowing, the mean daily maximum and minimum temperatures were 19.4 and 6.1^{0}C respectively.

In Experiment 1 no seedlings emerged from the October 2014 sown treatments at both sowing depths as well surface sown seed in November. Soil moisture was limiting during these months due to an extremely dry winter prior to sowing and below average rainfall in the months of sowing. In addition, above average maximum and minimum temperatures would have resulted in above average potential evapotranspiration and the hand-applied water to assist establishment would have been ineffective especially for the surface sown treatments where there was no ground cover or litter to reduce soil temperature or retain soil moisture (Cook 1980; Campbell and Swain 1973).

Although temperature is important, soil moisture is a key factor for successful establishment of desmanthus. Ideally desmanthus should be sown into a moist soil profile (Becerra Stiefel *et al.* 1998) with follow-up rainfall (Becerra Stiefel *et al.* 1998; Brandon and Jones 1998); preferably 1–2 days of good soil moisture (Brandon and Jones 1998).The importance of moisture was highlighted in December 2014–January 2015 (Experiment 1) where above average rainfall was received post the December and January sowings. Seedling emergence, seedling survival and herbage mass production in the first growing season after establishment was highest for these sowing months especially the December sowing time. In Experiment 2, seedling emergence, survival and herbage production in the following growing season was highest for the two January sowings and these sowings also coincided with above average rainfall.

Due to space and time constraints there were only a small number of temperature regimes tested in Experiment 3 and these temperature regimes could not be replicated. Although the experiment was limited by the achievable temperature range of the glasshouses (15°C–30°C) and additional representative spring and autumn sowing times could not be investigated the trend for declining root and shoot development at lower temperatures was clear and consistent.

9.4.2 Sowing depth

Tropical legume establishment can be affected by the depth from which species can emerge (Cook *et al.* 1987). The rate and ability of a species to emerge not only affects its reliability to establish, but also has important implications for the type and adjustment of machinery used for planting, and the time of planting (Brandon and Jones 1998). Small-seeded species such as desmanthus should be sown shallow, but not on the soil surface where establishment is likely to fail (Brandon and Jones 1998). In this study, both the field (Experiment 1) and glasshouse experiments (Experiment 3) confirmed this finding and hence surface sowing desmanthus is not recommended for Northern Inland NSW. Cook *et al.* (1987) and Brandon and Jones (1998) concluded that the optimum sowing depth range for desmanthus was 5–20 mm; establishment reduced for seed sown outside of this range. In the glasshouse study, neither seedling emergence nor their shoot and root characteristics were affected by sowing depths between 5 and 30 mm. This result was not expected as small seeded species such as desmanthus exhibit poorer establishment from greater depths (Mayer and Poljakoff-Mayber 1982, Brandon and Jones 1998) and may be due to the type of growing medium used (i.e. high sand content) compared to the clay soil used in Brandon and Jones (1998). Although the data from this study were not conclusive, combined with the evidence from Queensland and the experience with tropical perennial grasses in Northern Inland it is recommended that desmanthus seed be sown between 5 and 15 mm.

9.4.3 Future research

1. Sowing between November and January into moisture may be suitable for optimum emergence and seedling development, but soil temperatures at that time may be detrimental to the survival of the rhizobia suitable for desmanthus. Bacteria survive in sufficient numbers for effective nodulation when temperature is at 25°C, but when temperature rises above this, combined with dry conditions, nodulation failure can occur (Becerra Stiefel *et al.* 1998). Under controlled conditions freeze-dried rhizobia had higher survival rates than those in peat form and at 70°C the number of rhizobia that survived on the seed were substantially reduced and were only detected on desmanthus seed for 7 days (McInnes and Date 2005). Additionally the number of rhizobia on seed from freeze-dried inoculants fell when humidity increased from 5–8 to 31–63% (McInnes and Date 2005). Alternative methods that place rhizobia deeper into the soil, such as granules or water injection have been found to be more effective than traditional seed inoculation (Johnson *et al.* 2015) however, more work is required. In subtropical regions, summer rainfall is typically highly variable and with protracted dry conditions, therefore when sowing a desmanthus pasture, sowing early (i.e. November) when temperatures are lower and when there is rainfall forecast will increase likelihood of rhizobia survival. The viability and effectiveness of the rhizobia on desmanthus in this environment at a range of temperatures and moisture levels requires evaluation, as does alternative inoculation or rhizobia placement methods.
2. These field experiments were conducted on one soil type; the effect of different soil types particularly lighter textured soils on emergence and seedling survive would strengthen sowing guidelines.
3. The window of sowing and sowing depth recommendations for desmanthus is similar to that for sowing tropical perennial grasses (Lodge and Harden 2009, Harris *et al.* 2014). However, limited research has been conducted on the agronomy of sowing these species together in Northern Inland NSW and on the best spatial arrangement of these species to maximise both the grass and legume production.
4. As outlined in Chapter 5, techniques and guidelines on how to establish desmanthus into existing tropical perennial grass pastures where there is no reliable companion legume warrants further investigation.

9.5 Conclusions

In Northern Inland NSW sowing time and depth have a significant impact on seedling emergence and survival, and establishment of desmanthus. In this region, the optimum time to sow desmanthus was November to late January, but was most effective when soil moisture is non-limiting. The ideal sowing depth was 5–15 mm and sowing desmanthus seed on the soil surface should be avoided.

A number of factors were identified for further investigation including the impact of the soil and moisture conditions at sowing on rhizobia survival and effectiveness, establishment of desmanthus on different soil types, establishing desmanthus in competition with newly sown and established tropical perennial grasses.

10 Optimum plant density of *Digiteria eriantha* for dry matter production and hydrological performance in a summer dominant rainfall zone

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Submitted to *Grass and Forage Science*

10.1 Introduction

Tropical perennial grass species have become increasingly sown in Northern Inland NSW over the last 10–15 years. Since about 2003, evidence emerged of the superior persistence and productivity of tropical compared with temperate grasses in this frost prone, summer dominant rainfall zone (Boschma *et al.* 2009), and subsequently considerable effort has been given to understand their requirements for establishment and productivity. These include weed control (Lodge *et al.* 2010a), seed quality (McCormick *et al.* 2009b), sowing time and depth (Lodge and Harden, 2009), also their growth rates (Boschma *et al.* 2016), response to nitrogen (Boschma *et al.* 2014a, 2016), rooting depth (Murphy *et al.* 2008a), water use and rainfall efficiency (Murphy *et al.* 2008b, 2010), and species persistence (McCormick *et al.* 1998; Boschma *et al.* 2009).

This knowledge resulted in successful establishment and has led to wide spread adoption of tropical pastures across the region. Establishment has been so successful that anecdotal evidence suggests that pastures commonly have densities greater than 26 plants/m^2 which are perhaps higher than necessary for maximum plant production and water use efficiency, and so represent over investment. Conversely, it is not known at what density a pasture should be considered a ‘failure’ because plant density is too low and re-establishment considered.

In the 400–600 mm winter dominant rainfall zone of South Coast and Northern Agricultural Regions of Western Australia, studies found that the optimum plant density for pasture production of *Megathyrsus maximus* (panic grass) was 4–8 plants/m^2 (Nichols *et al.*, unpublished data). In a higher rainfall, more summer dominant rainfall zone in Queensland it is recommended that established tropical grass pastures have a minimum plant density of 10 plants/m^2 (W Scattini, pers. comm. 2005). This is based on the experience of scientists, but it is not known if this density is relevant for NSW and should potentially be lower, since the amount and proportion of summer rainfall in Northern Inland NSW is less than regions where the Queensland recommendation were developed. Relevant information on the optimum density of plants required in an established pasture to maximise herbage production and achieve efficient use of water would assist producers and advisors to plan establishment and provide knowledge of intraspecific competition between digit plants in pure swards.

A spaced plant field study was conducted over 3.5 years to determine the optimum plant density for herbage production and hydrological performance of a digit grass pasture on a brown Vertosol in northern NSW. In this study we define optimum to be high pasture production, persistence and ground cover, in conjunction with high soil water extraction so that winter rainfall can be stored in the soil profile to reduce deep drainage and nutrient loss below the rooting zone and support spring growth with high water use efficiency.

10.2 Methodology

10.2.1 Site description

The experiment was conducted at Tamworth Agricultural Institute (31°08'43"S 150°58'06", 400 m above sea level) on a brown Vertosol (Isbell, 1996). The annual average rainfall at Tamworth is 674 mm with 60% falling in the warm season between October–March. In the 2 years prior to establishing the experiment, the site had been sown to forage oat (*Avena fatua*) each autumn and fallowed each summer. In September 2011, the forage oat was sprayed with glyphosate (450 g/L a.i. at 1.5 L/ha) to accumulate soil water in preparation for establishing the experiment and in November residual plant material was removed.

An automatic weather station located at the site recorded rainfall (0.5-mm pluviometer) at 30-min intervals.

10.2.2 Access tube installation and soil profile description

On 1 October 2011 an aluminium access tube (49 mm extern. diam. sealed at the bottom) was installed in the centre of each plot to 1.9 m depth after removing a soil core (51 mm extern. diam.) with a hydraulic push-coring machine and pouring about 1 L of a clay slurry (1 kg kaolinite: 1 L water) to fill voids and ensure good contact between the access tube and soil (White and Ridley, 1998). A cap was placed over the top of the tube to prevent rainwater entering. The soil cores were used to calibrate the neutron moisture meter (NMM) using the method described in Murphy *et al.* (2017) and analyse soil characteristics.

Soil water content (g/g^3) was determined by dividing each soil core into segments (0–0.1, 0.1–0.3, then 0.2 m intervals to 1.9 m) and retaining the samples in plastic bags, which were weighed in the field to obtain a wet weight and then again after drying at 105°C for 48 h. Soil bulk density (Mg/m^3) was calculated using the soil dry weight and volume of each soil core segment. Three samples at each depth were air dried and analysed to determine soil pH_{Ca}, electrical conductivity ($EC_{1:5}$ dS/m) and soil particle size distribution.

The soil profile was non-saline ($EC_{1:5}$ <0.3 dS/m) with a neutral-alkaline pH trend; values being near neutral in the upper layer (pH_{Ca} 6.9–7.6), and alkaline at depths greater than 0.9 m (pH_{Ca} ≥8.0, Table 10.1). Proportion of soil particles (sand 20–2000 µm, silt 2–20 µm, clay <2 µm, gravel >2000 µm) for each layer were determined using the laser diffraction method (Malvern Mastersizer 2000) and showed that the proportion of clay was greater than for either sand or silt for all sampling layers, apart for 0–0.3 m where silt was abundant (Table 10.1). Clay content was highest (36–43%) for soil layers below 0.3 m depth, while silt content declined with depth (39.0 to 31.1%). Soil texture at the surface (0–0.1 m) was silty clay loam and trending to silty clay for soil layers deeper than 0.5 m.

10.2.3 Neutron probe calibration

The NMM was calibrated by determining the relationship between neutron count and values of volumetric soil water content (SWC, θ_{vol} m^3/m^3), collected when access tubes were installed and at other times with conditions of high (near drained upper limit) and low (near crop lower limit) SWC, giving a range of values (n = 140) as per Lodge *et al.* (2010b). When access tubes were installed SWC was allowed to equilibrate for 24 h before taking neutron counts. Gravimetric SWC was determined on retained soil cores sampled at 0.2 m depth intervals from 0.1 m to a maximum depth of 1.9 m and converted to volumetric SWC by using bulk density values for each layer (Table 10.1).

For each soil layer, values of volumetric SWC (θ_{vol}) and neutron count were plotted and logarithmic regression best explained the relationship between SWC and neutron count (R^2 = 0.87, n = 140).

Table 10.1. Mean proportion (% ± s.d.) of soil particle size (sand 20–2000 µm, silt 2–20 um, clay <2 µm, gravel >2000 µm), soil salinity (dS/m) and soil pH (pH_{Ca}) of the brown Vertosol sampled in nine layers.

Sampling layer (m)	Sand (%)	Silt (%)	Clay (%)	Soil Nitrate (kg/ha)	Soil salinity (dS/m)	Soil pH_{Ca}
0–0.1	30.6	39.0	30.4	14.1	0.24	6.9
0.1–0.3	28.5	37.3	34.2	30.9	0.12	7.0
0.3–0.5	20.9	38.7	40.3	28.1	0.11	7.0
0.5–0.7	22.4	35.9	41.7	20.9	0.12	7.2
0.7–0.9	28.8	34.4	36.7	19.3	0.23	7.6
0.9–1.1	29.0	35.0	36.0	18.0	0.42	8.0
1.1–1.3	25.2	32.8	42.0	20.3	0.72	8.2
1.3–1.5	24.7	32.6	42.7	17.9	1.05	8.2
1.5–1.7	28.1	31.1	40.8	11.1	1.23	8.2
1.7–1.9	30.6	39.0	30.4	4.5	1.23	8.2

10.2.4 Experimental design and plant establishment

The experiment was a randomised complete block design with three replicates. There were five plant density treatments (total of 15 plots), each plot 4 x 4 m: 0 (i.e. bare, D0), 1 (D1), 4 (D4), 9 (D9) and 16 plants/m^2 (D16).

On 15–16 November 2011, 6 week old seedlings grown in small pots (50 x 50 x 150 mm) in a glasshouse and fertilised fortnightly with a soluble fertiliser [Yates Thrive®, 25:5:8.8:4.6% nitrogen (N):phosphorus (P):potassium (K):sulfur (S)] were transplanted into the experiment and each plant given two applications of about 2.5 L of water. The plants at each density were uniformly spaced across the plot and around an aluminium access tube (described above) centrally located in each plot.

Herbicides were applied throughout the experiment to control weeds and prevent digit grass seedling establishment. Broadleaf weeds were sprayed with MCPA (750 L/ha a.i. at 0.75–1.2 L/ha), clopyralid (300 g/L a.i. at 50 ml/ha), 2,4-D amine (625 g/L a.i. at 1.7 L/ha), dicamba (500 g/L a.i. at 150 ml/ha), diuron (900 g/kg a.i. at 1.4 L/ha) and paraquat-diquat mix (135 and 115 g/L respectively at 2.0 L/ha) in December 2011, April and June 2012, July 2013, and June and July 2014 respectively. Either metsulfuron-methyl (600 g/kg a.i. at 5 g/ha) or S-metalachlor (960 g/L a.i. at 0.25–1.2 L/ha) were applied 9 times over the course of the experiment to provide residual weed control and prevent digit grass seedling recruitment; once during year 1 (December 2011), twice during year 2 (November 2011, March 2012), three times during year 3 (September and December 2013, February 2014) and once each winter period (June 2012, July 2013, June 2014). The experiment was also sprayed with insecticides chlorpyrifos (500 g/L a.i. at 0.3 L/ha) and omethoate (290 g/L a.i. at 0.2 L/ha) to control two spotted spider mite (*Tetranychus urticae*) in November and December 2012 respectively, and omethoate (at 0.2 L/ha) in October 2013.

The experiment was fertilised each growing season from 2012–13; 250 kg/ha single superphosphate (8.8% P, 11% S) applied in spring and 110 kg/ha urea (46% N) applied in both spring and summer (total of 220 kg/ha, timed to occur with rainfall to minimise volatilisation).

10.2.5 Data collected

Soil water content (SWC) was assessed with a neutron moisture meter (NMM, CPN 503DR Hydroprobe; Boart Longyear Co., Martinez, CA, USA) on 21 October 2011 prior to establishing the experiment, then every three weeks from 9 December 2011 to 27 April 2015; a total of 62 times. Counts were taken over a 16-s period at 0.2 m intervals down the soil profile with readings representing soil layers 0.2 m thick to 1.9 m (i.e. 0.1–0.3, 0.3–0.5,…1.7–1.9 m). Values of volumetric SWC for each layer were summed to obtain values for stored soil water (SSW, mm) for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and full profile (0.1–1.9 m).

Herbage production was assessed every six weeks over the growing season by collecting all herbage to 50 mm height in a 1 x 1 m quadrat rotated through four locations around the access tube. Herbage was assessed a total of 23 times; 3 times in year 1 (2011–12), 7 times in years 2–3 (2012–13, 2013–14) and 6 times in year 4 (2014–15). The herbage was dried at 80^{o}C for 48 hr and dry weight (kg/ha) determined. After each assessment the experiment was crash grazed by sheep to about 50 mm height. Any residual stem >50 mm in height was manually removed.

Plant frequency (Brown, 1954) was assessed twice a year; after the start and prior to the end of each growing season (*c*. November and May). The number of cells (0.1 x 0.1 m) containing a live plant were counted in two fixed quadrats (1.0 x 1.0 m, total 100 cells) in each plot about 7 days after grazing and the proportion of occurrence determined.

10.2.6 Data analysis

Maximum extractable soil water. SWC data for each plant density were plotted for each soil layer and sampling time. Periods of 3 months or more during each growing season (September–May) when SWC declined without interruption by complete profile rewetting (i.e. significant rainfall) were visually identified to demonstrate the capacity of digit grass at each plant density to extract soil water (Murphy and Lodge 2006). The differences between high and low values of SSW (i.e. start and end date of the periods identified respectively) for each soil layer and plant density (Neal *et al.* 2012) were calculated as the maximum extractable water (MEW, mm). Values of MEW for each depth were determined for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and total (0.1–1.9 m) soil profile. Analysis of variance was used to model values of MEW for each part of the soil profile with plant density as an explanatory factor and replicate as a block term. The least significant difference (LSD) was calculated using a 5% level of significance.

Plant root depth. This was estimated using changes in soil water content values between the dates identified to determine MEW (Murphy and Lodge, 2006). On each date, the trend in SWC over depth among the three replicate values was modelled using a linear mixed model for each plant density. The model included depth as a covariate and a fixed term to model the linear trend over depth and the spline term [spline(depth)] as a random effect to model a smooth curvilinear trend (Verbyla *et al.* 1999). A smooth curve for each plot was fitted by adding plot as a random term and its interaction with depth and the spline term. Predicted values of SWC with depth (± s.e. of prediction) at both dates were plotted for each plant density and plant root depth identified as the greatest depth at which the two curves +/- standard error did not overlap (Murphy and Lodge, 2006).

Soil profile refill. Soil moisture accumulation, or refill (mm), was calculated during the winter period (typically May to July–August) when digit grass was frosted and not growing. Using plots of SWC data for each soil layer and sampling times, periods when SWC changed from lowest to highest were identified. The difference between the values of low and high SSW for each soil layer were calculated as change in stored soil water (mm) for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and total (0.1–1.9 m) soil profile. Analysis of variance with density as an explanatory factory and replicate as a block term was used to determine significant differences among start and end stored soil water, change in SSW in each soil layer, and rainfall refill efficiency. The least significant difference (LSD) was calculated using a 5% level of significance.

Water use efficiency (WUE, kg DM/ha/mm) was calculated by dividing herbage production (kg DM/ha) by actual evapotranspiration (ETa, mm) (Allen *et al.* 1998). Actual evapotranspiration was determined by water balance with the assumption that no water was lost by runoff or deep drainage. WUE of dry matter production for each growing season was calculated for each treatment in each year, also total for the duration of the experiment. Significant differences were determined by analysis of variance and least significant difference (LSD) calculated using a 5% level of significance.

Herbage production. Cumulative herbage production (kg DM/ha) over the three and a half years of the experiment was modelled using a linear mixed model similar to that for SWC versus depth describe above. The terms in the model (day, spline(day), plot and the interaction of plot with day and spline(day) were added to produce a smooth curve of cumulative herbage production versus time for each plot. Adding a term for the density treatments to the model and its interaction with day and the spline term produced a smooth curve for each of treatment. Fitted curves for each treatment ± s.e. of prediction were produced.

Plant frequency. Trend in plant frequency (%) was modelled similar to cumulative herbage production. Fitted curves for each treatment +/- s.e. were produced.

10.3 Results

10.3.1 Rainfall and temperature

Annual rainfall was average (2012) to below the long term average (2013–14) during the 3.5 years that the experiment as conducted. Monthly rainfall was below average 8–9 months each calendar year during the period 2012–14, and above average the other 3–4 months (Fig. 10.1). Subsequently, growing season (September–May) rainfall was below average 2012–13 and 2013–14, but well above average in 2014–15, due to 2.5–3 times the long term monthly average rainfall in December 2014 and January 2015. Mean monthly temperatures were generally similar to the long term average (LTA) with a few exceptions, such as maximum temperatures were below LTA December 2011–March 2012 (2.1–5.4^{o}C) and above the LTA January–February 2014 (1.6–2.8^{o}C) and October–November 2014 (3.4–4.2^{o}C). Minimum temperatures were below average December 2011–October 2012 (0.8–3.8^{o}C) and October–December 2013 (1.3–2.7^{o}C) (Fig. 10.1).

10.3.2 Soil water content

At the start of the experiment, all plant density treatments had high soil water content (>0.45 m^3/m^3) at all depths (Fig. 10.2). The bare plot (D0) maintained these levels throughout the duration of the experiment (Fig. 10.2a), while soil water content in the other treatments showed progressively drier values at greater depths in each successive growing season (Fig. 10.2b–e). Soil water content increased during May–September each year while the grasses were inactive, before declining again after growth recommenced.

In 2012, small declines in soil water content were observed in the upper part of the profile during the growing season, followed by near complete rewetting of the profile during winter (Fig. 10.2b–e). In 2013, greater changes in soil water content were observed to greater depths in the profile during the growing season with the D16 treatment declining to about 0.25 m^3/m^3 at the surface and ≤0.45

m^3/m^3 at 1.0 m (Fig. 10.2e). The soil profile for the D1–D16 treatments did not completely rewet during winter in either 2013 or 2014 (Fig. 10.2b–e).

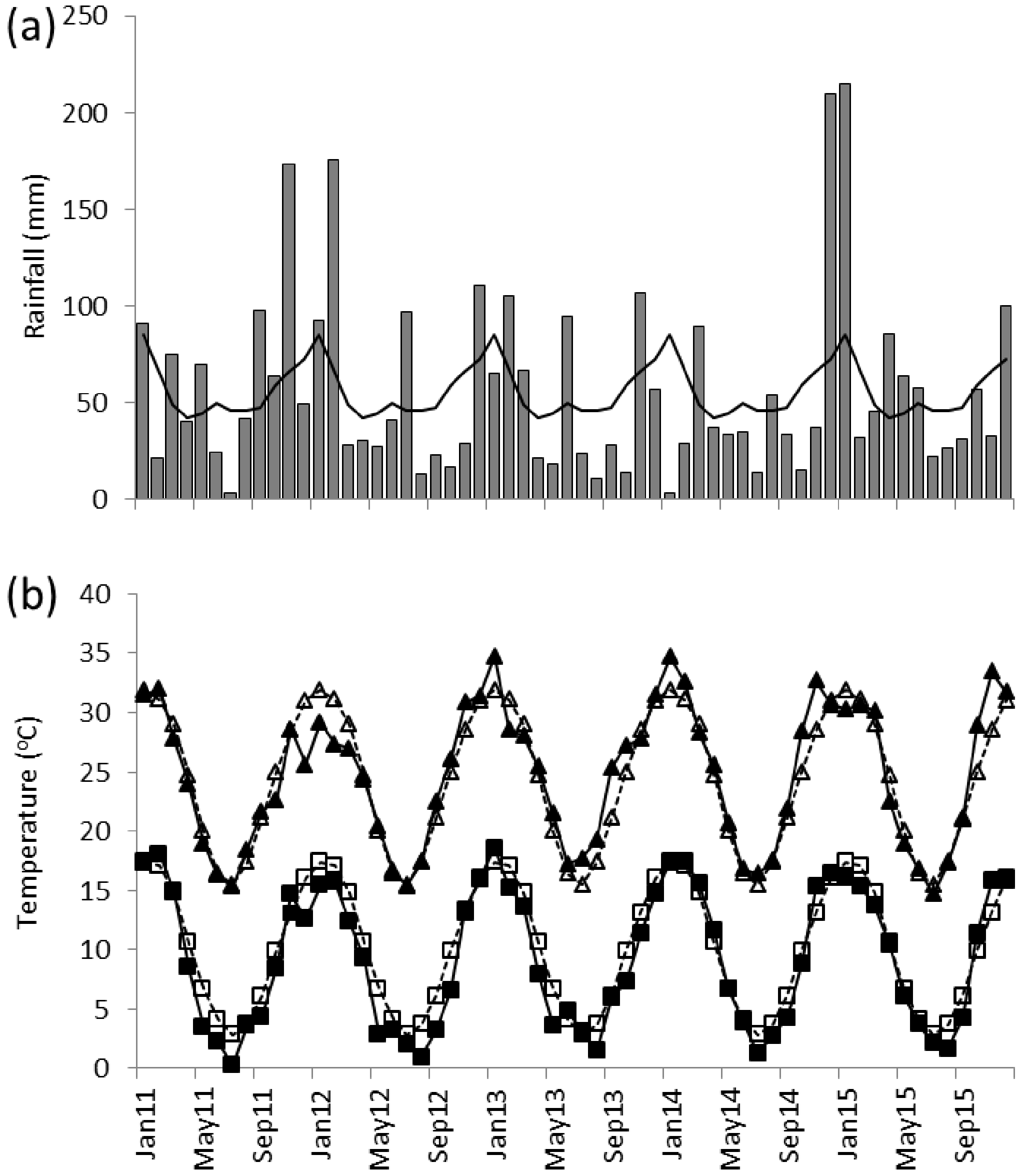

Fig. 10.1 (a) Mean monthly (mm, bars) and long term average (LTA, solid line) rainfall (mm), and (b) mean monthly maximum and minimum temperatures (▲,■, solid lines) and long term average monthly maximum and minimum temperatures (△,□, broken lines). Mean values for rainfall and temperature are for Tamworth Airport (1876–1992; BOM site 55054).

In 2014, extensive drying of the soil profile was observed in all treatments containing digit grass (Fig. 10.2b–e). The D9 treatment, particularly, showed large parts of the profile with soil water content ≤0.30 m^3/m^3 for most of 2014. There were only minor increases in profile soil water during winter 2014 with water contents returning to 0.4 m^3/m^3 only near the surface. Over 400 mm rainfall in December 2014–January 2015 increased soil water content in all treatments containing digit grass (Fig. 10.2); levels increasing to >0.4 m^3/m^3 at more depths in the higher plant density treatments (D9–D16).

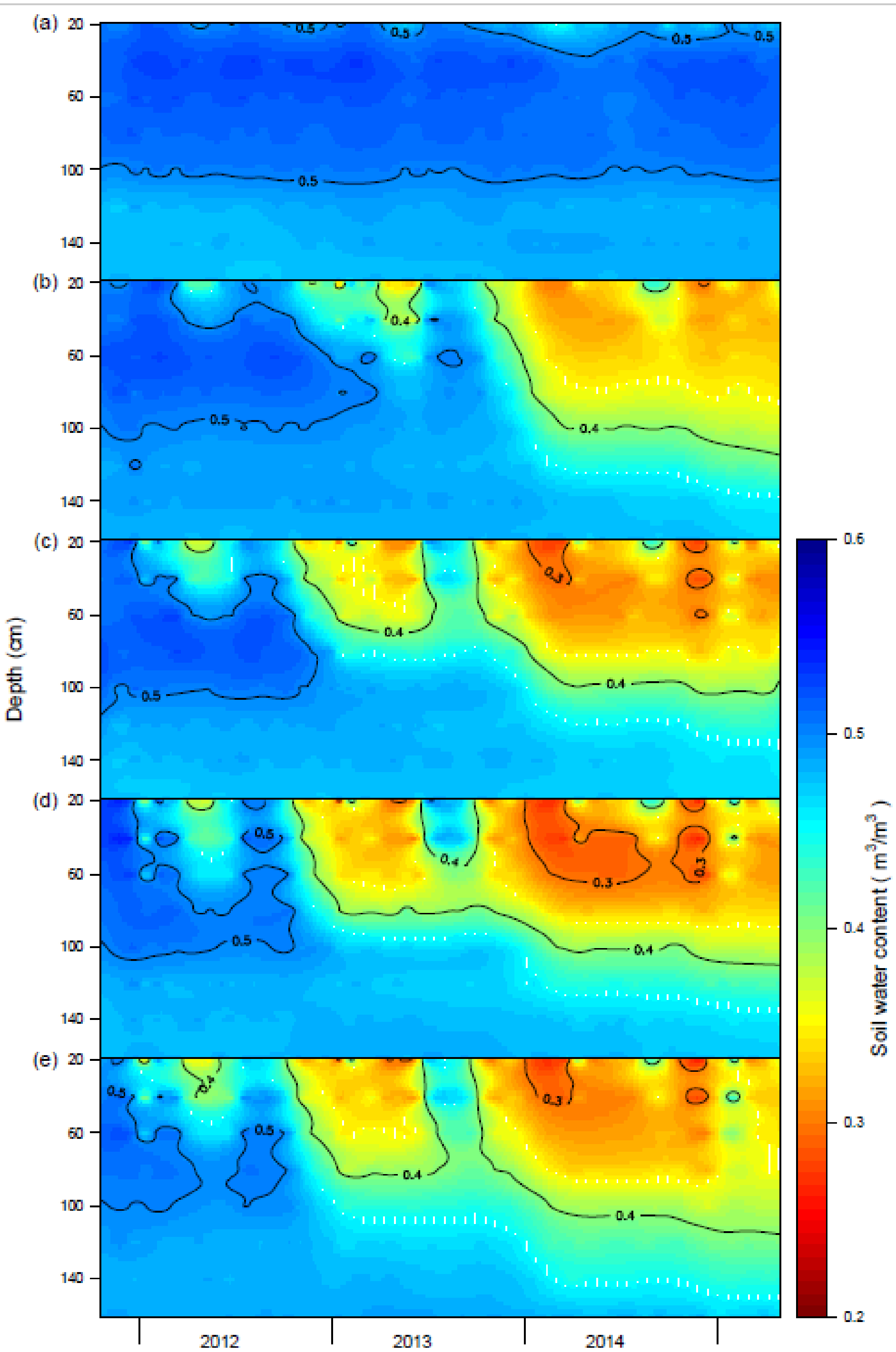

Fig. 10.2. Profile (20–160 cm) soil water content (m^3/m^3) from November 2011–May 2015 for digit grass with plant densities (a) 0, (b) 1, (c) 4, (d) 9, and (e) 16 plants/m^2.

10.3.3 Maximum extractable water

In the establishment year maximum and minimum SSW occurred on 9 December 2011 and 14 May 2012, at the start and end of the growing season, respectively (Table 10.2, Fig. 10.3). Profile SSW was not different at the start of the season, but by the end of the season SSW values were different and they decreased with increasing plant density order with the bare treatment (D0) having the highest

SSW (903 mm, $P < 0.05$) and D16 the lowest (834 mm, $P < 0.05$). Differences in MEW were only significant in the upper profile (0.1–0.7 m), which reflected differences observed for the total profile (0.1–1.9 m). In the upper profile the D9 treatment had the highest MEW (71 mm, $P < 0.05$) which was similar to that for D16. Total profile extraction values reflected those in the upper profile with D9 and D16 having the highest MEW ($P < 0.05$) followed by the other densities in decreasing order. The bare treatment showed a small increase in SSW in all profile zones creating a negative MEW value.

In year 2 (2012–13) maximum SSW values were observed in August and minimum values in May. There were no significant differences in maximum SSW, while minimum SSW values decreased in plant density order: D0 was wetter than D1, which had higher SSW content than the D4–D9 treatments ($P < 0.05$). The majority of the changes occurred in the upper profile with D4–D16 density treatments having MEW values >98 mm *cf.* 72 and 9 mm for D1 and D0, respectively (Table 10.2). Significant differences were also evident in the middle profile with the treatment ranking being similar, but MEW values ranged from 0.2 mm to 37 mm for D0 and D16, respectively ($P < 0.05$, Table 10.2). Differences in values of MEW for the total profile, again reflected those in the upper profile, with D4–D16 treatments (124–135 mm) being significantly greater than for D0 or D1 (6 or 81 mm, respectively).

In year 3 (2013–14) there were significant differences among treatments for both maximum (23 July 2013) and minimum SSW (25 March 2014). The bare (D0) and D1 treatments were wetter, while treatments with densities D4–D16 were drier ($P < 0.05$, Table 10.2). Similar to year 2, significant differences in MEW occurred in the total profile and both the upper and middle profile. Total profile MEW was smallest in the bare treatment plots (15 mm), followed by D4–D16 (127–158 mm). The greatest MEW occurred at D1 with 158 mm water extracted ($P < 0.05$), with the majority of this water extracted from the upper profile. In the upper profile, the bare treatment (D0) had the lowest MEW value ($P < 0.05$), significantly less than the other four treatments which were not significantly different from one another (88–104 mm, Table 10.2).

In the final year of the experiment (year 4, 2014–15), maximum SSW occurred in August 2014 and varied with D0 (bare) plots having the highest SSW and D4–D16 the lowest SSW ($P < 0.05$). Minimum SSW occurred in December 2014. Again the plots were in density order with the bare treatment being wettest and higher density treatments being driest ($P < 0.05$). The greatest values of MEW occurred in the upper profile, with small but significant changes occurring in the middle profile and non-significant changes in the lower profile. Total profile MEW values reflected those in upper profile with the bare plot having negligible water extracted (1 mm) and the other four treatments extracting similar volumes ranging 43–63 mm (Table 10.2).

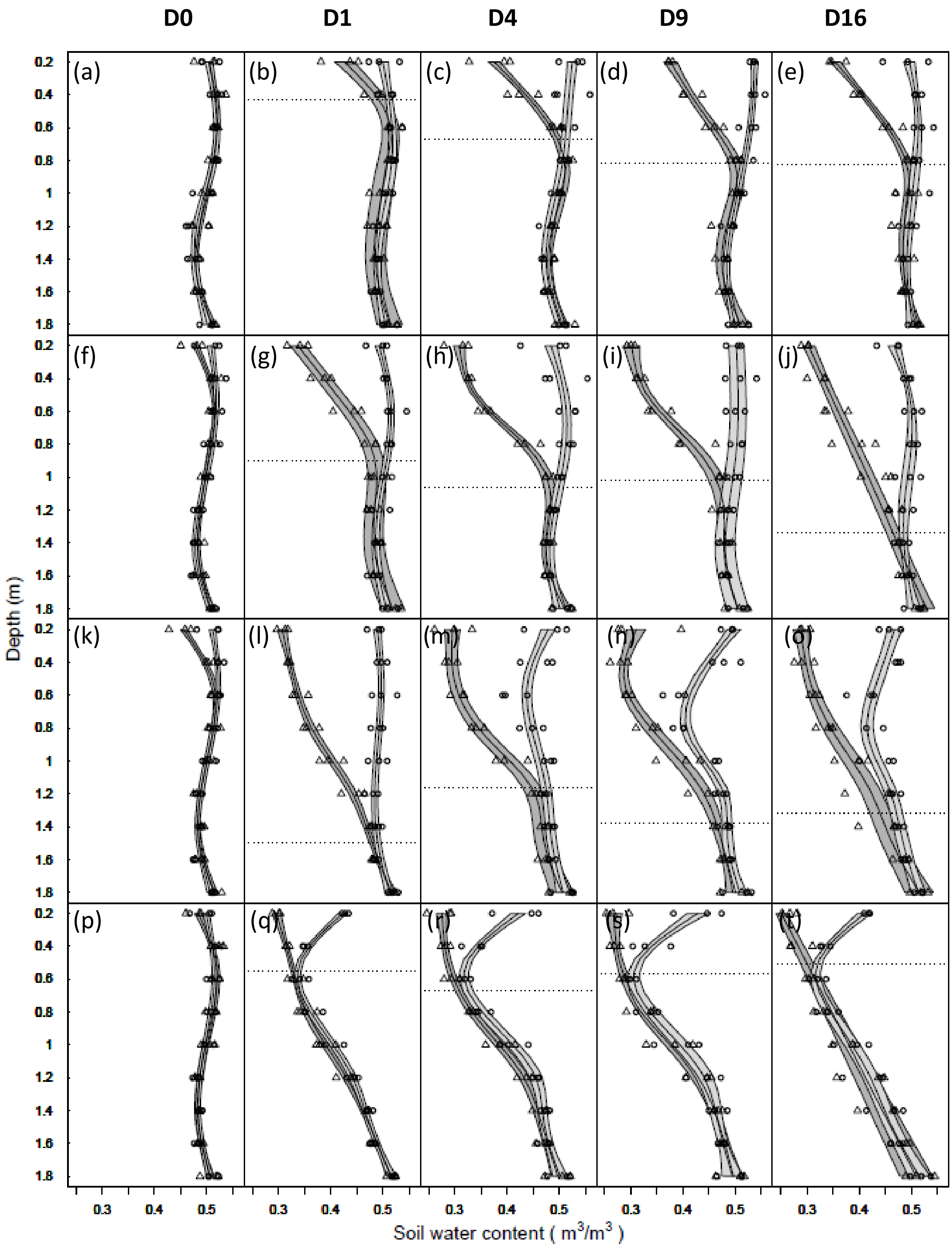

Fig. 10.3. Observed replicate values of maximum (○) and minimum (Δ) volumetric soil water content (m^3/m^3) for each treatment at times indicating maximum extractable water (mm) for D0 (a, f, k, p), D1 (b, g, l, q), D4 (c, h, m, r), D9 (d, i, n, s), and D16 (e, j, o, t) in 2011–12 (a–e), 2012–13 (f–j), 2013–14 (k–o) and 2014–15 (p–t), respectively (dates as per Table 10.2). Solid and dashed lines represent the predicted soil water content ± s.e. of prediction, respectively. Root depth is indicated by the horizontal dot line.

Table 10.2. Mean values of profile maximum and minimum stored soil water (SSW, mm), maximum extractable water (mm) for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and the total soil profile (0.1–1.9 m), and estimated plant root depth (m) for digit grass at each density for each growing season. Least significant differences (LSD; *P* = 0.05) among treatment means for each season is provided where it is appropriate.

Density	Max SSW (mm)	Min SSW (mm)	Maximum extractable water (mm) 0.1–0.7 m	0.7–1.3 m	1.3–1.9 m	0.1–1.9 m	Root depth (m)
			Year 1 – 2011–12 (9 Dec 11–14 May 12)				
D0	899	903	-1	-1	-2	-4	-[a]
D1	908	884	20	5	-1	24	0.43
D4	901	854	52	-4	-2	46	0.67
D9	917	840	71	6	0	77	0.82
D16	902	834	62	6	0	68	0.83
LSD	NS	19.4	16.4	NS	NS	19.4	-
			Year 2 – 2012–13 (27 Aug 12–21 May 13)				
D0	902	895	9	0	-2	6	-
D1	901	821	72	10	-2	81	0.90
D4	896	773	103	22	-1	124	1.06
D9	894	759	109	25	1	135	1.02
D16	884	750	98	37	-1	134	1.34
LSD	NS	30.2	20.2	22.1	NS	33.4	-
			Year 3 – 2013–14 (23 Jul 13–25 Mar 14)				
D0	907	893	17	0	-2	14	-
D1	888	730	104	51	4	158	1.50
D4	847	711	91	40	5	136	1.16
D9	833	703	93	32	6	130	1.38
D16	826	699	88	31	8	127	1.32
LSD	32.9	23.1	26.8	15.1	NS	20.0	-
			Year 4 – 2014–15 (21 Aug 14–1 Dec 14)				
D0	900	898	2	-1	0	1	-
D1	760	717	35	7	0	43	0.55
D4	751	688	46	13	3	63	0.67
D9	735	680	49	7	-1	55	0.57
D16	732	682	43	6	1	50	0.51
LSD	24.5	22.3	15	6.7	NS	21.3	-

[a]Nil plants

10.3.4 Plant root depth

In the first two years of the experiment, plant root depth was ranked in plant density order with D16 having the greatest rooting depth and D1 the shallowest (Table 10.2, Fig. 10.3). The rooting depths of digit grass generally reached their maximum in year 3 with digit at D1 being the deepest rooted (1.50 m) and D4 the shallowest (1.16 m). The rooting depth of digit grass with densities D9–D16 were intermediate ranging 1.32–1.38 m. In year 4, rooting depth during the 3.5 month profile drying period was similar for all plant densities and ranged from 0.51 m (D16) to 0.67 m (D1).

10.3.5 Soil profile refill

Stored soil water replenished during winter when digit grass was inactive, commonly from mid–May to end–July or August (Table 10.3 and Fig. 10.2). At the start of replenishment period, values of profile SSW were different ($P < 0.05$), reflecting the amount of extraction achieved over the summer (Table 10.3). At the end of the refill period in 2012, all treatments had similar SSW, but the amount of refill achieved by the treatments was different ($P < 0.05$, Table 10.3). The greatest change in profile SSW occurred in the D4–D16 density treatments with SSW increasing 42–54 mm, but negligible change occurred in the bare (D0) treatment. Change in SSW was primarily observed in the upper profile (0.1–0.7 m) with significant differences among treatments reflecting those for the total profile (Table 10.3).

Table 10.3. Mean values of profile start and end stored soil water (SSW, mm), change in SSW for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and the total soil profile (0.1–1.9 m), rainfall (mm) and rainfall refill efficiency (%) for each plant density and rainfall refill period. Least significant differences (LSD; $P = 0.05$) among treatment means for each season is provided where it is appropriate.

Density (plants/m^2)	Start SSW (mm)	End SSW (mm)	0.1–0.7 m	0.7–1.3 m	1.3–1.9 m	0.1–1.9 m	Rainfall (mm)	Rainfall refill efficiency (%)
				Year 1 – 2012 (14 May 12–28 Aug 12)				
D0	903	902	0	-1	-1	-2	168	-1.1
D1	884	901	18	0	-1	18		10.5
D4	854	896	42	1	-1	42		24.8
D9	840	894	53	1	1	54		32.1
D16	834	883	49	1	-1	49		29.4
LSD	19.4	NS	13.1	NS	NS	14.9	-	8.87
				Year 2 – 2013 (21 May 13–23 Jul 13)				
D0	895	907	11	1	0	12	125	9.5
D1	821	888	65	3	0	68		54.2
D4	773	847	72	0	2	75		59.7
D9	759	833	78	-6	3	74		59.2
D16	750	826	74	3	-1	76		60.9
LSD	30.2	32.9	16.0	NS	NS	20.2	-	16.14
				Year 3 – 2014 (26 May 14–21 Aug 14)				
D0	893	900	8	-1	0	7	102	6.8
D1	734	760	26	1	-2	25		24.9
D4	724	751	28	2	-2	27		26.8
D9	708	735	33	0	-6	27		26.2
D16	703	732	28	-1	2	29		28.8
LSD	21.9	24.5	10.3	NS	4.2	11.2	-	11.01

In 2013, starting SSW levels were lowest in the D4–D16 treatments (750–773 mm) and highest in the bare treatment (D0, 896 mm). At the end of the refill period D0–D1 treatments had the highest SSW levels (888–907 mm). The other three treatments refilled to lower ($P < 0.05$), but similar levels (826–847 mm, $P > 0.05$), which reflected their starting values of SSW. Similar to the previous year the changes in SSW only occurred in the upper profile (0.1–0.7 m) with D1–D16 treatment gaining more soil water (66–78 mm) than the D0 (11 mm).

At the commencement of the refill period in 2014, digit grass with densities D4–D16 were significantly drier than the bare treatment plot (703–724 mm *cf.* 893 mm). All treatments containing plants (i.e. D1–D16) accumulated similar quantities of soil water (25–29 mm), the vast majority in the upper profile ($P < 0.05$), however unlike previous years, D1 treatment did not completely refill, but had less SSW than the D0 treatment (760 mm *cf.* 900 mm). The D4–D16 treatments refilled to similar levels (732–751 mm, $P > 0.05$), but lower than D0 ($P < 0.05$).

10.3.6 Rainfall refill efficiency

Refill efficiencies varied significantly each year; all treatments containing digit grass plants having higher efficiency than the D0 treatment plots (Table 10.3). In the first refill period, the D1 density treatment was intermediate, but in the two latter years there were no significant differences between the four densities which contained plants (i.e. D1–D16). Efficiencies were highest during the 2013 refill period (averaging 59% *cf.* 10% for the bare treatment) and similar during the 2012 and 2014 refill periods (average 24 and 27% respectively).

10.3.7 Herbage production

During the establishment year (2011–12), total growing season herbage production ranged from 4809 to 8381 kg DM/ha (D1 and D9, respectively), but were not significantly different ($P > 0.05$; Table 10.3). Similarly, in year 2 (2012–13) production ranged from 8164 to 10426 kg DM/ha (D1 and D4, respectively), but differences were not significant ($P > 0.05$, Table 10.4). In year 3, the D4 treatment had the highest herbage production (4887 kg DM/ha), being significantly greater than the other three treatments containing plants ($P < 0.05$, Table 10.4). In year 4, treatments with densities ≥D4 had similar herbage production (9800–9470 kg DM/ha), but significantly greater than those with D1 (5913 kg DM/ha) (Table 10.4).

Over the 4 years of the experiment, total herbage production was highest for D4 (32557 kg DM/ha; $P < 0.05$), although there was no significant difference between treatments with densities ≥D4 (Table 10.4, Fig. 10.4a; $P > 0.05$).

10.3.8 Water use efficiency

In the first 2 years of the experiment, SSW values at the start of the growing season were similar for the five treatments and although the total volume of water used by digit grass at the different plant densities varied ($P < 0.05$), WUE for the four treatments containing plants did not ($P > 0.05$) ranging 8.5–13.6 kg DM/ha/mm (Table 10.4). In year 2 (2012–13), treatments D4–D16 had the highest and similar water use (572–583 mm), followed by D1 ($P < 0.05$). D0 treatment plots had less total water use than those containing plants (454 mm, $P < 0.05$). Water use efficiency values ranged 14.4–18.2 kg DM/ha/mm and were the highest achieved during the experiment.

In year 3 (2013–14), D0 and D1 treatments had the highest starting SSW levels, while the other densities were similar. The highest total was use was D1 (551 mm), while D4–D16 were similar (519–521 mm). D4 had the highest WUE, being significantly greater than the other three densities (9.4 *cf.* 6.5–7.5 kg DM/ha/mm).

Table 10.4. Herbage production (kg DM/ha) and components of the water balance used to calculate water use efficiency (kg DM/ha/mm) for treatments in each growing season and the total experimental period. Least significant differences (LSD; *P* = 0.05) among treatment means for each season are provided where it is appropriate.

Density (plants/m^2)	Herbage (kg DM/ha)	Rainfall (mm)	Start SSW (mm)	End SSW (mm)	Total Water Used (mm)	WUE (kg DM/ha/mm)
			Year 1 – 2011–12			
D0	-[a]	545	900	903	541	-[a]
D1	4809		905	884	566	8.5
D4	6669		901	854	592	11.3
D9	8381		910	840	614	13.6
D16	6764		899	834	609	11.1
LSD	NS	-	NS	19.4	14.8	NS
			Year 2 – 2012–13			
D0	-	448	902	895	454	-
D1	8164		901	821	529	15.5
D4	10426		896	773	572	18.2
D9	9489		894	759	583	16.3
D16	8404		883	750	581	14.4
LSD	NS	-	NS	30.2	33.4	NS
			Year 3 – 2013–14			
D0	-	396	907	893	411	-
D1	4111		888	734	551	7.5
D4	4887		847	723	520	9.4
D9	3549		833	708	521	6.8
D16	3381		826	703	519	6.5
LSD	751.8	-	32.9	21.9	19.1	1.39
			Year 4 – 2014–15			
D0	-	684	900	903	680	-
D1	5913		760	724	719	8.2
D4	9800		751	726	709	13.8
D9	9470		735	718	701	13.5
D16	9831		732	720	696	14.2
LSD	1422.1	-	24.5	40.7	20.2	2.40
			Total (2011–15)			
D0	-	2073	900	903	2649	-
D1	23438		905	724	2649	8.9
D4	32557		901	726	2644	12.3
D9	31498		910	718	2660	11.8
D16	29019		899	720	2646	11.0
LSD	3920.4	-	NS	40.7	NS	1.45

[a]Nil plants

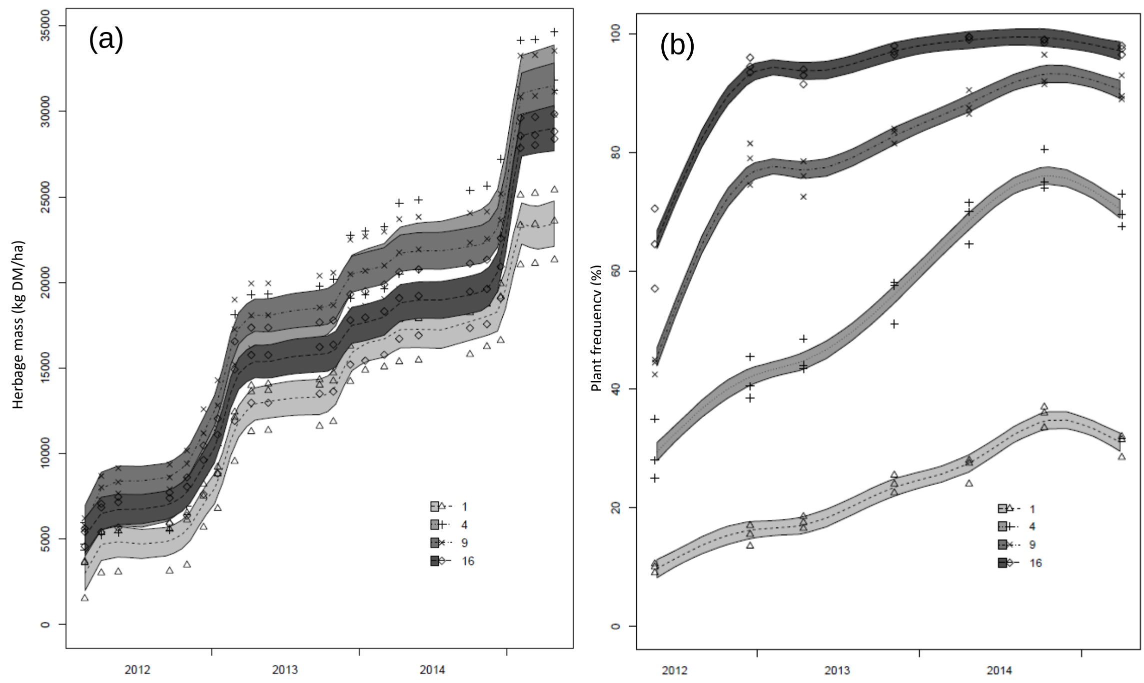

Fig. 10.4. (a) Cumulative herbage production (kg DM/ha) of digit grass from February 2012–May 2015 at 1, 4, 9, and 16 plants/m^2. (b) Plant frequency of digit grass from May 2012–May 2015 at 1, 4, 9, and 16 plants/m^2. In both figures, solid and dashed lines represent the predicted value ± s.e. of prediction, respectively.

In the final year of the experiment (2014–15), SSW values at the start of the growing season were again highest in the bare treatment plots (D0, 900 mm, $P < 0.05$), with SSW values decreasing with increasing plant density such that D4–D16 had the lowest SSW levels (732–751 mm). Similar to the previous year, D1 had the highest total water use (719 mm, $P < 0.05$), but was not significantly different to the D4 and D9 densities treatments. At the end of the growing season SSW levels were highest in D0 treatment plots (903 mm) and all treatments containing plants had lower but similar SSW content (718–726 mm, $P < 0.05$). Despite having the highest total water use, D1 had the lowest water use efficiency (8.2 kg DM/ha/mm, $P < 0.05$) and there were no significant differences between the other three plant densities (13.5–14.2 kg DM/ha/mm).

Total water use calculated over the entire period of the experiment was similar for the four treatments containing plants (i.e. D1–D16). Water use efficiency over this period was lowest for D1 (8.9 kg DM/ha/mm, $P < 0.05$), and while D4 had the highest WUE, it was similar for D4–D16 (11.0–12.3 kg DM/ha/mm; Table 10.3).

10.3.9 Plant frequency

The initial assessment conducted at the conclusion of the establishment year showed plant frequency of digit grass at the four densities were different and ranked in plant density order ($P < 0.05$) with D1 having the lowest plant frequency (10%) and D16 the highest (29%, Fig. 10.4b). The rate of increase in plant frequency for each treatment and the level that they appeared to plateau varied, with D1 increasing in a linear trend over the 4 years of the experiment, reaching a maximum in year 4 of 35%. Digit grass with D4 also reached maximum plant density (77%) in year 4. Plant frequency of digit grass at D9 and D16 increased rapidly in the first year; reaching 78 and 94% respectively by December 2012. Plant frequency in the D9 treatment had a further linear increase, reaching maximum in year 4 (94 %), while D16 plateaued at about 98% (Fig. 10.4b).

10.4 Discussion

This experiment has shown that in a frost prone summer dominant rainfall zone the largest differences between plant densities of digit grass for the metrics assessed occurred during the first 2 years. After this period there were few differences between densities 4–16 plants/m^2 for any of the hydrologic or agronomic measures, with the exception of plant frequency. The differences between treatments only occurring for a limited period of time is related to the finite resources that were available (e.g. stored soil water) and the rate they were utilised by the establishing pasture; the higher plant density (16 plants/m^2) utilising the resources fastest until all densities ≥4 plants/m^2 responded similarly and were dependent on rainfall for continued production.

A density of 1 plant/m^2 was too low to effectively and efficiently use the resources available (e.g. Hayes *et al.*, 2010b; McCaskill and Kearney, 2016) and was ranked lowest for all parameters measured throughout the experiment (with the exception of root depth during year 3). Digit grass with a plant density of 4 plants/m^2 was ranked low during year 1 and 2, but as the plants developed and crowns increased, this density had similar hydrologic and growth responses to the higher density swards, and over the duration of the experiment showed the highest WUE. The exception was plant frequency which peaked at 77% during year 4. At 9 plants/m^2, digit grass performed well each year for all metrics, including plant frequency which was greater than 80% from the beginning of year 3. In the establishment year, digit grass at the highest density, 16 plants/m^2, was the most productive with the greatest soil water extraction and rooting depth, and achieved plant frequency values greater than 90% by the start of year 2, however, in subsequent years it tended to lag behind those with 4–9 plants/m^2 (although not always significant) except following periods of above average rainfall.

A grazed perennial pasture requires a sufficient plant population to achieve both production and sustainability goals: high production and persistence (Hayes *et al.* 2010a,b; McCaskill and Kearney, 2016) also high ground cover to reduce weeds (Dear *et al.* 2007; McCaskill and Kearney, 2016), minimise runoff (Murphy *et al.* 2004a), and maximise infiltration (Murphy *et al.* 2010), maximise soil water usage (extraction and rooting depth) to produce a dry profile to store winter rainfall (Hayes *et al.*, 2010b; McCaskill and Kearney, 2016). There is also a threshold plant density below which a species is not able to achieve these goals (e.g. Virgona, 2003; Hayes *et al.* 2010a,b; Dolling *et al.* 2011; McCaskill and Kearney, 2016). Our data indicated that digit grass with density of 1 plant/m^2 was below this threshold, while densities ≥4 plants/m^2 achieved both production and sustainability goals. Practically, however, there are advantages of targeting 4–9 plants/m^2 because these densities also allow soil water resources to be available for 18–24 months beyond establishment, and so potentially provide the opportunity for establishment of legumes before resources are exhausted.

Optimum plant density of a species is known to vary with climate (rainfall and temperature), soil fertility, plant available water and cultivar (Palmer and Wynn-Williams, 1976) with the optimum density for maximum herbage production likely to be lower in a lower rainfall environment than a higher rainfall environment (Dear *et al.* 2007). Our data suggest that in this summer dominant rainfall zone the optimum plant density is 4–9 plants/m^2 and therefore intermediate to those suggested for southern WA and Queensland. Our lower density range is similar to that for the 400–600 mm zone in southern WA (Nichols *et al.*, unpublished data), while our upper range is similar to Queensland (W Scattini, pers. comm.). However, it is pertinent to note that our experiment is the first to empirically evaluate the impact of plant density on herbage production and water use efficiency in this environment.

The majority of soil water extraction occurred in the upper soil profile (i.e. 0.1–0.7 m) and although water content declined in the middle profile (0.7–1.3 m) in year 2 and 3, the values were smaller and similar among treatments. Similar results have been found for tropical grasses, including digit grass on a brown Vertosol in this region (Lodge *et al.* 2010b) and Hayes *et al.* (2010b) also reported little change in soil water content below 0.75 m for a range of summer and winter active forage species.

The soil profile of all treatments refilled to the same level of the bare plots during the winter in year 1, however, only the low density (1 plant/m^2) plots refilled fully during winter in year 2. This was due to a combination of greater rooting depth and total soil water extraction of the higher density treatments during the second growing season (Table 10.2) and lower winter rainfall (Table 10.3). However, rainfall refill efficiencies were higher in winter year 2 (>50% *cf.* 11–32% in first winter), presumably due to higher ground cover (as indicated by higher plant frequency values). These rainfall efficiencies are similar to those reported for digit grass on a red Chromosol soil (Murphy *et al.* 2010).

Several studies conducted in the even/winter dominant rainfall zones have tested the ability of summer active species to utilise summer rainfall and so create a dry soil 'buffer' to store winter rainfall, which can minimise soil water leaking below the rooting zone into the water table (e.g. White *et al.* 2000; McCaskill and Kearney, 2016). Here, our aim was also to prevent water and nutrient moving below the rooting zone, but importantly also to provide a reservoir of stored soil water to be used by the tropical pasture during spring.

Maximum rooting depth of digit at all plant densities was achieved during year 3, with the exception of the highest density treatment (16 plants/m^2) which achieved a maximum and similar rooting depth during both year 2 and 3. Digit grass has been reported to have an active rooting depth of 1.2 m during year 2 on a red Chromosol soil (Murphy *et al.* 2008a, 2018) which is comparable with rooting depth of digit grass with a density of 16 plants/m^2 at the same age on a brown Vertosol. By year 4 when the initial stored soil water resources had been utilised and the pasture was dependent on winter and summer rainfall for soil water accumulation and plant growth, maximum plant root depth was restricted to <0.7 m (Table 10.2).

A study conducted on a red Chromosol soil in this zone reported digit grass produced over 16 t DM/ha, utilised incident rainfall and extracted 137 mm of soil water to achieve a WUE of 32.4 kg DM/ha/mm (Murphy *et al.* 2008b), which is almost double the WUE values determined in our study. This large difference may be partly due to different herbage mass assessment methods (i.e. calibration quadrat cutting height of 10 mm in previous study *cf.* 50 mm in this study), however the difference is most likely due to WUE being a complex interaction of soil type, rainfall distribution, water availability and nutrition.

Our data show that WUE in year 3 (2013–14) was distinctly lower than other years yet starting stored soil water and total amount of water used were similar. The contrast was herbage production in year 3 was less than half of the previous years, despite similar total water use. The difference between years was the timing of rainfall received during the growing season. In year 3, rainfall during the key months of December, January and February when high growth rates may be expected, was well below average, which forced plants to actively extract stored soil water to meet their requirements rather than extracting freely available water (Bennett and Doss, 1963).

In comparison, during year 4 starting stored soil water values were the lowest of the experiment suggesting tough growing conditions, however, rainfall during the growing season was the highest received during the experiment, allowing grasses to express their high growth rate potential resulting in higher herbage production and higher values of WUE.

An advantage of digit grass pastures with densities 4–9 plants/m^2 is the opportunity to establish a legume. If temperate legumes are to be added to digit grass, our results indicated that they would be best sown during the first 1–2 winters following tropical grass establishment as the soil profile has a generally higher level of SSW and is more easily replenished over winter. After that time, the soil profile is drier, particularly at the end of the grass growing season (May), and especially in the upper soil profile (0–0.7 m). These findings support research conducted in southern NSW which indicated that perennial pastures with high plant densities dry the soil, limiting persistence of temperate annual legumes (Dear and Cocks, 1997; Dear *et al.* 1998). To establish companion legumes after the initial soil water resources have been depleted, a wet winter would be required to provide good rainfall for the legumes and refill the soil profile or alternative strategies that promoted soil water accumulation such as plant or soil disturbance (Peck *et al.* 2015; O'Reagain *et al.* 2015). Lower plant densities (e.g. 4–9 plants/m^2) provide suitable ground cover conditions for establishment (e.g. Boschma *et al.* 2018a) and greater opportunity to build populations of companion legumes, and recruit more grass plants.

Our study was somewhat artificial and unlike a commercial pasture because we did not allow recruitment of other plants. Some species, including digit grass, readily recruit seedlings, however, low densities (e.g. 1 plant/m^2) require both time and management for the plant population to build. In addition, while digit seedlings may be recruiting, there is a risk that weeds will also recruit and dominate a pasture (e.g. Dear *et al.* 2007).

The optimum plant density determined in this study was for a single species and while the results may be similar for other bunch grasses, they are not likely to be applicable to rhizomatous or stoloniferous grasses, such as *Chloris gayana* (Rhodes grass), *Bothriochloa insculpta* (creeping bluegrass) or *Pennisetum clandestinum* (kikuyu). An alternative method that may have made results applicable to more species would be assessing tiller density.

10.5 Conclusions

Our study has shown that in this environment, digit grass pasture with a density of 4 or more plants/m^2 will have high herbage production, soil water extraction and plant frequency, while 1 plant/m^2 is insufficient to achieve either production or sustainability goals. We recommend that a digit pasture with 4–9 established plants/m^2 is optimum for herbage production and hydrological performance with the advantage of having sufficient carryover initial resources to establish a legume over the following 24 months.

11 Alternative sowing times of temperate annual legumes to improve their establishment and productivity in tropical perennial grass mixtures

S. P. Boschma and M. A. Brennan

11.1 Introduction

Temperate annual legumes and tropical grass pastures have alternate growth patterns, that is, the legumes regenerate in autumn, growing through winter and senesce in spring, while the grass recommences growth in spring, continues throughout summer and ceases when frosts start in autumn. In a mixed pasture, these species offer the opportunity to provide almost year round feed.

Maintaining a seed bank is considered important for persistence of temperate legumes (e.g. Hagon 1974; Lodge 1993) but the optimum time to sow to legume and the grass to achieve this is unknown (Chapter 4). In a highly variable environment with increased risk of regeneration failure in a tropical grass pasture (Chapter 4), development of a seedbank in the establishment year is highly beneficial so that pasture management for livestock production can commence within the shortest timeframe without fear of compromising legume persistence.

In Northern Inland NSW, temperate annual legumes are commonly sown in autumn following establishment of the tropical grass. Two alternative sowing technique developed in Western Australia (WA) are twin and summer sowing (Loi and Nutt 2010; Loi *et al.* 2012; Loi and Yates 2018). In both cases pod of hard seeded legumes are sown in either autumn (twin sowing) or summer (summer sowing). The hard seeded legumes breakdown over the summer period and germinate the following autumn. These techniques have the advantages of allowing legume pod grown and harvested on-farm to be sown (reduced seed cost) either in autumn with a crop (single sowing operation) or in summer (when there are no/fewer conflicting farming-grazing operations) (Loi and Nutt 2010; Loi and Yates 2018). In WA, the twin and summer sown legumes commonly germinate earlier than those sown in the traditional sowing window, and provide quality forage earlier in the season and greater total productivity over the entire growing season (Loi and Nutt 2010; Loi and Yates 2018). These techniques have been trialled in southern NSW and found to have potential in mixed farming systems (e.g. Hackney and Quinn 2015; Howieson and Hackney 2018), but neither technique has been tested in a summer dominant rainfall zone. A derivative of these techniques could however be useful for establishing hard seeded legumes into tropical grass pastures, that is, sowing pod of hard seeded legumes in spring at the same time as the tropical grass so that the legume can soften over the summer and germinate in autumn.

This study evaluated a combination of three temperate annual legumes sown before, at the same time (spring sowing) or after digit grass (traditional method) to determine the optimum sown time as determined by legume (seedling establishment and regeneration, and herbage production) and grass productivity (establishment, herbage production and plant persistence).

11.2 Methodology

11.2.1 Site and treatments

The experimental site was located about 18 km west of Manilla (30°42’11” S, 150°30’10” E, elevation 412 m ASL) on a brown Chromosol soil (Isbell 1996) and co-located with the temperate grass evaluation experiment described in Chapter 4. Average annual rainfall at Manilla is 576 mm. Soil pH_{Ca} was 6.1 and 7.3 for 0–0.1 m and 0.1–0.2 m depths respectively (detailed nutrient analyses are provided in Chapter 4. In the two winters prior to sowing the first treatment, the area was sown to forage oat (*Avena sativa*) during the winter period and fallowed over the summer period. Weeds were controlled during the fallow periods and the unsown plots with glyphosate (450 g/L a.i. at 1–1.5 L/ha), 2,4-D (400 g/L a.i. at 1.7–2 L/ha) and/or paraquat (135 g/L a.i. at 2.5 L/ha) and diquat (115 g/L a.i. at 2.5 L/ha).

The experiment consisted of five treatments based on the sowing time of the temperate legumes and tropical grass arranged in a randomised complete block design with three replicates. The treatments were:

1. *Legumes sown in autumn 2013 and grass sown in spring 2014* to allow the legumes to establish a seed bank in 2013, but remove the legumes in late winter-early spring 2014 (e.g. glyphosate) to allow soil water accumulation prior to sowing the grass.

2. *Legumes sown in autumn 2014 and grass sown in spring 2014* to allow the legumes to set seed prior to sowing the grass.
3. *Scarified legume seed and grass seed sown simultaneously in spring 2014* to allow the legumes and grass to establish in spring.
4. *Legumes in pod and grass seed sown simultaneously in spring 2014* to allow the grass to establish in spring and the legume in pod to soften over summer to germinate in autumn 2015.
5. *Grass sown in spring 2014 and legumes sown in autumn 2015* to allow the grass to establish prior to sowing the legumes.

The legumes were combined and consisted of: bladder clover (*Trifolium spumosum*) cv. Bartolo, French serradella (*Ornithopus sativus*) cv. Margurita, and biserrula (*Biserrula pelecinus*) cv. Casbah. Sowing rates and dates are provided in Table 11.1 and seeds were inoculated with the appropriate strain of commercial rhizobia and permethrin 25:75 (250 g/kg a.i. at 5 g/kg seed). Digit grass (*Digitaria eriantha*) cv. Premier was sown at 4 kg/ha coated seed adjusted for germination on 18 November 2014. The grass failed to establish due to poor seasonal conditions and was resown in February 2015. The resown experiment was covered with insect-proof netting from March to June 2015 to protect the seedlings from grasshoppers (*Austroicetes* sp.).

Table 11.1. Treatment identification number (ID), sowing details, also sowing date and seed rate (kg/ha) for the legumes (bladder clover, biserrula, French serradella) and digit grass.

ID	Treatment sowing time	Pasture	Autumn 2013 (21 May 13)	Autumn 2014 (13 May 14)	Spring 2014 (18 Nov 14)[5]	Autumn 2015 (24 April 15)
T1	Autumn 2013 legume-	Legume	6[1],1[2],3[3]			
	spring 2014 grass	Grass			4[4]	
T2	Autumn 2014 legume-	Legume		6,1,3		
	spring 2014 grass	Grass			4	
T3	Spring 2014 scarified	Legume			6,1,3	
	legume and grass	Grass			4	
T4	Spring 2014 podded	Legume			10,4,5	
	legume and grass	Grass			4	
T5	Spring 2014 grass-	Legume				6,1,3
	autumn 2015 legume	Grass			4	

[1]Bladder clover (coated seed 1:1); [2]Biserrula (bare seed); [3]French serradella (bare seed; [4]Digit grass (coated seed 1:3); [5]Digit grass was resown 19 February 2015

The plots were either sown by a disk seeder or by hand in 6 rows/plot (each 0.25 m apart) at about 10 mm depth into plots 6.6 m x 1.5 m wide. The hand sown rows were created by forming a furrow, sowing seed and rolling with a light roller to bury the seed and improve seed soil contact.

Due to dry conditions, 20 mm irrigation was applied on 5 September 2013 to ensure seed set of the temperate legumes (T1 plots only). All plots were also irrigated with 85 mm during February–March 2015 (37 day period) to assist establishment of digit grass.

Single superphosphate (8.8% phosphorus, 11% sulfur) was applied annually to all sown plots; 150 kg/ha in October 2013, 80 kg/ha May 2014, 75 kg/ha in October 2015, November 2016 and April 2017). Rainfall was collected from a manual gauge installed at the experimental site. Long term average (LTA) rainfall data were used from Bureau of Meteorology Station 55274 (number 55274, 1909–2013) located near Kelvin, approximately 20 km south west from site.

11.2.2 Data collection

11.2.2.1 Seedling density

Digit grass seedling density counts were conducted in May 2015, 10 weeks after the second sowing. Counts of legume seedling density (seedlings/m^2) were conducted 6–10 weeks after sowing or regeneration. Seedling densities were determined by counting the number of seedlings in six random quadrats (0.1 x 0.5 m) in each plot and converting to plants/m^2.

11.2.2.2 Herbage production

Herbage mass (kg DM/ha) was generally assessed in all sown plots by a single operator by visually estimating total herbage mass (continuous 0–5 scale where 0 = nil and 5 = high) and the proportion (%) of the total herbage mass of each sown legume and sown grass in three equal strata per plot (i.e. three assessments per plot). Twenty calibration quadrats (0.4 x 0.4 m) representing the range of herbage mass in the experiment were also scored. These quadrats were harvested to about 10 mm above ground level, sorted into the three legume species and digit grass components, dried at 80^{o}C for 48 h then weighed. Herbage mass scores and percentage estimates were regressed (linear or quadratic $R^2 > 0.80$) against actual herbage mass (kg DM/ha) and percentage of each component to determine the herbage mass of each sown species. After each assessment the plots were mown with a rotary mower and the herbage removed from the plots, with the exception of the final legume assessment in spring each year when mowing was delayed until the legumes had senesced and the material deposited onto the ground. Herbage mass of both legumes and digit grass were assessed three times per year during their respective growing seasons. The experiment was mown with a rotary mower and the herbage removed from the plots after each herbage mass assessment, with the exception of the spring assessment when the temperate legumes were allowed to flower and senesce.

11.2.2.3 Grass persistence

Digit grass plant frequency was assessed in a permanent quadrat (1.0 x 0.5 m) centrally located in each plot. Estimates were generally taken 0–7 days after the experimental area was mown. For each plot, cells containing a portion of a live digit grass plant were recorded (presence) and the proportion (%) of occupied cells used to estimate frequency of occurrence (plant frequency, Brown 1954). Plant frequency was assessed three times: November 2015, May 2016 and June 2017 at the start and end of the second grass growing season and end of third growing season.

11.2.3 Statistical analyses

Differences among treatments for seedling density (plants/m^2; for individual legume species and total legume), growing season herbage mass (sum of all assessments conducted within a growing season for individual legume species, total legume and digit grass) and plant frequency (%) were examined using a linear model. Statistical analyses were conducted using R (R Core Team 2017). The data did not warrant transformation to stabilise the variance, with the exception of legume herbage mass in 2014 and 2015 which were log and square root transformed respectively. A least significant difference of means (LSD; $P = 0.05$) was calculated for all significant effects for comparison among treatments.

11.3 Results

11.3.1 Environmental conditions

Monthly rainfall was below average 36 of the 52 months (69% of LTA rainfall) that this experiment was conducted and 18 of the 20 month period (90% of LTA, August 2013–April 2015) the five treatments were sown (Fig. 11.1). The two legume growing seasons (May–September 2015 and 2016) following establishment of all treatments received near average and above average rainfall respectively (92% and 127% of LTA respectively). Rainfall received during the two grass growing seasons (October–April 2015–16 and 2016–17) was well below average (46% and 57% respectively).

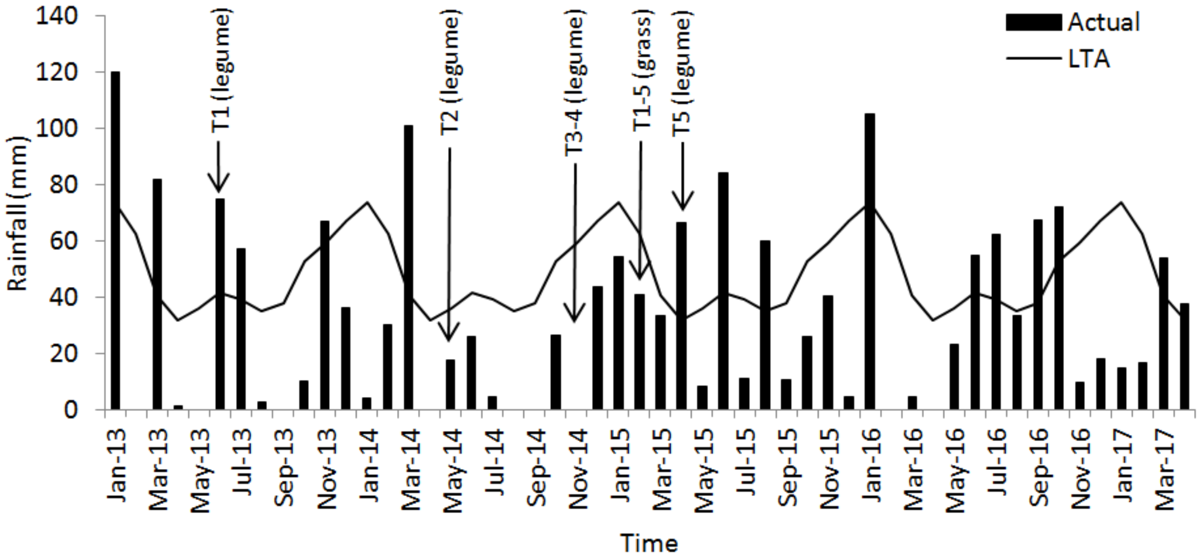

Fig. 11.1. Actual and long term average (LTA) monthly rainfall (mm) at the Manilla site, January 2013–April 2017. Month of sowing and resowing of the treatments are indicated. Treatment identifications (T1–T5) are summarised in Table 11.1.

11.3.2 Seedling density

In 2013 when the T1 legumes were sown, total seedling density was 84 plants/m^2 (Fig. 11.2a) with near equal densities of bladder clover and serradella. In 2014, T2 seedling densities were higher than those for regenerating in T1 (149 *cf.* 49 plants/m^2; $P < 0.05$; Table 11.2). T2 had similar densities of bladder clover and serradella, while in T1 the proportion of serradella had declined to be similar to biserrula; a third of the density of bladder clover.

In autumn 2015, all treatments had been sown. Of these, the two regenerating treatments (T1 and T2) had similar legume seedling densities to the autumn sown legumes (T5; 151–172 plants/m^2; Table 11.2). Similar to previous years, the newly established legume treatments (T5) had similar proportions of bladder clover and serradella, while the regenerating treatments (T1 and T2) were dominated by bladder clover and serradella was almost non-existent (<2% of total seedling population). The three autumn sown treatments (T1, T2 and T5) had higher seedling densities than T3 and T4 which were spring sown as either scarified or hard seed (7–10 plants/m^2; $P < 0.05$).

In autumn 2016, the legumes in all treatments regenerated, although the two spring sown treatments (T3 and T4) had significantly lower seedling densities (1718–2189 *cf.* 404–614 plants/m^2; Table 11.2). Bladder clover was the dominant species in all treatments.

Digit grass established when it was resown in February 2015 but with low seedling densities ranging from 5 (T4) to 12 plants/m^2 (T3)($P > 0.05$).

Table 11.2. Establishment and regeneration seedling densities (plants/m^2) of three legumes assessed autumn each year (2013–2016) for sown treatments. Treatment identifications (T1–T5) are summarised in Table 11.1.

Treatment	Bladder	Biserrula	Serradella	Total
		2013		
T1	43	4	37	84
		2014		
T1	30	9	10	49
T2	71	17	61	149
LSD	*14.5*	*NS*	*4.3*	*72.2*
		2015		
T1	128	44	1	172
T2	118	30	3	151
T3	5	2	0	7
T4	3	6	1	10
T5	58	43	56	157
LSD	*98.7*	*16.0*	*10.3*	*92.6*
		2016		
T1	1577	126	16	1718
T2	1849	336	5	2189
T3	79	325	0	404
T4	116	480	19	614
T5	1427	428	21	1876
LSD	*622.1*	*NS*	*NS*	*502.2*

11.3.3 Herbage production

In 2014 total legume productivity was higher for the regenerating legume treatment (T1) than the newly sown treatment (T2) with 3197 and 3340 kg DM/ha respectively ($P < 0.05$; Fig. 11.2a). Herbage production during the 2015 season, after all legumes had been sown, ranged from 1665 (T3) to 5944 kg DM/ha (T1, $P > 0.05$, Fig. 11.2c). In 2016, winter rainfall was above average allowing the legumes to show their potential productivity; the three autumn sown treatments (T1, T2, T5) had significantly higher production than the spring sown (T3, T4) legume treatments (7476–8019 *cf.* 3541–4714 kg DM/ha; Fig. 11.2e).

The contribution of French serradella to total herbage mass declined with each successive regeneration during the experiment. Biserrula contributed a significant proportion of herbage in 2015 (average about 50%), but in 2016, contributed ≤10% in the autumn sown treatments (T1, T2 and T5) compared to >50% in the spring sown treatments.

Digit grass was slow to establish and produced <500 kg DM/ha in the establishment year (2014–15; $P > 0.05$; Fig. 11.2b). Productivity was highest during the 2015–16 season with digit grass in the early established legume treatments (T1 and T2) having the highest productivity (2887–3825 kg DM/ha); both significantly greater than digit grass in the autumn sown legume treatment (T5; 1450 kg DM/ha). T5 was similar to the summer sown treatments (T3 and T4; Fig. 11.2d). In the final summer (2016–17) total productivity was <2500 kg DM/ha (Fig. 11.2f), but reflected the previous year with digit grass herbage mass highest in T1 and T2 (1679–2243 kg DM/ha) and the other treatments significantly lower (690–885 kg DM/ha; *P* < 0.05).

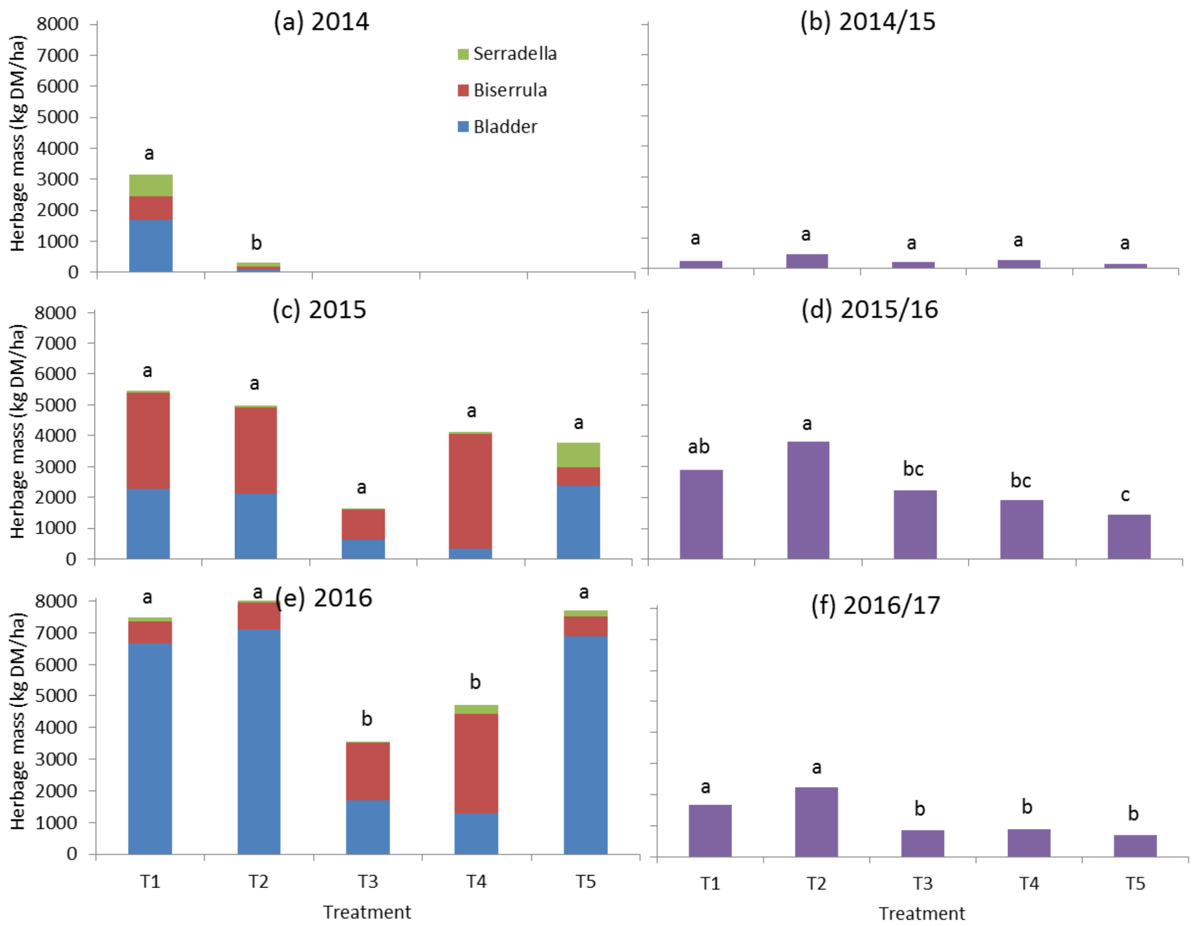

Fig. 11.2. Total growing season herbage mass (kg DM/ha) of the three legumes (a,c,e) and digit grass (b,d,f) from 2014 to summer 2016/17. Legumes are bladder clover, biserrula and French serradella. Bars with the same letter indicate total legume herbage mass values which are not significantly different (*P* = 0.05). Treatment identifications (T1–T5) are summarised in Table 11.1.

11.3.4 Grass persistence

Digit grass plant frequency increased slightly over the 18 months it was assessed. There were significant differences between treatments at each assessment (*P* < 0.05), however the treatment order was consistent: digit grass in T2 was highest (average 57%; Table 11.3) and similar to T1 (38%), while digit in T5 had the lowest plant frequency (average 12%) and was similar to T4 and T3 (15–22%).

Table 11.3. Plant frequency (%) of digit grass in the five treatments assessed three times from November 2015–June 2017. Treatment identifications (T1–T5) are summarised in Table 11.1.

Treatment	November 2015	May 2016	June 2017	Average
T1	39	34	42	38
T2	56	53	62	57
T3	20	23	22	22
T4	13	14	17	15
T5	10	12	13	12

LSD (P = 0.05)	*20.6*	*19.3*	*23.2*	*-*[1]

[1]Not analysed

11.4 Discussion

This study has shown that sowing temperate legumes in autumn, whether before or after the grass is sown will provide the greatest seedling densities and herbage production. In contrast, there was no benefit to sowing podded legumes in spring. The importance of choosing species adapted to the soil type was also evident in this study; soil pH suiting bladder clover which excelled and being unsuitable for biserrula and serradella.

11.4.1 Legume productivity

For the two treatments where legumes were established in autumn prior to grass establishment, seedling densities and herbage production were simular after the first year (i.e. regenerating legumes). Interestingly, the legumes sown in autumn after the grass (T5) had similar densities and productivity to the regenerating legumes. This may be because the establishing legumes benefitted from the cover provided by the grass (Boschma *et al.* 2018a) in addition to good rainfall during establishment. Boschma *et al.* (2018a) found that subterranean clover (*Trifolium subterraneum*) seedling densities were highest when ground cover was greater than 75% and the tropical grass pasture canopy cover was short (0.1 m tall) to minimise shading.

11.4.2 Sowing time

This experiment showed that there was no benefit of spring sowing. However, seedling density increased each year and herbage production in 2016 was >3500 kg DM/ha for all treatments and seed set high for all treatments (not quantified). This large seed set may have equalised the differences between the five sowing times tested, and regeneration and productivity in 2017 may have shown no or little differences. If this was the case spring sowing could be used in this environment, however the pasture would need to be managed to maximise legume seed set for at least 2 years after sowing to be equivalent to the first year of an autumn sowing. In studies conducted on the South Coast of Western Australia, Sanford *et al.* (2017, 2018) also reported no consistent benefit of summer sowing compared with autumn sowing and suggested that serradella can be successfully established at either time (Sanford *et al.* 2017).

Establishing legumes 1–2 autumns before sowing the tropical grass could be an alternative to forage oats which is commonly sown as part of the summer weed control program recommended for successful establishment of a grass pasture (Harris *et al.* 2014); the relative suitability of legumes or forage oat may depend on the enterprise. For example in mixed farming systems, sulfonylurea herbicides are commonly used which can persist many months and severely affect legume establishment/regeneration and growth (Noy 1996; Peck and Howie 2012). In these enterprises, a summer weed program incorporating cereals would be more beneficial. Legumes also have fewer broad leaf weed herbicide options than cereals.

Boschma *et al.* (2018b, Chapter 10) suggested that the best time to establish a companion legume into a new grass pasture is 1–2 years after sowing grass while initial soil water reserves are available. In this experiment, average rainfall in autumn 2015 allowed establishment of all legumes. In a separate experiment co-located at the site (reported in Chapter 4), this sowing technique was also used to establish temperate annual legumes into a digit grass pasture. In the experiment few legumes established well, including the three legumes used in this study. This shows the importance of planning and preparation to maximise success irrespective of the season.

The rapid decline in French serradella plant density and productivity clearly showed that it is not suited to this site. Biserrula also declined but to a lesser extent. French serradella and biserrula are best suited to soils with a pH_{Ca} of 4.0–7.0 (Hackney *et al.* 2012a,c) and the soil at this site was near and exceeded the upper limit in the 0–0.1 and 0.1–0.2 m depths respectively. It has been noted across the region that biserrula has greater tolerance of near neutral pH soils than serradella, however the pH at this site would have most likely disadvantaged both species, especially serradella. In contrast, the soil pH was within the suitable range for bladder clover (pH_{Ca} 4.8–8.0; Hackney *et al.* 2012b). Bladder has larger seed size than biserrula (500,000 *cf.* 1,000,000 seeds/kg; Hackney *et al.* 2012a,b), but higher seed yields than biserrula (700–800 *cf.* 300–500 kg/ha in southern NSW); both species are much higher yielding than subterranean clover (about 100 kg/ha)(Hackney *et al.* 2012a,b). A current study in Northern Inland NSW has shown that while both bladder and biserrula have high initial hard seed levels (>95%), the breakdown rate for biserrula is much greater (Chapter 13). All of these factors are likely to have contributed to the increase in proportion of bladder clover seedlings and herbage mass, and corresponding decrease of biserrula and serradella. Development of large seed banks of adapted hard seeded legumes is important for their long-term persistence.

11.4.3 Digit grass

Digit grass was inflicted with dry weather conditions throughout the entire experiment and this was clearly reflected in establishment plant densities and herbage production. Despite low establishment plant densities, if each seedling were to mature, the mature plant density is within the optimum range of 4–9 plants/m^2 suggested by Boschma *et al.* (2018b; Chapter 10). The adaptation of digit grass to this environment was demonstrated by increasing plant frequency, albeit small increases. Digit grass has proven itself on many occasions, and while establishment can be slow, once it is established it is persistent.

Digit grass established similarly in all treatments, but there were differences in the grass productivity and plant frequency during the 2015–16 and 2016–17 growing seasons. Over time plant frequency is a good measure of persistence, and in younger stands it also reflects crown development, especially when plant densities are low. Both herbage production and plant frequency measures showed that T1 and T2 were clearly superior sowing strategies and this may reflect greater availability of nitrogen fixed during the 6–18 months the legumes were established prior to the grass.

11.5 Conclusion

This study has shown that the optimal time to sow temperate annual legumes is autumn. The legumes can be sown 1–2 autumns before the grass is sown or autumn following grass establishment; the choice depending on enterprise and individual situation. In contrast, there was no benefit to spring sowing in this environment. Bladder clover was productive at this site and dominated both biserrula and French serradella which were not well adapted.

12 Can manipulating lucerne winter activity rating and spatial configuration in mixtures with digit grass affect agronomic and hydrological performance?

S.R. Murphy, S.P. Boschma and S. Harden

12.1 Introduction

Tropical perennial grass species can be productive, persistent and have become increasingly sown in Northern Inland NSW. These grasses are highly responsive to nitrogen (N) fertiliser application; with improvement in both dry matter production and forage quality (Boschma *et al.* 2014a, 2016). However, sole reliance on N fertiliser application on a pure grass sward can be expensive while companion legumes provide both a cost effective and sustainable option.

In Northern Inland NSW, temperate legumes have traditionally been sown; subterranean clover (*Trifolium subterraneum*) and medics (*Medicago* spp.) the main annual legumes and lucerne (*Medicago sativa*) the dominant perennial legume. As companion species, temperate legumes offer the potential to extend the growing season of a summer growing pasture from 6–8 months to 12 months of the year.

Northern Inland NSW presents a challenging environment for temperate annual legumes to germinate, grow and set seed within a sown summer active perennial grass sward. The grasses will utilise all, or the majority of plant available soil water over summer (Murphy *et al.* 2008a), leaving little in the soil profile for the regenerating legume. In this environment, autumn is the season with lowest rainfall, and springs often become hot and dry before temperate annual legumes can set seed large quantities of seed, thereby leading to depletion of the seedbank and limiting long-term persistence of these legumes. Lucerne is the most persistent (Lodge 1991a; Boschma *et al.* 2011) and widely grown legume in this region (Lodge *et al.* 1991), and is generally accepted by growers as a valuable forage. However, lucerne has potential to cause bloat in cattle, which can be mitigated by its incorporation with perennial grasses in a mixed pasture.

Lucerne is known to be a highly competitive plant species in mixed swards with tropical grasses; competition commencing at the seedling stage when its early growth rates exceed that of the more slowly establishing tropical grasses (Boschma *et al.* 2010a). However, once plants are established, lucerne is also widely reported as a strong user of stored soil water (e.g. Lolicato 2000), and can dominate herbage production within the sward (e.g. Hill 1991). Altering the spatial arrangement of the components in the mixed sward to separate the grass and legume is a possible way to alleviate the interspecific competition between the tropical grass and lucerne, both at seedling stage and as a mature sward (Boschma *et al.* 2010a). Further, while the main growth period of lucerne is during spring-summer (when tropical grasses also are active), cultivars have varying levels of winter activity rating (WAR) ranging from inactive (WAR 1; not available in Australia) to highly active (WAR 10). Therefore, it is possible that lucerne WAR may influence the interspecific competition due to differences in cool season growth and so soil water use.

A field experiment was established to examine the effects of both lucerne WAR level and spatial configuration in mixed swards with digit grass (*Digitaria eriantha*) on soil water dynamics, rooting depth, plant frequency, herbage production, and water use efficiency over four years at Tamworth NSW Australia. The studies examined the hypotheses; that greater spatial separation of digit grass and lucerne favour both grass and lucerne production and; that greater winter activity rating favours the production of lucerne in mixed swards at the expense of grass.

12.2 Methodology

12.2.1 Site description

The experiment was conducted at Tamworth Agricultural Institute (31°08’43”S 150°58’06”, 400 m above sea level) on a brown Vertosol (Isbell 1996). The annual average rainfall at Tamworth is 674 mm with 60% falling in the warm season between October–March. In the 2 years prior to establishing the experiment, the site had been sown to forage oat (*Avena fatua*) each autumn and fallowed each summer. In 2011, the forage oat was sprayed with glyphosate (450 g/L a.i. at 1.5 L/ha) 3 months prior to establishing the experiment to accumulate soil water and residual plant material was removed in November.

An automatic weather station located at the site recorded rainfall (0.5-mm pluviometer) at 30-min intervals.

12.2.2 Neutron probe access tube installation and soil profile description

On 1 October 2011 an aluminium access tube (49 mm extern. diam. sealed at the bottom) was installed in the centre of each plot to 1.9 m depth after removing a soil core (51 mm extern. diam.) with a hydraulic push-coring machine and pouring about 1 L of a clay slurry (1 kg kaolinite: 1 L water) to fill any voids and ensure good contact between the access tube and soil (White and Ridley 1998). A cap was placed over the top of the tube to prevent rainwater from entering. The soil cores were used to calibrate the neutron moisture meter (NMM) using method described in Murphy *et al.* (2017) and to analyse soil characteristics.

Soil water content (g/g^3) was determined by dividing each soil core into segments (0–0.1, 0.1–0.3, then 0.2 m intervals to 1.9 m), retaining the samples in plastic bags, which were weighed wet in the field and then again after drying at 105°C for 48 h. Soil bulk density (Mg/m^3) was calculated using the soil dry weight and volume of each soil core segment. Three additional samples at each depth were air dried (40°C) and analysed to determine soil pH_{Ca}, electrical conductivity ($EC_{1:5}$ dS/m) and soil particle size distribution.

The soil profile was non-saline ($EC_{1:5}$ <1.3 dS/m) with a neutral pH trend with values being neutral at the soil surface, 0–0.1 m, (pH_{Ca} 6.9), neutral in the upper profile, 0.1–0.9 m, (pH_{Ca} 7.0–7.6) and slightly alkaline in the middle and lower profile, 0.9–1.9 m, (pH_{Ca} 8.0–8.2, Table 12.1). Proportion of soil particles (sand 20–2000 µm, silt 2–20 µm, clay <2 µm, gravel >2000 µm) for each layer were determined using the laser diffraction method (Malvern Mastersizer 2000) and showed that the proportion of clay was greater than for either sand or silt for all sampling layers, apart for 0–0.3 m where silt was abundant (Table 12.1). Clay content was highest (36–43%) for soil layers below 0.3 m depth, while silt content declined with depth (39.0 to 31.1%). Soil texture at the surface (0–0.1 m) was silty clay loam and trending to silty clay at depths > 0.5 m.

The soil physical data were used in pedotransfer functions (McKenzie and Cresswell 2002) to predict soil water content values for the drained upper limit (*c*. -10 kPa) and crop lower limit (*c*. -1500 kPa) for each soil layer. The profile stored soil water at drained upper limit and crop lower limit were 817±1.1 and 439±0.1 mm, respectively providing a maximum plant available water content (PAWC, mm) of 378±1.0 mm.

Table 12.1. Proportion of sand (20–2000 μm), silt (2–20 μm) and clay (<2 μm) soil particles, soil salinity and soil pH of the brown vertosol sampled in 10 layers at Tamworth Agricultural Institute.

Sampling layer (m)	Sand (%)	Silt (%)	Clay (%)	Soil salinity (dS/m)	Soil pH_{Ca}	Soil bulk density (g/g^3)
0–0.1	30.6	39.0	30.4	0.24	6.9	0.68
0.1–0.3	28.5	37.3	34.2	0.12	7.0	1.10
0.3–0.5	20.9	38.7	40.3	0.11	7.0	1.14
0.5–0.7	22.4	35.9	41.7	0.12	7.2	1.16
0.7–0.9	28.8	34.4	36.7	0.23	7.6	1.14
0.9–1.1	29.0	35.0	36.0	0.42	8.0	1.30
1.1–1.3	25.2	32.8	42.0	0.72	8.2	1.34
1.3–1.5	24.7	32.6	42.7	1.05	8.2	1.33
1.5–1.7	28.1	31.1	40.8	1.23	8.2	1.33
1.7–1.9	30.6	39.0	30.4	1.23	8.2	1.40

12.2.3 Neutron probe calibration

The NMM (CPN503–DR Hydroprobe, Boart Longyear Co., Martinez, CA) was calibrated by determining the relationship between neutron count (16 s) and values of volumetric soil water content (SWC, θ_{vol}, m^3/m^3), collected when access tubes were installed and at other times with conditions of high (near drained upper limit) and low (near crop lower limit) SWC, giving a range of values (n = 140) (Lodge *et al.* 2010a). When access tubes were installed SWC was allowed to equilibrate for 24 h before taking neutron counts. Gravimetric SWC was determined on retained soil cores sampled at 0.2 m depth intervals from 0.1 m to a maximum depth of 1.9 m and converted to volumetric SWC by using bulk density values for each layer (Table 12.1).

For each soil layer, values of volumetric SWC (θ_{vol}) and neutron count were plotted and logarithmic regression best explained the relationship between SWC and neutron count (R^2 = 0.87, n = 140).

12.2.4 Experimental design and establishment

The experiment was a randomised complete block design with sowing configurations allocated to a row for ease of sowing, with three replicates of 15 treatments making 45 plots in total, with each plot 4 x 4 m. The 15 treatments (Table 12.2) included; digit grass and three lucerne cultivars in pure and mixed grass-lucerne swards at three spatial configurations, together with a mulch covered fallow. Winter activity rating (WAR) levels were used to select the three lucerne cultivars with contrasting cool season growth patterns: Q31 (WAR 3, dormant), Venus (WAR 5, semi-dormant) and Pegasis (WAR 9, highly active). The spatial configurations of lucerne and digit grass were alternating single rows of each species (1:1), alternating bands of 3 rows of each species (3:3) and alternating bands of 6 rows of each species (6:6).

Treatments were sown on 8 November 2011 at a total rate of 2 kg/ha of viable seed into a prepared seed bed at 0.17 m row spacing using a cone seeder with press wheels. Lucerne seed was inoculated with commercial Rhizobia and kept cool prior to sowing. For mixture treatments, digit grass and lucerne were sown at 1 kg/ha each. For the different spatial configurations, seed delivery tubes were allocated to drill rows to deliver the desired configurations of lucerne and digit grass (i.e.1:1, 3:3 or 6:6 rows). The fallow treatment was maintained weed free and covered with mulch at 1500 kg DM/ha.

To maintain moist surface soil conditions and ensure seed germination and establishment of the treatments, the site was irrigated using sprays to apply 5 mm of water per day for 6 days between 10 and 15 November 2011. Soon after this in a 4 day period (23–26 November 2011), 130.5 mm of rainfall occurred which resulted in minor soil erosion and displacement of digit grass seed and seedlings. On 5 December 2011, where deemed necessary, additional digit grass seed was hand sown into drill rows at the same rate as previous.

The sowing configurations (C1, C2 and C3) were readily apparent soon after the species were established (Plate 1).

12.2.4.1 Treatment plots and experimental management

Herbicides were applied throughout the experiment to control annual broadleaf and grass weed seedlings. Broadleaf weeds and grass weeds were sprayed with 2,4-DB amine (500 g/L a.i. at 2.0 L/ha) and diuron (900 g/kg a.i. at 1.4 L/ha) in July 2013 and paraquat-diquat mix (135 and 115 g/L respectively at 2.0 L/ha) in July 2014. Weeds on fallow plots were sprayed with glyphosate (450 g/L a.i. at 1.5 l/ha) in December 2013. The experiment was also sprayed with insecticides chlorpyrifos (500 g/L a.i. at 0.3 L/ha) and omethoate (290 g/L a.i. at 0.2 L/ha) to control two spotted spider mite (*Tetranychus urticae*) in November and December 2012 respectively, and omethoate (at 0.2 L/ha) in October 2013.

The experiment was fertilised each growing year from 2012–13; 250 kg/ha single superphosphate (8.8% phosphorus, 11% sulfur) applied in spring and 110 kg/ha urea (46% N) applied in both spring and summer (total of 220 kg/ha, timed to occur with rainfall to minimise volatilisation).

12.2.5 Data collected

12.2.5.1 Soil water content

Soil water content (SWC) was estimated at about 3-week intervals using the NMM described above. Counts were taken over a 16-s period with readings taken at the midpoint of 0.2 m-deep soil layers from 0.1–0.3 m to a maximum depth of 1.9 m. Values of volumetric SWC for each layer were converted to stored soil water (SSW, mm) and summed to obtain values for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) profile and the total profile (0.1–1.9 m). Soil water content was assessed a total of 60 times, during the experimental period, October 2011–April 2015.

12.2.5.2 Herbage production

Green herbage mass was assessed at approximately 6-week intervals to a height of 0.1 m above ground level by two observers. Each observer assessing four quadrats (0.4 x 0.4 m) spaced equally along separate transects in each half of the plot, avoiding edge effects (total of eight quadrats assessed in each plot). Total herbage production was assessed using a continuous 0–5 scale (0, nil; 5, high) and the percentage of lucerne and grass was estimated (on a dry-weight basis). At each sampling time, the green materials from 20 calibration quadrats (0.4 x 0.4 m), selected to cover the range of herbage mass and proportion of lucerne and grass at the site, were harvested to about 10 mm above ground level, sorted into lucerne, grass and other, and each portion dried at 80^oC for 48 h before weighing.

Scores and percentage estimates for these calibration quadrats were regressed (linear or quadratic $R^2 > 0.75$, $n = 20$) against actual herbage mass (kg DM/ha) and percentage (%) of lucerne and grass to determine the herbage mass of each component in each strata per treatment plot. Herbage production was assessed a total of 28 times, during the experimental period, October 2011–April 2015 Values of herbage production were accumulated for each of 4 growing years beginning on 1 June–31 May.

Table 12.2. Descriptions of 15 treatments applied in the lucerne variety x sowing configuration experiment at Tamworth Agricultural Institute.

	Treatment	Code	Cultivar	Sowing rate (kg/ha)	Configuration	Description
1	Digit grass	D	Premier	2	All rows	Grass sown in every drill row
2	Lucerne 1	L1	Q31	2	All rows	Lucerne sown in every drill row
3	Lucerne 2	L2	Venus	2	All rows	Lucerne sown in every drill row
4	Lucerne 3	L3	Pegasis	2	All rows	Lucerne sown in every drill row
5	Lucerne 1:Digit grass	L1C1	Q31	1 + 1	1:1	Lucerne and digit grass in alternate rows
6	Lucerne 2:Digit grass	L2C1	Venus	1 + 1	1:1	"
7	Lucerne 3:Digit grass	L3C1	Pegasis	1 + 1	1:1	"
8	Lucerne 1:Digit grass	L1C3	Q31	1 + 1	3:3	Three rows lucerne to three rows grass
9	Lucerne 2:Digit grass	L2C3	Venus	1 + 1	3:3	"
10	Lucerne 3:Digit grass	L3C3	Pegasis	1 + 1	3:3	"
11	Lucerne 1:Digit grass	L1C6	Q31	1 + 1	6:6	Six rows lucerne to 6 rows grass
12	Lucerne 2:Digit grass	L2C6	Venus	1 + 1	6:6	"
13	Lucerne 3:Digit grass	L3C6	Pegasis	1 + 1	6:6	"
14	Lucerne 2:Digit mix	L2MIX	Venus	1 + 1	Mixed in rows	Lucerne and grass sown mixed in all rows
15	Fallow	F	-	-	-	Mulch covered control

C1 (1:1) sowing configuration C2 (3:3) sowing configuration C3 (6:6) sowing configuration

Plate 12.1. An example of treatment plots from October 2012 showing each sowing configuration; C1 (1:1), C2 (3:3) and C3 (6:6).

Plots were crash grazed (*i.e.* high stock density for a short period of several hours up to 1 d depending on quantity of herbage) by wether sheep to a residual height of about 50 mm after each assessment and any residual stem >50 mm in height was removed using either a flail or rotary mower equipped with a catcher.

12.2.5.3 Plant frequency

Plant frequency (Brown 1954) was assessed twice a year; after the start and prior to the end of each growing year (c. November and May). The number of cells (0.1 x 0.1 m) containing a live plant of either digit grass or lucerne were counted in two fixed quadrats (1.0 x 1.0 m, total 100 cells) in each plot about 7 days after grazing and the proportion of occurrence determined. Plant frequency was assessed a total of 7 times during the experiment.

12.2.6 Statistical analysis

Main effects and interaction. Significant differences among treatments were determined by analysis of variance for data from all treatments (n = 15). To study the main effects of lucerne type (n = 3; L1, L2, L3) and sowing configuration (n = 3; C1, C2, C3) and their interactions, data for treatments 5–13 (Table 12.2) were analysed separately. Significant differences among the main effects and interactions were determined by analysis of variance and least significant difference (LSD) calculated using a 5% level of significance.

Maximum extractable water. SWC data for each treatment were plotted for each measurement layer and sampling time. Periods of 3 months or more during each growing year (September–May) when SWC declined without interruption by complete profile rewetting (*i.e.* significant rainfall events) were identified as they demonstrated the capacity of the treatments to extract soil water (Murphy and Lodge 2006). The difference between the high and low values of SSW (i.e. start and end date of the periods identified respectively) for each depth and treatment (Neal *et al.* 2012) was calculated as the maximum extractable water (MEW, mm). Values of MEW for each depth were summed to values for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and full (0.1–1.9 m) soil profile layers. Analysis of variance was used to model values of MEW for each soil layer against the factors treatment, year and their interaction. Replicate effects within each year were accounted for in the model by including the interaction of year by replicate as a block term. The least significant difference (LSD) was calculated using a 5% level of significance.

Plant root depth. Changes in soil water content values between the dates identified to determine MEW were also used to estimate plant rooting depth (Murphy and Lodge 2006). On each date, the trend in SWC over depth among the three replicate values was modelled using a linear mixed model for each treatment. The model included depth as a covariate and a fixed term to model the linear trend over depth and a spline term as a random effect to model a smooth curvilinear trend (Verbyla *et al.* 1999). Terms to account for differences between the replicates were included as random effects in the model. Predicted values of SWC with depth (±standard error of prediction) were plotted for each plant density and plant root depth identified as the greatest depth where the modelled decrease in soil water content was >0.01 m^3/m^3 (Murphy and Lodge 2006).

Soil profile refill. Soil moisture accumulation, or refill (mm), during the winter period (typically May to September) when digit grass was frosted and not growing was calculated. Using plots of SWC data for each soil layer and sampling times, periods when SWC changed from lowest to highest were identified. The difference between the low and high values of SSW for each soil depth was calculated as soil water refill (mm). Values of soil water refill were summed to values for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and full (0.1–1.9 m) soil profile layers. Analysis of variance was used to determine significant differences among start and end stored soil water, SWR in each soil layer, and rainfall refill efficiency. The least significant difference (LSD) was calculated using a 5% level of significance.

Plant production. Trend in herbage production (kg DM/ha) and lucerne percentage were modelled using a linear mixed model for each treatment. The model included sample date as a covariate and a fixed term to model the linear trend over time and a spline term as a random effect to model smooth curvilinear trend. Predicted values of herbage production were accumulated for each growing year. Analysis of variance was used to model values of accumulated plant dry matter against the factors of treatment, year and their interaction. The least significant difference (LSD) was calculated using a 5% level of significance. Replicate effects within each year were accounted for in the model by including the interaction of year by replicate as a block term.

Water use efficiency (WUE, kg DM/ha/mm) for each 6-week interval during the growing year was calculated by dividing herbage production (kg DM/ha) by actual evapotranspiration (ETa, mm) (Allen *et al.*, 2008). Actual evapotranspiration was determined by water balance, under the assumption that no water was lost by runoff or deep drainage. WUE of dry matter production for each growing year (2012–15) was calculated for each treatment in each year and for total dry matter production over 4 years. Significant differences were determined by analysis of variance and least significant difference (LSD) calculated using a 5% level of significance.

Plant frequency. Trend in plant frequency (%) was modelled using a linear mixed model for each plant density. The model included sample date as a covariate and a fixed term to model the linear trend over time and a spline term as a random effect to model smooth curvilinear trend. Predicted values of plant frequency with time (±standard error of prediction) were plotted to determine changes for each pasture type.

12.3 Results

12.3.1 Rainfall

Rainfall received at the experimental site was generally below the long-term average in most seasons, apart from the summers of 2011–12, 2012–13, and 2014–15, and the autumns of 2014 and 2015 (Fig. 12.1). Monthly rainfall values are reported in Fig. 10.1 (Chapter 10).

12.3.2 Summary of significant effects

In two of the four growing years (2012 and 2013), stored soil water at the start of the extraction period was similar among treatments ($P > 0.05$), while that at the end of the extraction period was different in all years ($P < 0.01$, Table 12.4). In all growing years, treatment had a significant effect on total profile MEW ($P < 0.05$) and a highly significant effect on upper profile MEW ($P < 0.001$, Table 12.4). Treatment had a significant effect on middle and lower profile MEW ($P < 0.05$) in 3 of the 4 growing years (Table 12.4). Neither lucerne type nor sowing configuration had any effect ($P > 0.05$) on either start or end SSW during each extraction period, but lucerne type affected middle and upper profile MEW in 2012 and 2013, respectively ($P < 0.05$, Table 12.4). Sowing configuration had a significant effect on upper profile MEW in 2013 and 2014 ($P < 0.05$) and a significant effect on middle profile MEW in 2012 and 2014 ($P < 0.05$, Table 12.4). Sowing configuration had an effect on total profile MEW in 2014 only ($P < 0.01$), while there was no interaction between lucerne type and sowing configuration ($P > 0.05$, Table 12.4).

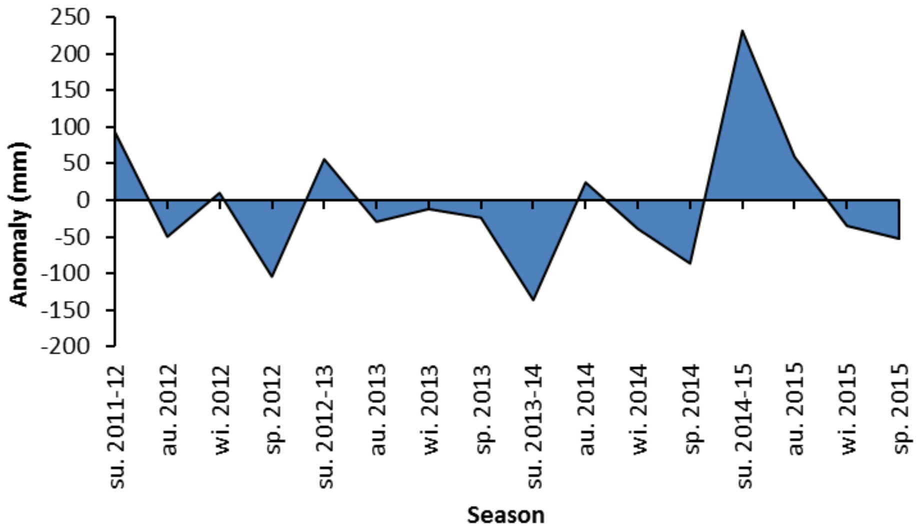

Fig. 12.1. Seasonal (su. – summer; au. – autumn; wi. – winter; sp. – spring) rainfall anomaly for the experimental site compared with the long-term average for Tamworth NSW for the period 2011–2015.

In each of the three winter rainfall refill periods, treatment had a significant effect ($P < 0.01$) on starting SSW and total profile SSW, which led to significant differences in ($P < 0.01$) the rainfall refill efficiency (Table 12.5). In each refill period, treatment had a significant effect ($P < 0.001$) on change in upper profile SSW, but only in 2014 were significant changes ($P < 0.05$) observed in the middle and lower profile. Neither lucerne type nor sowing configuration had any effect on any variable related to rainfall refill efficiency (Table 12.5).

Levels of stored soil water among treatments at the beginning of the experiment were similar ($P >$ 0.05), but were different among treatments at the start of each subsequent growing year ($P < 0.01$, Table 12.6). Stored soil water levels among treatments at the end of each growing year were significantly different ($P < 0.01$) with significant changes ($P < 0.01$) occurring in the upper profile in three of four growing years (Table 12.6). Fewer significant differences among treatments were observed for changes in SSW in the middle and lower profile, but treatment had a significant effect ($P < 0.05$) on change in total profile SSW in three of four growing years (Table 12.6). Subsequently, treatment total water use was significantly different ($P < 0.05$) in three of four growing years (Table 12.6).

Treatment had a highly significant effect ($P < 0.001$) on each of the herbage mass variables of grass, lucerne and total herbage mass in all growing years, which translated into highly significant effects (P < 0.01) on the proportion of legume (%, Table 12.6). The fact that treatment affected both total water use and total herbage mass resulted in highly significant effects ($P < 0.001$) on WUE in each growing year (Table 12.6).

Sowing configuration had an effect ($P < 0.05$) on total water use in 2013 only (Table 12.6). Both lucerne type and sowing configuration and their interaction had significant effects on the lucerne herbage mass variables in all growing years (Table 12.6). In the first two growing years, lucerne type produced differing proportions of legume (%, $P < 0.001$), while in the latter two seasons sowing configuration produced differing proportions of legume (%, $P < 0.001$, Table 12.6). Neither lucerne type nor sowing configuration and/or their interaction produced differing WUE ($P < 0.05$, Table 12.6) in all growing years (Table 12.6).

Table 12.4. Significance levels of fitted terms (T – treatment, L – lucerne type, C – sowing configuration) from the variables related to maximum extractable water analysed each growing year; start profile stored soil water (SSW mm), end profile SSW (mm), maximum extractable water (MEW, mm) for upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and total (0.1–1.9 m) profile.

			F-probability					
					Maximum extractable water			
Growing year	Fixed terms	Degrees of freedom	Start SSW (mm)	End SSW (mm)	Upper profile (mm)	Middle profile (mm)	Lower profile (mm)	Total profile (mm)
2012	T	14		***	***	***	*	***
	L	2				*		
	C	2				**		
	L:C	4						
2013	T	14		**	***			*
	L	2			*			
	C	2			*			
	L:C	4						
2014	T	14	**	**	***	**	*	***
	L	2						
	C	2			**	*		**
	L:C	4						
2015	T	14	***	***	***	**	*	***
	L	2						
	C	2						
	L:C	4						

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 12.5. Significance levels of fitted terms (T – treatment, L – lucerne type, C – sowing configuration) from the variables analysed each winter rainfall refill period; start profile stored soil water (SSW mm), end profile SSW (mm), change in SSW for upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and total (0.1–1.9 m) profile, together with rainfall refill efficiency (%).

			F-probability						
					Change in profile SSW				
Winter	Fixed terms	Degrees of freedom	Start SSW (mm)	End SSW (mm)	Upper profile (mm)	Middle profile (mm)	Lower profile (mm)	Total profile (mm)	Rainfall refill %
2012	T	14	***		***			**	**
	L	2							
	C	2							
	L:C	4							
2013	T	14	**	**	***			**	**
	L	2							
	C	2							
	L:C	4							
2014	T	14	***	**	***	***	*	***	**
	L	2							
	C	2							
	L:C	4							

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 12.6. Significance levels (*F*-probability) of fitted terms (T – treatment, L – lucerne type, C – sowing configuration) from the variables related to herbage production and water use efficiency analysed each growing year; total water use (mm), herbage mass of grass (kg DM/ha), lucerne (kg DM/ha), total herbage mass (kg DM/ha), proportion of legume (%) and water use efficiency (WUE, kg DM/ha/mm).

Growing year	Fixed terms	Degrees of freedom	Total water use (mm)	Herbage mass (kg DM/ha)				WUE (kg DM/ha/mm)
				Grass	Lucerne	Total	Legume (%)	
2012	T	14	***	***	***	***	**	***
	L	2			***	***	***	
	C	2		***	**	***		
	L:C	4				*		
2013	T	14	***	***	***	***	**	***
	L	2		*	***	***	***	***
	C	2	*	***	***	***		***
	L:C	4		*	***	**		**
2014	T	14		***	***	***	**	***
	L	2			**	**		**
	C	2			***	***	***	***
	L:C	4				*		
2015	T	14	*	***	***	***	***	***
	L	2			*	**		*
	C	2		***	*	***	***	**
	L:C	4				*		*
2012-15	T	14	**	***	***	***	*	***
	L	2		*	***	***	**	***
	C	2		***	***	***	***	***
	L:C	4		*	**	**	*	***

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

12.3.3 Soil water content through time

Soil water content of the treatments varied with time and depth, but similar patterns emerged among the treatments (Fig. 12.1 and 12.2). All sown treatments showed a decline in SWC through time and depth in the months and years after establishment, with the extent and depths that changes occurred, generally increasing with each successive growing year from 2011–12 to 2013–14 (Fig. 12.1 and 12.2). All the treatments showed increasing SWC in the winter periods of 2012 and 2013, but to a lesser extent in 2014 (Fig. 12.1 and 12.2).

Soil water content in the upper profile (0.1–0.7 m) for digit grass (Fig. 12.1d) was < 0.30 m^3/m^3 for most of 2014, which contrasted to fallow (Fig. 12.1e), which was consistently > 0.40 m^3/m^3, apart from a short period of drying in the upper profile in early 2014. The three lucerne cultivars, showed very similar patterns of SWC through time (Fig. 12.1a–c) apart from cv. Pegasis (Fig. 12.1c) showing lesser drying of the profile compared with cvv. Q31 and Venus (Fig. 12.1a, b). Of the three treatments with a 1:1 sowing configuration (Fig. 12.2a–c), the L2C1 mixture (Fig. 12.2b) showed lower SWC during 2013–14. The SWC of the three treatments with a 3:3 sowing configuration appeared similar as a group (Fig. 12.2d–f) and similar to the 1:1 group of treatments. The SWC of the three treatments with a 6:6 sowing configuration appeared similar as a group (Fig. 12.2g–i), but with lower values of SWC compared with the other configurations (Fig. 12.2a–f) and more similar to digit grass (Fig. 12.1d).

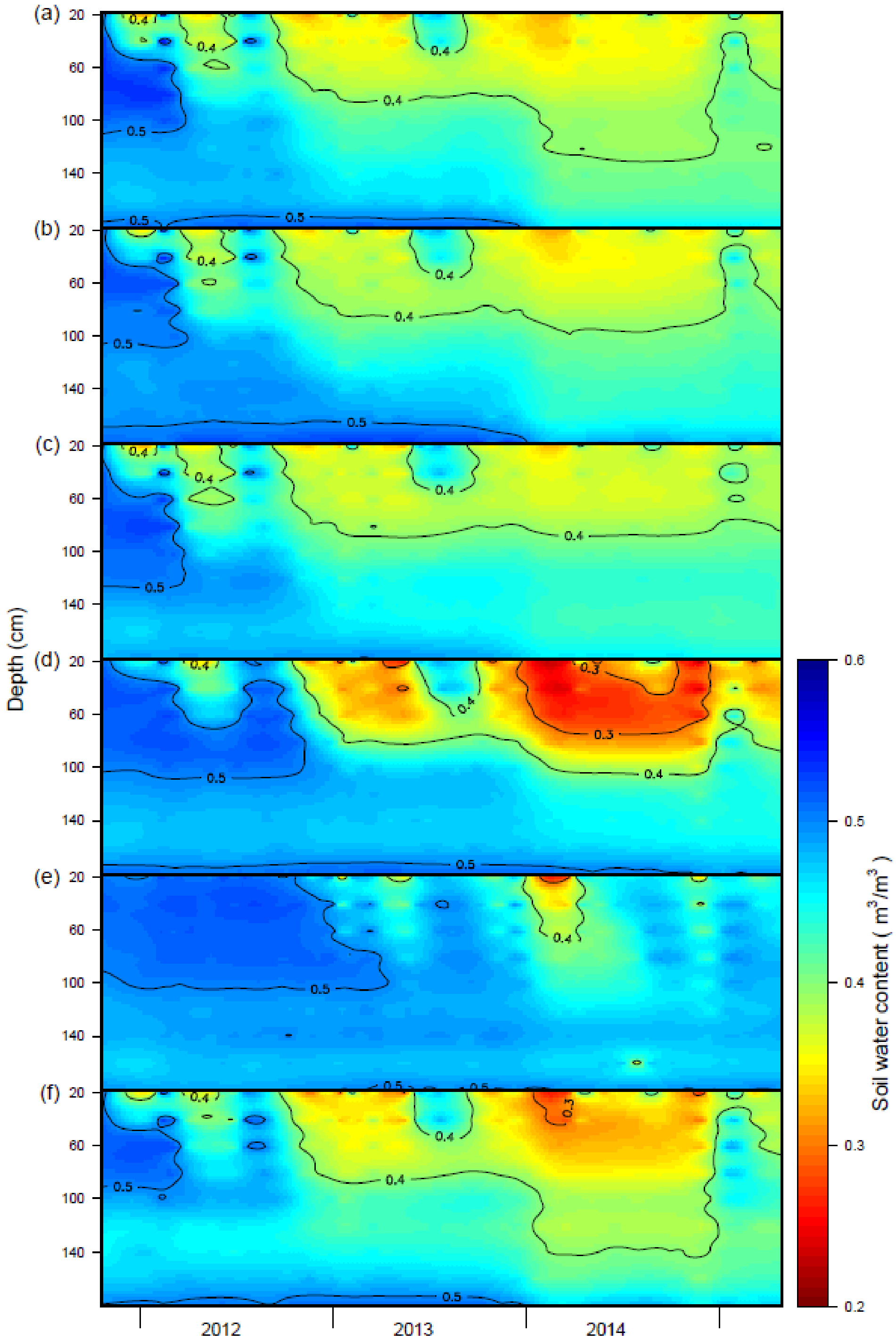

Fig. 12.1. Patterns of distribution of profile (20–180 cm) soil water content (m^3/m^3) through time for (a) L1-Q31 (b) L2-Venus, (c) L3-Pegasis, (d) D-Premier, (e) F-Fallow, and (f) L2-MIX from October 2011 to April 2015.

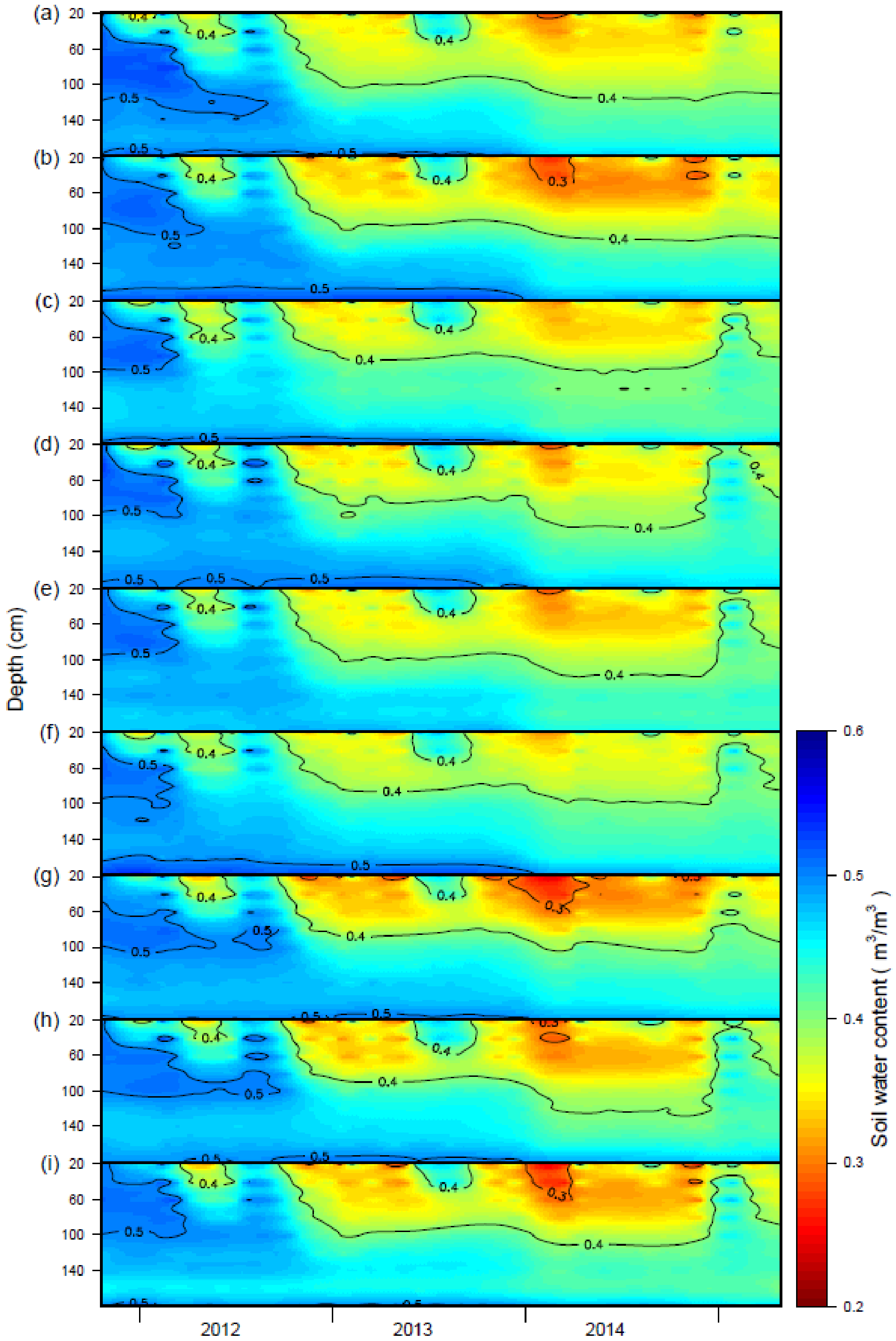

Fig. 12.2. Patterns of distribution of profile (20–180 cm) soil water content (m3/m3) through time for (a) L1C1 (b) L2C1, (c) L3C1, (d) L1C2, (e) L2C2 (f) L3C2, (g) L1C3, (h) L2C3, and (i) L3C32 from October 2011 to April 2015.

12.3.4 Plant root depth and maximum extractable water

Plant root depth varied considerably between treatments and years, with all treatments achieving maximum depths in either 2013 (1.19–1.58 m) or 2014 (1.11–1.90 m, Table 12.7). Plant root depth and maximum extractable water achieved by each treatment in each growing year is shown in Table 12.7 and represented graphically for 2013 growing year in Figure. 3. In 2012, lucerne, as pure stands had the maximum values of root depth (1.14–1.22 m), while digit grass (0.73 m) and the three treatments with C3 configuration were all < 1.00 m (0.81–0.91 m, Table 12.7). In 2013, digit grass (1.08 m) had a shallower rooting depth than all treatments containing lucerne (1.19–1.58 m, Table 12.7). In 2014 the greatest range in root depth values were obtained, with L3 and L3C3 (0.72 m) being the shallowest while L1 and L2MIX (1.90 m) were the deepest. Values of root depth in 2015 were less than half the values achieved in any other year (Table 12.7) and ranged 0.34 (L1) to 0.54 m (L2C1 and L3C3).

In 2012, pure stands of lucerne extracted significantly higher amounts of water from the soil profile than digit grass (108–121 *cf.* 75 mm, Table 12.7). Interestingly, in 2013–15, digit grass extracted greater amounts of water from the soil profile compared with any of the pure stands of lucerne (72–168 *cf.* 6–124 mm, Table 12.7, $P < 0.05$).

The sowing configuration treatments generally extracted similar amounts of water each year of the study: 2012 (81–112 mm), 2013 (113–156 mm), 2014 (100–144 mm), and 2015 (25–49 mm, Table 12.7), apart from some isolated and specific differences. For example, L3C2 extracted less water in both 2013 (113 mm) and 2014 (100 mm) compared with either L2C3 (156 and 144 mm) or L3C3 (156 and 136 mm, respectively). In 2015, L3C1 (25 mm), extracted less water than L1C3 (46 mm) and L3C3 (49 mm, $P < 0.05$).

The main effects of lucerne type and sowing configuration, and their interaction were non-significant each year, except for sowing configuration in 2014 ($P < 0.01$, Table 12.7) with the C3 treatments (136.3 mm) extracting greater amounts of soil water compared with C1 and C2 treatments (117.6 and 112.3 mm).

Table 12.7. Plant root depth (m) and maximum extractable water (MEW, mm) for treatments in each of four growing years. Least significant differences (LSD; $P = 0.05$) among all treatment means (T), main effects of lucerne type (L) and sowing configuration (C), and their interaction (L:C) for each year is provided where significant.

Treatment	Root depth (m)				MEW (mm)			
	2012	2013	2014	2015	2012	2013	2014	2015
D	0.73	1.08	**1.11**	0.45	75	167	168	72
L1	1.22	1.41	**1.90**	0.34	120	124	112	6
L2	1.17	**1.52**	0.76	0.39	108	108	107	12
L3	1.14	**1.50**	0.72	0.35	121	115	81	19
L1C1	1.09	**1.55**	0.85	0.50	101	155	114	40
L2C1	1.06	**1.46**	0.86	0.54	84	155	133	41
L3C1	1.09	**1.58**	1.22	0.44	112	126	107	25
L1C2	1.08	1.27	**1.64**	0.39	97	146	126	37
L2C2	1.09	**1.43**	0.75	0.47	89	149	111	33
L3C2	1.17	**1.19**	0.72	0.44	93	113	100	31
L1C3	0.81	1.26	**1.34**	0.46	83	153	132	46
L2C3	0.82	1.35	**1.68**	0.51	81	156	144	41
L3C3	0.91	**1.43**	1.42	0.54	89	156	136	49
L2MIX	1.02	1.24	**1.90**	0.46	90	140	152	49
F[1]	0.20	1.10	1.08	0.77	7	78	140	65
Mean	1.01	1.38	1.24	0.48	90	136	124	38
LSD T	-[2]	-	-	-	27.9	42.8	28.7	20.0
LSD L	-	-	-	-	NS[3]	NS	NS	NS
LSD C	-	-	-	-	NS	NS	6.8	NS
LSD L:C	-	-	-	-	NS	NS	NS	NS

[1] nil plants [2]not statistically analysed [3]NS - not significant

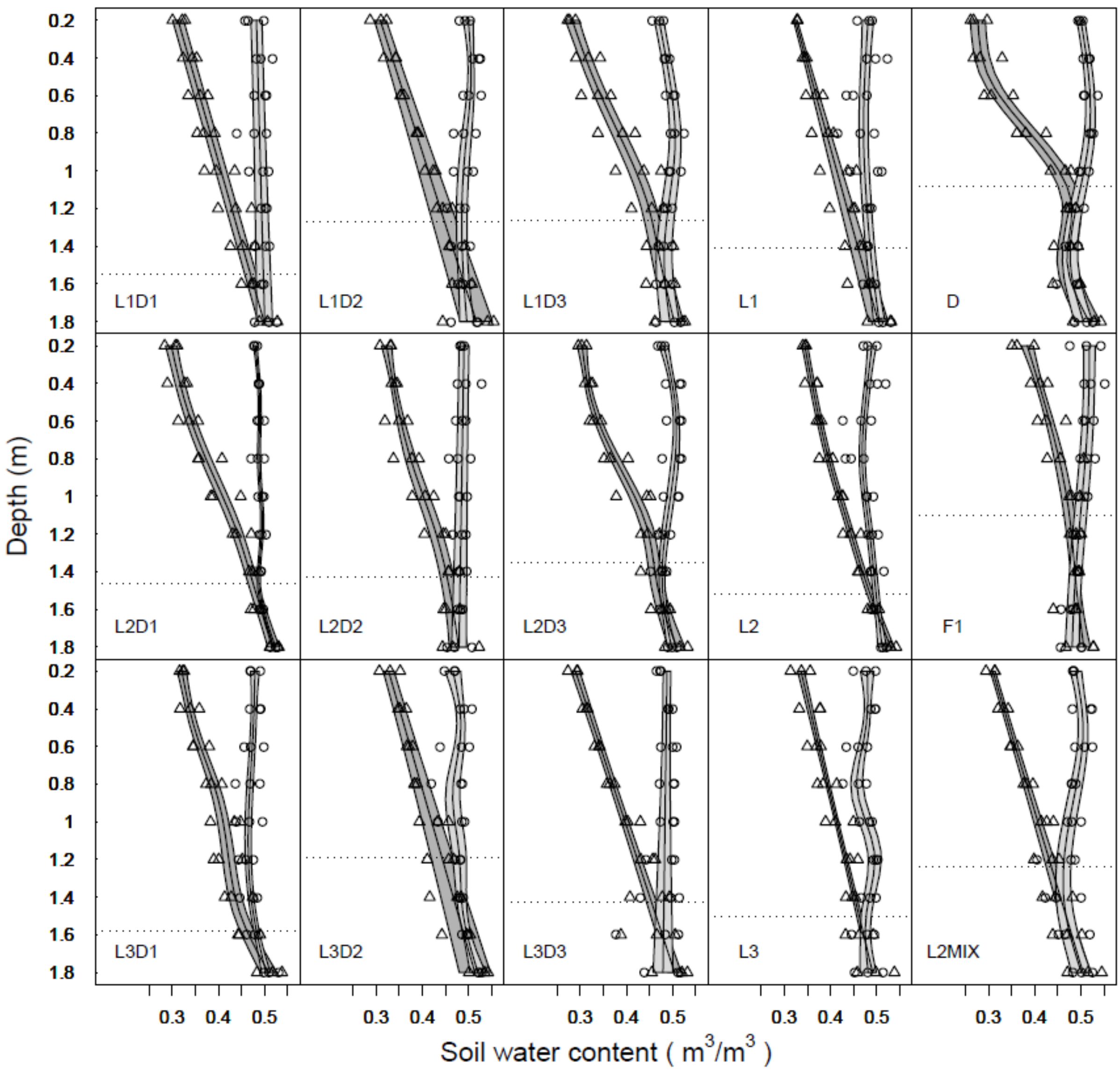

Fig. 12.3. Observed replicate values of maximum (○) and minimum (Δ) volumetric soil water content (m^3/m^3) for each treatment (as labelled, L = lucerne type, D = sowing configuration) in the 2013 growing year. Solid and dashed lines represent the predicted soil water content ± s.e. of prediction, respectively with the difference between the lines indicating maximum extractable water (mm). Root depth is indicated by the horizontal dot line.

12.3.5 Rainfall refill efficiency

Change in stored soil water (mm) and rainfall refill coefficient (%) for 2012 were similar among all the treatments apart from fallow, which only had a small increase in stored soil water (6 mm) as stored soil water was already high and therefore low rainfall refill efficiency (4%, Table 12.8). The other treatments stored about 45% of rainfall during winter 2012. In 2013 and 2014, digit grass captured a higher proportion of rainfall (78 and 29%, $P < 0.01$) than did any pure lucerne stand (43–48 and 12–15%, respectively, Table 12.8). The majority of lucerne mixtures captured similar amounts of winter rainfall in 2013 and 2014 (i.e. 48–59% and 16–22%, respectively) apart from L3C2 which captured less rainfall than L2C1 (45 and 10 *cf.* 59 and 21% respectively). There was no effect of lucerne type, sowing configuration or their interaction in any year ($P > 0.05$).

Table 12.8. Change in profile stored soil water (SSW, mm) and rainfall refill efficiency (%) in each winter rainfall refill period. Least significant differences (LSD; *P* = 0.05) among treatment means (T), lucerne type (L), sowing configuration (C), and their interaction (L:C) for each year is provided where appropriate.

Treatment	Change in SSW (mm)			Rainfall refill coefficient (%)		
	2012	2013	2014	2012	2013	2014
D	64	97	30	41	78	29
L1	71	57	15	46	46	15
L2	70	53	16	45	43	15
L3	71	60	12	46	48	12
L1C1	72	66	16	46	53	16
L2C1	66	74	21	43	59	21
L3C1	77	66	21	50	53	21
L1C2	78	68	16	50	54	15
L2C2	70	61	18	45	48	18
L3C2	60	57	10	38	45	10
L1C3	71	68	19	46	54	19
L2C3	70	80	23	45	64	22
L3C3	71	71	18	45	57	18
L2MIX	67	74	27	43	59	26
F	6	61	52	4	49	51
LSD T	28.8	17.4	9.9	0.185	0.139	0.097
LSD L	NS	NS	NS	NS	NS	NS
LSD C	NS	NS	NS	NS	NS	NS
LSD L:C	NS	NS	NS	NS	NS	NS

12.3.6 Herbage production, total water use and water use efficiency

12.3.6.1 Herbage mass

In each of the growing years, the pure swards of both digit grass and the three lucerne cultivars had significantly higher productivity than their relevant components in any of the mixtures (Table 12.9). Digit grass grown in any C3 configuration (6:6 rows) contributed < 50% of pure digit grass in all years of the experiment (Table 12.9) and this trend extended to all configurations in 2013 and 2014. However, in both the establishment (2012) and final year (2015), digit grass grown in either C1 or C2 (1:1 or 3:3) contributed > 50 % of pure digit grass. For lucerne, the opposite was true in the majority of years; lucerne grown in most configurations contributing > 50% of the herbage mass of pure lucerne in the same year (Table 12.9).

In all growing years, the total herbage production of mixtures in any configuration, apart from L1C3 in 2015, was significantly greater than that of pure digit grass ($P < 0.001$, Table 12.9). However, the productivity of lucerne mixtures was significantly less than those of pure lucerne for the majority of lucerne types and configurations (24/36 occurrences across three lucerne types x three configurations x four growing years) across growing years ($P < 0.001$, Table 12.9). The total herbage production of mixtures sown in the C3 (6:6) configuration were always smaller than the production of the corresponding pure lucerne type ($P < 0.001$, Table 12.9). The total productivity of mixtures containing L3 was always significantly smaller than that of pure L3 (cv. Pegasis) in all growing years and configurations, apart from L3C1 in 2015 (Table 12.9).

12.3.6.2 Total water use

Differences in total water use of all treatments were significant in all years except 2014. Interestingly, digit grass used less water than each of the pure lucerne cultivars in 2012, but used more than each lucerne cultivar in 2013 ($P < 0.001$, Table 12.9). The mixtures used similar or more water in most growing years and configurations than the pure lucerne treatments. Exceptions were in 2012, when L2C3 used less water than pure L2 treatment and in 2015, three various configurations containing either L1 or L2 used less water than pure stands of L1 and L2, respectively (Table 12.9).

12.3.6.3 Water use efficiency

Digit grass had the lowest WUE in each growing year (9.8–15.2 kg DM/ha/mm, $P < 0.001$) apart from 2015 when it was similar to L1C3 (15.4 kg DM/ha/mm, Table 12.9). The WUE values of lucerne mixtures were significantly lower than those of pure lucerne for the majority of lucerne types and configurations (21/36) across growing years ($P < 0.001$, Table 12.9). The WUE values of mixtures sown in the C3 (6:6) configuration were lower than the WUE of the corresponding pure lucerne type (27/36) of the time ($P < 0.001$; Table 12.9). The WUE values of mixtures containing L3 (cv. Pegasis) were always smaller than those of pure L3 in all growing years and configurations, apart from L3C1 in 2015 (Table 12.9).

12.3.6.4 Lucerne type and sowing configuration main effects and interactions

The main effect of lucerne type demonstrated consistent effects across growing years 2012–14 with mixtures containing L3 having greater total herbage mass and WUE compared with mixtures containing the other lucerne types (Table 12.9). Mixtures containing L3 had greater legume herbage mass across growing years 2012–14. In the final year, however, mixtures containing L2 had greater herbage production and WUE compared with mixtures containing L1. Similarly, in each growing year the mixtures sown in the C3 (6:6) configuration had lower herbage mass and WUE compared with the mixtures sown in other configurations.

There was a significant interaction ($P < 0.05$) between lucerne type and sowing configuration for total herbage mass in all growing years; L3C1 outperformed the other combinations, while L1C3 underperformed the other combinations. Herbage mass tended to increase with WAR (i.e. with increasing winter activity) and decreased as species were separated (i.e. across configurations C1 to C3).

There was a significant interaction ($P < 0.01$) between lucerne type and sowing configuration for WUE in 2013 and 2015, indicating that in those years, L3C1 outperformed and L1C3 underperformed all the other combinations, respectively. Sowing configuration had no effect on WUE of L2 mixtures (19.7 kg DM/ha/mm). However, the WUE of L1 mixtures was significantly lower in C3 compared with either C1 or C2 configurations (15.4 c. 18.0–19.8 kg DM/ha/mm) and the WUE of L3 mixtures was significantly higher in C1 configuration compared with either C2 or C3 configurations (20.4 c. 17.9–18.1 kg DM/ha/mm).

12.3.7 Plant frequency

Plant frequency of lucerne sown as pure swards declined for all types, but L3 (cv. Pegasis) declined more quickly and from 2014 onwards had a significantly lower frequency compared with the other types (Fig. 12.4a). Similarly, L3 (cv. Pegasis) grown in the C2 (3:3) configuration had significantly lower plant frequency compared with L1 (cv. Q31) from mid-2013 (Fig. 12.4c). Plant frequency of digit grass in the pure stand showed the largest increase of treatments, rising from 66 to 89% over the duration of the experiment (Fig. 12.4e). Plant frequency of digit grass in mixture treatments increased by a similar amount, but from a lower starting level, rising from about 35 to 50% over the duration of the experiment (Fig. 12.4f,g,h).

Table 12.9. Grass, lucerne and total herbage mass (kg DM/ha), also total water use (TWU, mm) and water use efficiency (WUE, kg DM/ha/mm) for treatments in each of four growing years. Least significant differences (LSD; *P* = 0.05) among treatment means (T), lucerne type (L), sowing configuration (C), and their interaction (L:C) for each year are provided where significant.

Treatment	Herbage mass (kg DM/ha)																			
	Grass				Lucerne				Total				TWU (mm)				WUE (kg DM/ha/mm)			
	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015
D	3484	8788	5914	10962	0	0	0	0	3484	8788	5914	10962	354	696	611	720	9.8	12.6	9.7	15.2
L1	0	0	0	0	5347	19253	11935	15306	5347	19253	11935	15306	381	648	598	741	14.1	29.7	20.0	20.7
L2	0	0	0	0	5677	17940	11371	15518	5677	17940	11371	15518	380	640	590	742	14.9	28.0	19.3	21.0
L3	0	0	0	0	9122	24764	13942	16554	9122	24764	13942	16554	384	640	565	759	23.8	38.7	24.8	21.8
L1C1	1688	3037	2039	7484	3581	13133	8556	6027	5269	16170	10596	13511	372	674	584	752	14.2	24.0	18.2	18.0
L2C1	2446	3770	2566	7549	3138	12038	7593	6680	5584	15808	10159	14229	374	675	601	737	14.9	23.4	16.9	19.3
L3C1	2457	3364	2444	7894	5166	16497	9328	7011	7623	19861	11772	14904	389	643	585	733	19.6	30.9	20.2	20.4
L1C2	1768	3391	2179	7664	3610	14045	9161	6911	5378	17436	11340	14575	366	663	581	737	14.7	26.3	19.6	19.8
L2C2	1949	3699	2219	7764	2742	13733	8929	7454	4692	17432	11148	15218	374	674	579	730	12.6	25.8	19.3	20.9
L3C2	1835	3048	1920	7875	4374	14157	9315	5726	6210	17205	11234	13602	374	644	577	751	16.6	26.7	19.5	18.1
L1C3	1628	2304	2133	4760	2808	9571	5826	6642	4436	11875	7959	11401	365	679	582	739	12.2	17.5	13.7	15.4
L2C3	1575	2286	2158	4646	2942	10841	6463	8865	4517	13127	8621	13512	355	678	593	713	12.7	19.4	14.6	19.0
L3C3	1616	2548	2398	5225	4177	14166	7330	7862	5794	16714	9729	13087	379	683	575	733	15.3	24.5	16.9	17.9
L2MIX	2307	3701	2008	6268	2277	13093	9876	9238	4584	16794	11883	15505	359	662	612	704	12.7	25.3	19.5	22.0
F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD T	554	878.1	422.7	771.8	758.6	2652.4	1306.3	1833.4	789.1	2595.4	1126.1	1700.4	25.2	26.2	NS	26.2	2.16	3.64	2.18	2.46
LSD L	NS[1]	234.6	NS	NS	366.5	1045.1	680	866.3	402.2	1160.8	553	796.7	NS	NS	NS	NS	1.28	1.56	1.06	1.27
LSD C	271.1	234.6	NS	203.6	366.5	1045.1	680	866.3	402.2	1160.8	553	796.7	NS	16.2	NS	NS	1.28	1.56	1.06	1.27
LSD L:C	NS	406.3	NS	NS	NS	1810.2	NS	NS	696.7	2010.6	957.8	1379.9	NS	NS	NS	NS	NS	2.7	NS	2.19

[1]NS- not significant

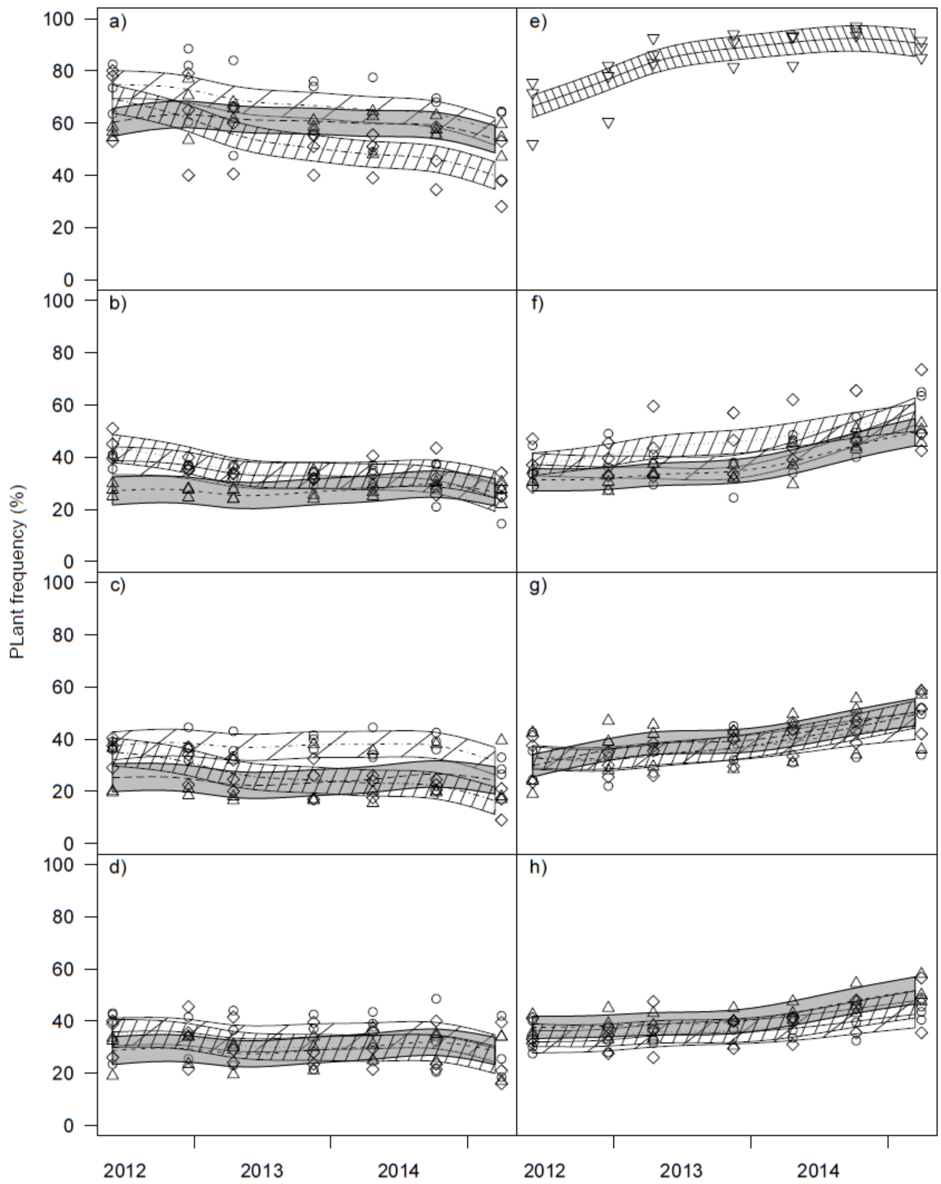

Fig. 12.4. Plant frequency (%) of lucerne (left hand side, a–d,) and digit grass (right hand side, e–h) in pure stands (a, e), and sowing configurations C1 (b, e), C2 (c, g), and C3 (d, h). Replicate values of plant frequency are shown for treatments according to each lucerne type (L1 - ○, L2 - Δ, L3 - ◊). Predicted plant frequency ± s.e. of prediction is represented by hatching/shading for treatments according to each lucerne type (L1 - light cross hatch, L2 - shading, L3 - heavy cross hatch).

12.3.8 Total herbage production, water use and efficiency

Over the 4 years of the experiment, the fallow treatment had the lowest total water use of all treatments (2260 mm, $P < 0.05$). L2C1 (2387 mm) had the highest water use, but was similar to all treatments containing plants except L2MIX (2337 mm, $P > 0.05$, Table 12.10). There were no main effects of lucerne type or sowing configuration on total water use.

All treatments with the C3 (6:6) sowing configuration had total herbage mass of grass that was < 50% of the productivity of pure digit grass (D, 10825–11788 < 29147 kg DM/ha) and significantly lower compared with herbage mass of grass in all the other mixtures (10825–11788 < 14248–16330 kg DM/ha, Table 12.10). Further, sowing configurations with L1 (cv. Q31) lucerne had significantly lower herbage mass of grass than configurations with the other lucerne types (13358 < 14209 kg DM/ha, Table 12.10). The significant interaction between lucerne type and sowing configuration indicated that grass productivity in L1C1 was less than other mixtures in C1 (1:1) configuration, but herbage mass of grass did not vary by lucerne type within other sowing configurations (Table 12.10).

Total lucerne production in mixtures was > 50% of that for each pure lucerne type in the mixture apart from L1C3 and L2C3 (Table 12.10). Lucerne cv. Pegasis (L1, 64381 kg DM/ha) had the highest herbage mass, while L1C3 (24846 kg DM/ha) had lower lucerne herbage mass than all treatments apart from L2C3 (29112 kg DM/ha) and L2C1 (29450 kg DM/ha). Subsequently, sowing configurations containing L3 (cv. Pegasis, 35036 kg DM/ha) contributed more lucerne herbage mass than configurations containing the other types (29957–30473 kg DM/ha, Table 12.10), while sowing configuration C3 (29164 kg DM/ha) contributed less lucerne herbage mass than the other configurations (32916–33386 kg DM/ha, Table 12.10). The significant interaction between lucerne type and sowing configuration indicated that herbage mass of lucerne in C2 was similar regardless of lucerne type, but in C1 and C3 configurations, lucerne herbage mass significantly increased with increasing WAR (i.e. from L1 to L3). Of the nine lucerne type-configuration combinations, lucerne herbage mass was highest in L3C1 (38001 kg DM/ha, $P < 0.05$) and lowest in L1C3 (24846 kg DM/ha, $P < 0.05$) (Table 12.10).

Total productivity was highest for pure lucerne stands (50506–64381 kg DM/ha) and all mixtures (35671–48729 kg DM/ha, $P < 0.001$) were significantly greater than that of pure digit grass (29147 kg DM/ha, Table 12.10). In general, mixtures with L3 (cv. Pegasis, 49245 kg DM/ha) had higher total herbage mass than those with the other lucerne types (43315–44682 kg DM/ha) and mixtures sown in C3 (6:6, 40257 kg DM/ha) had lower herbage mass than the other configurations (1:1 or 3:3, 48490–48495 kg DM/ha, Table 12.10). The lucerne type-sowing configuration interaction reflected the same pattern as for lucerne herbage mass; total yield in C2 was similar regardless of lucerne type, but in C1 and C3 configurations, total yield significantly increased from L1 to L3.

Overall for the 4 growing years WUE of digit grass (D, 12.2 kg DM/ha/mm) and cv. Pegasis (L3, 27.4 kg DM/ha/mm) were the lowest and highest, respectively, (Table 12.10, $P < 0.05$). WUE of mixtures sown in C3 configuration (15.1–19.1 kg DM/ha/mm) were significantly lower than those sown in the other configurations (19.1–23.0 kg DM/ha/mm, Table 12.10). Subsequently, sowing configurations containing L3 (cv. Pegasis, 20.9 kg DM/ha/mm) had higher WUE than those containing the other lucerne types (18.3–18.9 kg DM/ha, $P < 0.05$), while sowing configuration C3 (17.1 kg DM/ha) had lower WUE than the other configurations (20.5–20.6 kg DM/ha/mm, Table 12.10, $P < 0.05$). The significant interaction between lucerne type and sowing configuration for WUE reflected the same pattern as for lucerne type and total herbage mass; WUE in C2 was similar regardless of lucerne type, but in C1 and C3 configurations, WUE significantly increased with increasing WAR (i.e. from L1 to L3).

Table 12.10. Change in profile stored soil water (SSW, mm), total water use (mm), herbage mass (kg DM/ha) of grass, lucerne and total herbage mass, also proportion of legume (%) and water use efficiency (WUE, kg DM/ha/mm) of treatments for the duration of the experimental period (2012–15). Least significant differences (LSD; *P* = 0.05) among treatment means (T), lucerne type (L), sowing configuration (C), and their interaction (L:C) are provided where significant.

Treatment	Total water use (mm)	Herbage mass (kg DM/ha)			Legume (%)	WUE (kg DM/ha/mm)
		Grass	Lucerne	Total		
D	2381	29147	0	29147	0	12.2
L1	2368	0	51840	51840	100	21.9
L2	2351	0	50506	50506	100	21.5
L3	2348	0	64381	64381	100	27.4
L1C1	2382	14248	31297	45545	68.7	19.1
L2C1	2387	16330	29450	45780	64.3	19.2
L3C1	2350	16159	38001	54160	70.1	23.0
L1C2	2347	15002	33727	48729	69.3	20.8
L2C2	2357	15631	32858	48489	67.6	20.6
L3C2	2347	14679	33573	48251	69.5	20.6
L1C3	2365	10825	24846	35671	69.7	15.1
L2C3	2339	10665	29112	39776	73.1	17.0
L3C3	2371	11788	33535	45323	74.0	19.1
L2MIX	2337	14283	34484	48767	69.9	20.8
F*	2260	-	-	-	-	-
LSD T	45.6	1792.0	4868.7	4452.9	4.73	1.76
LSD L	NS	723.9	1802.9	1910.6	0.80	0.76
LSD C	NS	723.9	1802.9	1910.6	0.80	0.76
LSD L:C	NS	1253.8	3122.8	3309.3	1.38	1.32

[1]NS- not significant

12.4 Discussion

This study examined the hypotheses that in mixed species swards; greater spatial separation of digit grass and lucerne would favour grass production and; that greater winter activity rating would favour the production of lucerne at the expense of grass. These were found to be false and true respectively.

12.4.1 Hypothesis 1: Increasing spatial separation of grass and lucerne reduces grass production

In our experiment the C3 (6:6) configuration, which created 1.0 m wide bands of digit grass and lucerne, resulted in less total herbage mass of both grass and lucerne, compared with the closer spacings of C1 (alternate rows) and C2 (3:3, or 0.5 m bands). The lower productivity of lucerne under the C3 configuration was not expected.

Two factors were possibly at play. Firstly, with the greater spatial separation of the species, there was less likelihood of the grass accessing N fixed by the lucerne, as their respective root zones and canopies were spatially separated. Since our mixed sward treatments did not receive any N fertiliser, the only N available to digit grass was from that fixed by rhizobia on lucerne roots or by leaf fall from the lucerne. In the C3 configuration, the grasses in the sowing row immediately adjacent to the lucerne sowing row had the best chance of accessing N, while the other four intermediate rows of grass may have struggled.

Secondly, intraspecific competition by lucerne within the band may have limited total herbage production by the lucerne. Lucerne treatments on our site were sown at rates of 2 and 1 kg/ha for pure and mixed swards, respectively, resulting in mean plant frequency at 6 months after sowing of 67 and 34% for pure and mixed swards, respectively. At this young age, lucerne plants had small crowns, meaning that plant frequency at 6 months of age would be a conservative estimate of actual plant density. At a neighbouring experiment established in a similar manner on the same soil type, lucerne seedling density (plants/m^2) and plant frequency counts at 6 months were highly correlated (Frequency = 0.2172 x density – 2.05, R^2 = 0.782, n = 9; Murphy and Boschma unpublished data). Using this relationship, values of seedling plant density for our experiment may have been as much as 315 and 165 plants/m^2 for pure and mixes swards, respectively. Such a density in our environment is well above the recommendation for a dryland lucerne stand, where excellent establishment is considered to be 30 plants/m^2 with the sward thinning to 15–20 plants/m^2 in the following season to provide optimum production (McDonald *et al.* 2003). Our lucerne plant density may have been excessive for the water availability of our environment, thus resulting in lower yield potential (Pembleton *et al.* 2010b). The effect of the high plant density and intraspecific competition would be more evident as the width of the sowing band increased from alternate rows to 3 to 6 rows, respectively.

Pure digit grass had values of total herbage production (29147 kg DM/ha) and overall WUE (12.2 kg DM/ha/mm) that were similar to values recorded for digit grass during the same period on the same soil at a neighbouring experimental site (Chapter 10) and similar to the upper end of the range (7.5–15.6 kg DM/ha/mm) reported by Tow (1993). In the neighbouring experiment, digit grass swards growing at a range of plant densities (4–16 plants/m^2) yielded 29019–32557 kg DM/ha at 11.0–12.3 kg DM/ha/mm; the lower yield and lower WUE associated with higher plant density (Chapter 10). However, a study conducted on a red Chromosol soil in the same region reported digit grass produced over 17,000 kg DM/ha with a WUE exceeding 33 kg DM/ha/mm (Murphy *et al.* 2018), which is half the yield but more than double the WUE values determined in our study. This large difference may be partly due to different water availability (timing and amount), rainfall distribution and soil fertility leading to higher growth as WUE is highly correlated with herbage production (Neal *et al.* 2012; Pemberton *et al.* 2011).

12.4.2 Hypothesis 2: Higher lucerne winter activity rating increases WUE

The climate of our region is typical of moist mid-latitudes having a mild winter and hot humid summer (Köppen-Geiger class Cfa – humid subtropical, Peel *et al.* 2007), but with a summer dominant rainfall pattern mainly generated by thunderstorms. In this environment, lucerne maintains green leaf throughout the year and generates herbage growth year round, albeit with very low growth rates (<5 kg DM/ha/d) in winter. Therefore, lucerne genotypes with higher winter activity, are potentially a suitable fit with the climate to provide year round growth. However, previous studies have reported contradictory findings regarding the impact that lucerne winter activity rating has on WUE.

Research conducted in cool temperate Washington USA has shown that winter active genotypes of lucerne have lower WUE (e.g. Johnson and Tieszen 1994). However, field and modelling studies in cool temperate Australia have shown higher activity genotypes have higher WUE (Pembleton *et al.* 2010a). Our data supports the findings by Pembleton *et al.* (2010a), with lucerne cv. Pegasis, clearly having higher WUE. The difference between the overseas and Australian findings may be due to Australia's warmer climate with temperatures in Northern Inland NSW providing favourable growing conditions for lucerne, virtually year round.

Winters in Northern Inland NSW are mild by global standards, with July (mid-winter) mean maximum temperature rarely falling <15°C and global solar radiation 8–11 MJ/m^2/d, thereby comfortably exceeding base temperatures for lucerne growth (e.g. Sharratt *et al.* 1989). Lucerne genotypes with high winter activity rating, therefore, can grow throughout our entire winter, albeit with modest growth during the coldest weeks, giving them an opportunity to grow when evaporative demand is at its lowest and so achieve high WUE. However, later in the main growing season when water availability becomes more limiting, which may occur more quickly with the highly active types because they have exhausted plant available water, growth and thereby WUE is likely to decline (Pembleton *et al.* 2011). The winter dormant genotypes, with lower capacity for winter growth, may conserve soil water for a longer period into spring, but then peak growth occurs at a time when evaporative demand is greater resulting in a rapid decline in available soil water and so WUE.

12.4.3 Practical implications for industry

Spatial separation of tropical grass and lucerne plants within a mixed sward was shown to have significant effects on both soil water dynamics and herbage production resulting in significant differences in WUE; productivity and WUE higher in mixtures sown in alternating single rows or 0.5 m-wide bands compared with alternating 1 m-wide bands. Therefore, sowing mixed tropical grass lucerne pastures in either alternate rows or narrow bands of 0.5 m would be preferable, and practically, this type of seed tube configuration on a seeder may perhaps be easier than sowing in wider bands.

Our experiment was crash grazed at 6 week intervals at high stocking rates for short durations representing a rotational grazing system. Lucerne was commonly at or near to 20% flower at this time. This type of grazing management reduced selective grazing pressure on the lucerne within the mixed swards and ensured good utilisation of both grass and legume, something which can be difficult to achieve in mixed swards at commercial paddock scale (Boschma *et al.* 2014). A longer grazing period would have allowed selective grazing of lucerne which may have been exacerbated by sowing wider bands of lucerne and grass.

Lucerne genotypes with low winter activity rating are usually preferred in harsher environments where growing seasons are shorter or water availability is less (e.g. Pembleton *et al.* 2011). Conversely, highly active genotypes, offer the potential for high production and WUE where growing conditions are longer and water availability is higher. However, the more winter active types also tend to have poorer persistence (e.g. McDonald *et al.* 2003), with plant densities declining at a faster rate than those with lower winter activity ratings. While our plant frequency data showed few significant differences between the lucerne types sown as mixed swards, the highly active type cv. Pegasis as a pure sward and in C2 (3:3), had significantly lower plant frequency in the latter part of the experiment indicating that plant density had declined faster. This suggests that to maintain lucerne in the sward, cv. Pegasis would require re-sowing sooner compared with the other types.

The proportion of legume (64–74%) observed in these mixed swards of tropical grass-lucerne was very high and so may exceed what is necessary to meet livestock intake requirements. Certainly, the amount of legume produced was adequate to fix sufficient nitrogen to sustain production from the grass component of the sward. While legume pastures may fix up to 35 kg N/ha/t DM (taking into account N fixed on a whole plant basis; Peoples *et al.* 2012), with a lesser amount of 13 kg N/ha/t DM becoming available to the perennial pasture sward (Herridge 2004). These levels indicate that lucerne production of 4–8 t DM/ha per year would be required to achieve the 50–100 kg N/ha that is recommended to achieve optimum growth by the perennial grass (Boschma *et al.* 2016). Our data indicated that lucerne production over the 4 growing years within the various sowing configurations ranged from 29.2–33.4 t DM/ha, or 7.3–8.4 t DM/ha per year, which is at the upper end of the range suggested by Boschma *et al.* (2016). Therefore, within our swards there appears scope for the lucerne population to decline, and yet still fix sufficient N to sustain production from the grass component.

12.4.4 Unanswered questions

The data collected in this experiment, and earlier experiments conducted as part of our tropical grass research program, suggest that the density of lucerne in our mixed swards was excessive. Therefore, the proportion of lucerne and tropical grass that provides the multiple benefits of optimum herbage production, desired proportion of legume and high water use efficiency, is not known.

The proportion of legume (64–74% of total herbage production) observed in mixed swards of tropical grass-lucerne was very high. While ground cover was greater in the mixed swards compared to pure lucerne swards, the high proportion of lucerne production in the mixed swards would not necessarily have overcome the risk of bloat in cattle, which is a perennial concern of lucerne pastures. In light of this and the main role of lucerne in a mixed pasture is to fix sufficient N, we suggest that lucerne plant densities, and the proportion of lucerne in our treatments were too high. This was perhaps largely a result of our use of supplementary irrigation to establish the experiment, which favoured the establishment of lucerne more so than digit grass.

An experiment conducted to determine the optimum plant density of digit grass in a pure sward at an experimental site immediately adjacent our site, indicated that 4–9 plants/m^2 provides high levels of herbage production and water use efficiency (Chapter 10). Similar studies have been performed for lucerne in dryland farming systems (e.g. Dolling *et al.* 2011), but were conducted in lower rainfall environments. These results in conjunction with findings from our experiment suggest that further studies are required to help elicit the proportion of lucerne and tropical grass that provides the multiple benefits of optimum herbage production, desired proportion of legume and high water use efficiency.

12.4.5 Conclusion

Sowing mixed swards in alternating 1 m wide bands of tropical grass and lucerne, resulted in the lowest herbage production and WUE. Sowing in alternating 0.5 m wide bands of grass and lucerne had consistent production and WUE across the different lucerne activity types, while sowing in alternate rows favoured production from all genotypes compared with the other configurations.

13 Hard seed breakdown patterns of temperate and tropical legumes in Northern Inland NSW

S.P. Boschma, S. Harden and M.A. Brennan

13.1 Introduction

Northern Inland NSW is located at about latitude 29–30°S and is a frost prone region with a summer dominant rainfall pattern; 60% falling during the period October–March. In general, about 50 frosts occur annually over about a 145 day period (Hobbs and Jackson 1977). Monthly temperatures range from mean maximum and minimum of 32.8 and 17.5°C during summer (January) and 16.3 and 2.2°C during winter (July) (Bureau of Meteorology 2018).

Legumes have an important role in grazing systems in this region. They provide high quality forage for grazing livestock and fix N for companion grasses in longer-term or permanent pastures and/or for subsequent crops in ley farming systems (Harris *et al.* 2014). Previous studies (e.g. Archer *et al.* 1985; Moylan and Crocker 2000; Boschma *et al.* 2011, 2012) and those conducted during the course of this MLA project have shown that the region is suitable for both temperate and tropical legumes (e.g. Chapters 4, 5 and 11), but to be effective they need to be both productive and persistent; producing large quantities of forage to maximise N fixation and able to survive long term (e.g. Boschma *et al.* 2014b). This is achieved through adaptation strategies, which are many and varied, but two strategies used by many legume species are high seed production to produce a seed bank and producing seed with a seed coat which is impermeable to moisture, that is hard seed. Hardseededness is an innate mechanism of seed dormancy that regulates germination of legumes (Taylor 2005). Degradation of the hard seed coat until it becomes permeable, can imbibe water and germination commence is called hard seed breakdown or seed softening (Taylor 2005). The process of hard seed breakdown can take from weeks to many years (Taylor 2005) and in temperate legumes, has been the subject of multiple reviews including Taylor (2005) which focussed on research conducted in Australia.

The requirements for successful regeneration of a species has been described as having high seed production to maintain a sufficiently sized seed bank for insurance against poor seasons, with seed softening patterns that provide sufficiently high levels of hard seed which prevent germination during summer, but enough soft seed to allow good plant densities of regenerating legumes in autumn (Hagon 1974). Seed banks are important in all management systems (from permanent pastures to ley systems), but are particularly important in environments which experience frequent drought or systems with longer cropping phases (Taylor 2005).

Hardseed breakdown is a complex interaction of many factors including temperature, humidity and genetics (Taylor 2005), and in Australia, hard seed breakdown factors and patterns have been determined for a range of legumes, predominantly temperate species with the majority of studies conducted in a Mediterranean environment (i.e. WA) (e.g. Taylor 1984; Loi *et al.* 1999; Norman *et al.* 2002, 2006), and limited studies conducted in environments with winter-dominant (southern NSW; Howieson and Hackney 2018), aseasonal (central NSW; Howieson and Hackney 2018) and summer-dominant rainfall distributions (northern NSW and Southern Queensland; e.g. Lloyd *et al.* 1997; Bell *et al.* 2003).

Little is known of the hard seed breakdown of both temperate and tropical legumes in this frost prone summer dominant rainfall zone. Unanswered questions include: Is desmanthus as hard seeded in this environment as it is reported in field and controlled environment studies (e.g. Cook *et al.* 2005; McDonald 2000)? How does it compare with other tropical legumes that may have potential in this environment? Does location of seed production affect hard seed break down of tropical legumes? Medics (*Medicago* spp.) have been reported as hard seeded in this environment (Archer *et al.* 1985; Lodge 1993), how do their hard seed break patterns compare with the newer hard seed legumes, such as biserrula (*Biserrula pelecinus*) and serradella (*Ornithopus* spp.)?

The aim of this study was to quantify the hard seed breakdown patterns of a range of tropical and temperate legumes in the summer dominant rainfall zone in Northern Inland NSW. The experiments reported in this chapter were still being conducted and only results to May 2017 have been included in the analyses.

13.2 Methodology

Two separate experiments were conducted on a brown Chromosol soil at Tamworth Agricultural Institute (31°08’43”S 150°58’06”E, 400 m above sea level): tropical and temperate legumes. Both experiments were conducted in two phases: seed production then hard seed breakdown experiment.

13.2.1 Tropical legumes

13.2.1.1 Seed increase

On 12 December 2013, seed of eight tropical legumes (Table 13.1) were space planted into soil covered with weed mat. Seed was sown into holes (50 mm diameter) in the weed mat arranged on 0.3 x 0.3 m grid in plots 2 x 10 m (total of 238 plants/plot). Four plots were arranged in a block (2 x 2 array) covered with weed mat with 2 m buffers between each plot. The covered blocks were separated by 5.5 m. The blocks were irrigated from mid-December until late January (100 mm) to assist establishment and fertilised with soluble fertiliser [37.5 kg/ha nitrogen (N), 7.5 kg/ha phosphorus (P), 13.2kg/ha potassium (K), and 6.75 kg/ha sulfur (S)]. Holes without seedlings were transplanted with seedlings and all holes thinned to 1 plant/hole. Annual forage sorghum (*Sorghum × drummondii*) cv. Super Sweet Sudan was sown around each block and cages covered with small aperture insect netting (Saint-Gobain ADFORS fibreglass small insect screen FCS8503-M) erected over desmanthus plots to minimise air and pollen movement to prevent cross pollination. All plants were allowed to flower and set seed, and the annual species were allowed to senesce. Mature pod was harvested from late-March (desmanthus cv. Marc) until July (all legumes except desmanthus).

These legumes were also seed increased at Gatton, Queensland (27°32’22”S, 152°20’20”E, about 80 m alt) in 2013–14. A proportion of seed of all legumes from each site was swapped to test whether there was any effect of location of seed production on hard seededness or hard seed breakdown pattern. The Southern Queensland softening experiment was conducted 60 km west of Toowoomba (27°43’33”S, 151°21’00”, alt about 330 m). The Queensland experiment was not part of this project and the results are not reported.

Monthly rainfall (mm) at Tamworth and Gatton seed increase sites are presented in Fig. 13.1.

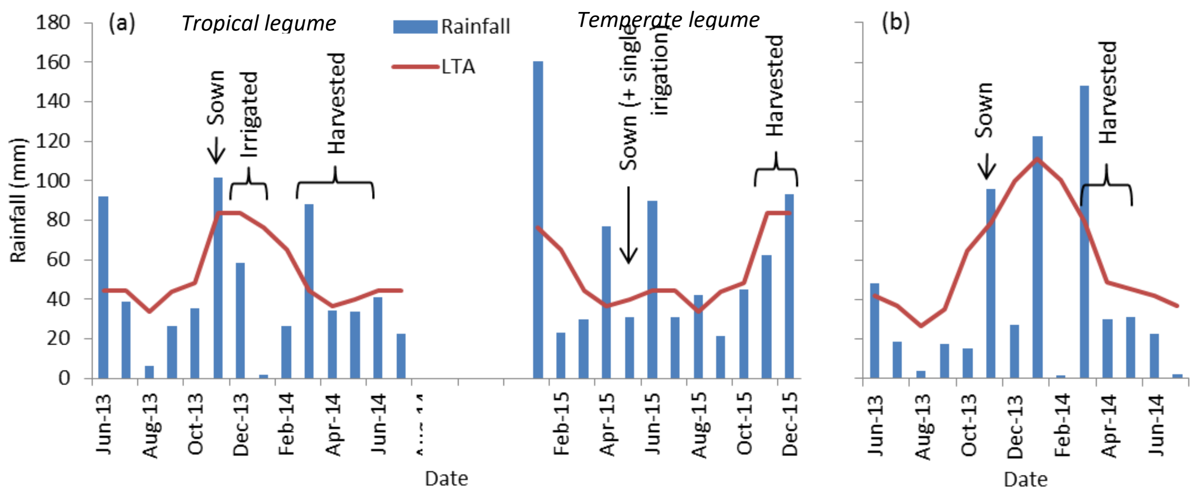

Fig. 13.1. Monthly and long term average (LTA) rainfall (mm) at (a) Tamworth, NSW and (b) Gatton, Queensland during the tropical (June 2013–July 2014) and temperate legume seed increase (January–December 2015).

13.2.1.2 Seed softening experiment

In September–October 2014, debris was removed from seed and pods by passing the material gently over a series of sieves then a vacuum separator (Kimseed; http://www.kimseed.com.au) taking care not to damage or scarify the seed.

Once cleaned, seed of each legume and seed in hull of the three stylo entries, were placed in individual nylon bags made of small insect flyscreen mesh (Adfors small insect flyscreen; 90 x 100mm); 500 seeds of all species except burgundy bean, butterfly pea and siratro which had 200 seeds per bag (Table 13.1). There was a total of 13 legume entries in the experiment (7 and 6 from the NSW and Southern Queensland seed increases respectively, Table 13.1). A nylon bag of each legume entry was strung onto 1 m lengths of wire, each wire representing a sampling time. The position of legumes along the wire was randomised.

Table 13.1. Tropical legumes seed increased in northern NSW (Tamworth) and Southern Queensland (Toowoomba) and included (✓) in the experiments at both locations.

Common name	Botanical name	Cultivar	Northern NSW	Southern Qld	Seeds /bag
Burgundy bean	*Macroptilium bracteatum*	B1	✓	-[4]	200
Butterfly pea	*Clitoria ternatea*	Milgarra	✓	✓	200
Desmanthus	*Desmanthus virgatus*	Marc	✓	✓	500
Desmanthus	*D. virgatus*	JCU2[1]	✓	✓	500
Caatinga stylo	*Stylosanthes seabrana*	Primar	✓	✓	500[3]
Caatinga stylo	*S. seabrana*	Unica	-[2]	✓	500[3]
Fine stem stylo	*S. guianensis* var. *intermedia*	-	✓	✓	500[3]
Siratro	*M. atropurpureum*	Aztec	✓	-[4]	200

[1]www.progardes.com.au; [2]Poor plant establishment; [3]seed in hull; [4] Insufficient seed produced

The bags strung on wire were placed in the field 0.6 m apart on 17 November 2014 in a randomised complete block design with 11 sampling times and four replicates. Each bag was placed 10 mm below the soil surface and lightly covered with soil. The sampling times were November 2014 (initial, S0 and not placed in the field), every 2 months to autumn 2015 (S1–S2; January and March 2015), every 3 months to spring 2015 (S3–S4; June and September 2015), every 4 months to autumn 2016 (S5–S6; January and May 2016), then every 5–7 months until spring 2018 (S7–S11; October 2016, May and October 2017, and May and October 2018).

At each sampling time, the wire and attached all nylon bags were removed from the field. Dirt was removed with water and the wires hung to dry overnight. Seed were removed from each packet and placed in sealable plastic containers lined with two sheets of unbleached paper towel (225 x 210 mm) wet with about 35 mm water. The containers were sealed and placed in a cabinet at 30/25^{o}C. After 4 days the number of seeds which had not imbibed (i.e. hard seeds) were recorded.

Two temperature probes (Tain Electronics; https://www.tain.com.au) were installed at 10 mm depth at the site in November 2014 and temperature recorded every 30 min. Rainfall (0.5 mm pluviometer at 30 min intervals) was recorded by an automatic weather station located 100 m from the site.

13.2.2 Temperate legumes

13.2.2.1 Seed increase

Seed of eight legumes (Table 13.2) were sown to produce spaced plants on 13 May 2015 into the area covered with weed mat used for the tropical legume seed increase, while two cultivars of subterranean clover were hand broadcast sown into plots 3 x 3 m next to the covered blocks. Seven days prior to sowing the subterranean clover plots were cultivated with a rotary hoe, trifluralin (480 g/L a.i. at 1.7 L/ha) applied and incorporated. The legumes were inoculated with commercial rhizobia, irrigated once in May to assist establishment, fertilised with liquid fertiliser (25.0 kg N, 5.0 kg/ha P, 8.8 kg/ha K and 4. 6 kg/ha S), and thinned to 1 plant/hole. Aphids (*Acyrthosiphon kondoi*) were sprayed with omethoate (290 g/L a.i. at 200 ml/ha) in September and October. The legumes senesced naturally and were harvested November (barrel medic)–December 2015 (remainder of legumes).

13.2.2.2 Seed softening experiment

In May–June, seed of each legume entry was prepared using similar techniques to tropical legumes except seed was not removed from the pod of the serradella or medic species. One hundred seeds, or an estimated equivalent number of seed in pod, of each legume were placed in individual nylon bags made from the same material as the tropical legume bags (100 x 100 mm and 140 x 120 mm for snail medic) and strung on 1 m length of wire in a randomised order. The number of pods or seed placed in each bag is shown in Table 13.2. On 15 June 2016, the seed bags strung on wire were placed in the field in a randomised complete block design with 10 sample times and four replicates. The sample times were June 2016 (initial, S0), then every 2.5 months during the first summer (S1–S4; October and December 2016, February and May 2017), then every 3.5 months over the second and third summers (S5–S10; October 2017, January, May and October 2018, January and May 2019).

Table 13.2. Temperate legumes and cultivars and the number of seeds or pod in each nylon bag.

Common name	Botanical name	Cultivar	Seeds or pods/bag[1]
Subterranean clover	*Trifolium subterraneum* subsp. *brachycalycinum*	Clare	100
Subterranean clover	*T. subterraneum* subsp. *subterraneum*	Dalkeith	100
Arrowleaf clover	*T. vesiculosum*	Cefalu	100
Bladder clover	*T. spumosum*	Bartolo	100
Biserrula	*Biserrula pelecinus*	Casbah	15[2]
Woolly pod vetch	*Vicia villosa*	Haymaker	100
Yellow serradella	*Ornithopus compressus*	Santorini	13[2]
French serradella	*O. sativus*	Margurita	100
Barrel medic	*Medicago truncatula*	Caliph	13[2]
Snail medic	*M. scutellata*	Silver	20[2]

[1]The number of seeds and pods/bag were doubled for S8–10 (October 2018, January and May 2019) [2]Pod

Similar to the tropical legumes, a wire and all attached nylon bags were removed from the field on the specified date. The seed bags were washed, dried overnight and seed or pod placed in plastic containers lined with paper towel and placed in a cabinet at 20°C for 14 days. The trays were checked after 4–5 and 8–10 days and germinated seedlings counted, recorded and removed. On day 14 seed were removed from pods, and the number of seed which had not imbibed (i.e. hard seeds) was recorded.

13.2.3 Statistical analyses

Data and statistical analyses reported are for tropical legumes S0–S8 (November 2014–May 2017) and temperate legumes S0–S4 (June 2016–May 2017).

Plots of proportion of hard seed *vs*. time for each legume showed a range of responses. There are many possible choices of curve that could be fit to these responses. Three and four-parameter Weibull models were chosen as they are asymmetric, can fit a range of response shapes and their parameters have meaningful interpretations. The four-parameter Weibull curve fitted hard seed breakdown patterns significantly better than the three-parameter model for the tropical legumes, however the three-parameter Weibull curve fitted the temperate legume data better. The three-parameter Weibull curve was chosen for ease of interpretation and consistency across both experiments.

The three-parameter Weibull equation used for the tropical legumes was:

$$\frac{HS}{total} = d * \exp\big(-\exp\big(b * (\log(time) - \log(e))\big)\big)$$

where *HS* is number of hard seeds, *total* is total number of seeds, *time* is number of days after seed were placed in the field, *d* is the initial hard seed value (i.e when time = 0), *b* is the Weibull slope or shape parameter, and *e* is the scale parameter. The model was fitted in R (R Core Team 2017) using package drc using the “drm” command with option type=”binomial” (Ritz *et al.* 2015).

The Weibull slope (*b*) is always >0. When *b* = 1 hard seed breakdown occurs at a constant rate, however, when *b* < 1 hard seed breakdown is initially high but decreases with time, while when *b* > 1, initial hard seed breakdown rate is slow and increases with time. The scale parameter (*e*) is also always >0 and has the same units as *time*. Higher relative values of *e* indicate the hard seed breakdown pattern is elongated and hard seed break down occurs over a long period of time while low values of *e* indicate that breakdown occurs over a shorter period of time. Legumes with low *e* values have more rapid breakdown rates than those with high values.

For the temperate legume data, the aim was to have 100 seeds in each nylon bag, however it was not possible to count the initial total number of seeds of the serradella and medic entries as they were in pods, therefore these data were modelled using the number of hard seeds (*HS*) in the equation:

$$HS = d * \exp(-\exp(-\exp(b * \log(time) - \log(e))))$$

The equation was fitted with the “drm” command but with option type=”continuous”. Estimates for *d*, *b* and *e* and their respective standard errors were determined for each response curve.

Predicted values of percent hard seed for the tropical legumes and the number of hard seed for temperate legumes and their standard errors were predicted at the last sampling date (i.e. 15 May 2017).

13.3 Results

13.3.1 Temperature and rainfall

Weekly average minimum, maximum and average temperatures are presented in Fig. 13.2. Weekly minimum temperatures ranged from 3.4°C during the winter months to 16.6–17°C during the summer months while maximum temperatures ranged 24.7–27.7°C to 46.9–50°C during winter and spring–summer respectively. Weekly rainfall totals are also presented in Fig. 13.2 and ranged from 6.3 mm during autumn 2016 to 26.0 mm in summer 2014–15.

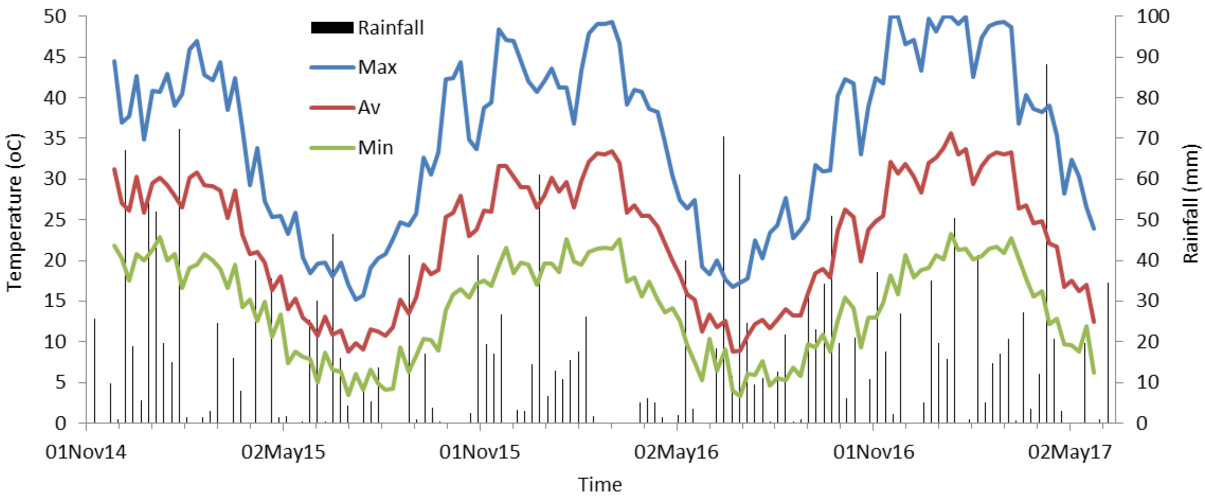

Fig. 13.2. Weekly average maximum, average and minimum temperatures (°C) and weekly rainfall (mm) at 10mm at the experimental site.

13.3.2 Tropical legumes

The initial hard seed values (*d*) ranged from 97% (desmanthus cv. Marc SQ) to 7% (caatinga stylo cv. Unica) (Table 13.3). Desmanthus cv. Marc SQ had significantly higher hard seed levels than the other legumes ($P < 0.05$), followed by fine stem stylo (95%), then Desmanthus cv. Marc NSW (88%; ranked third). Desmanthus cv. JCU2 SQ and NSW were ranked fifth and sixth (83 and 76% respectively). Butterfly pea NSW and SQ had the lowest initial hard seed values (29 and 19% respectively) and were ranked tenth and eleventh (Table 13.3).

Desmanthus cv. Marc NSW had the highest Weibull slope (*b*, $P < 0.05$) which was similar to slopes of both desmanthus cv. JCU2 entries ($P > 0.05$). All three had Weibull slope values >1 indicating a slow initial rate of hard seed breakdown that increased with time (Table 13.3, Fig. 13.3). These three lines also had high values of *e* (ranked 2nd to 4th) indicating an extended breakdown pattern. Interestingly desmanthus cv. Marc SQ had the lowest Weibull slope value ($b = 0.239$; $P < 0.05$) of all legume entries in the experiment, but a particularly high value of *e* (1.1×10^6; $P < 0.05$). These describe a fast initial decline in hard seed of desmanthus cv. Marc SQ followed by a long slow breakdown pattern (Fig. 13.3), although this is likely an artefact of the Weibull model used.

All other entries in the experiment had Weibull slope values (b) < 1 and low inflection points ($e < 410$ days) indicating an initial slow hard seed breakdown, which increased (Table 13.3, Fig. 13.3). Butterfly pea had the lowest inflection point (ranked 13th) of the lines tested ($e = 26$ days) indicating a rapid hard seed breakdown pattern compared to the other legume entries.

Although there were differences between lines in their initial hardseededness, the pattern of seed softening (Weibull slope and inflection points) are similar for burgundy bean, butterfly pea, caatinga stylo (all entries) and Siratro.

At the final assessment (May 2017, 926 days), 30 months after the experiment commenced, desmanthus cv. Marc SQ had the highest hard seed values (81%) followed by desmanthus cv. Marc NSW (69%). All other entries had predicted hard seed values ≤20%. Fine stem and caatinga SQ stylos, and butterfly pea had <1% hard seed (Table 13.3).

Desmanthus cvv. Marc and JCU seed increased in NSW had similar Weibull shapes (*b*) but cv. Marc had higher initial (*d*) and final (926 days) hard seed levels and higher coefficients for shape indicating that it was harder seeded with a slower breakdown pattern. Desmanthus cultivars which were seed increased in Southern Queensland were similar except cv. JCU2 had a higher Weibull coefficient. Caatinga stylo seed increased in Southern Queensland had similar coefficients for shape (*e*), but Weibull shape (*b*) coefficient was high for cv. Unica than cv. Primar while cv. Primar had higher initial (*d*) and final (926 days) hard seed levels than cv. Unica.

Desmanthus cvv. Marc and JCU2 seed increased in Southern Queensland had higher Weibull coefficient values for initial (*d*), shape (*e*) and hard seed level in May 2017 indicating that seed produced in Southern Queensland was harder with a slower hard seed breakdown pattern. Butterfly pea and caatinga stylo were the reverse suggesting seed produced in NSW had higher hard seed levels and slower breakdown pattern. Fine stem stylo was the exception with seed produced in NSW having higher initial (*d*) and final hard seed levels (May 2017) than that produced in Southern Queensland, but the Weibull slope (*b*) and shape (*e*) were similar.

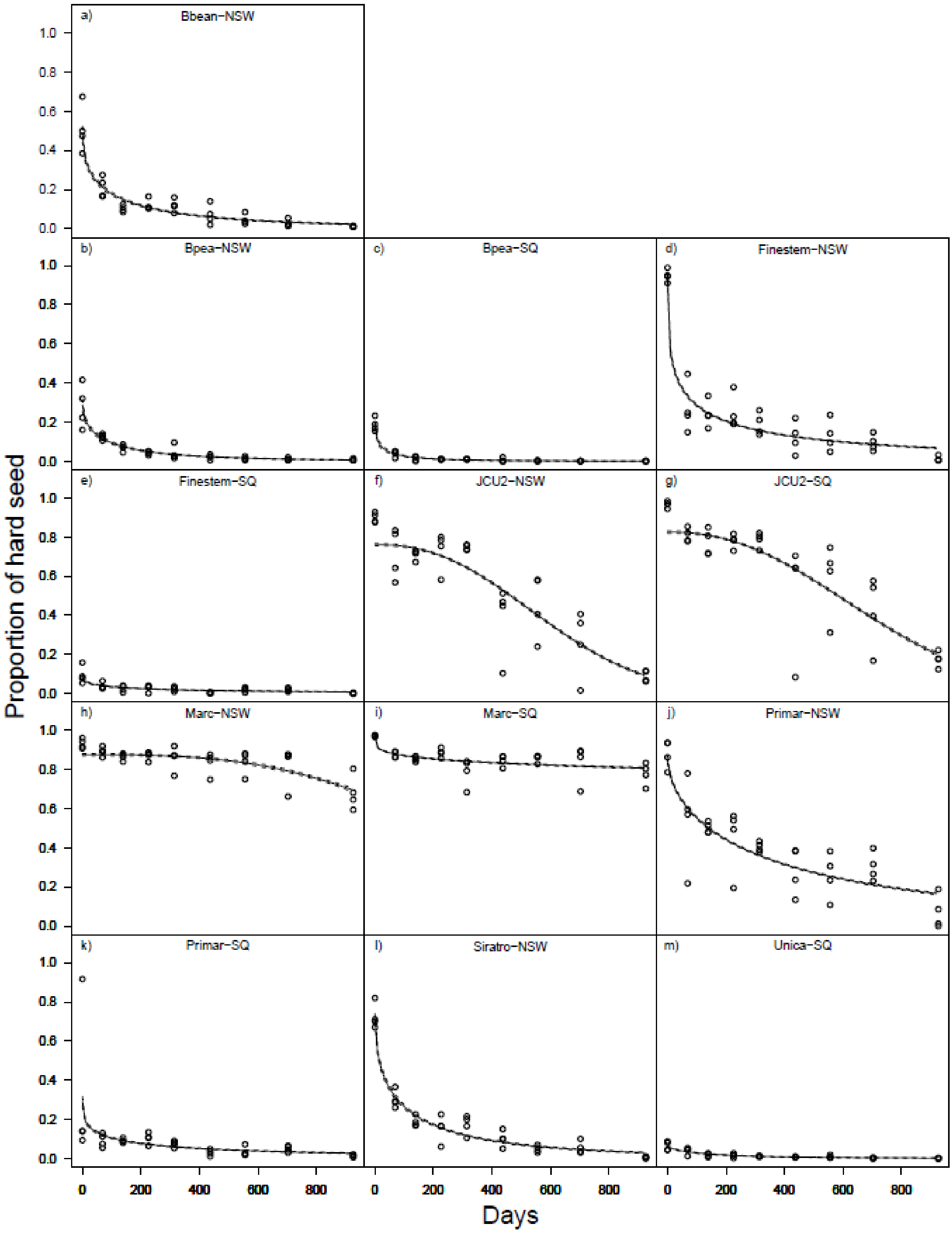

Fig. 13.3. Observed hard seed break down patterns of 13 tropical legumes at Tamworth, November 2014–May 2017: (a) burgundy bean NSW, (b) butterfly pea NSW, (c) butterfly pea SQ, (d) fine stem stylo NSW, (e) fine stem stylo SQ, (f) desmanthus cv. JCU2 NSW, (g) desmanthus cv. JCU2 SQ, (h) desmanthus cv. Marc NSW, (i) desmanthus cv. Marc SQ, (j) caatinga stylo cv. Primar NSW, (k) caatinga stylo cv. Primar SQ, (l) Siratro NSW, and (m) caatinga stylo cv. Unica. Solid and dashed lines represent the predicted hard seed ± standard error.

Table 13.3. Estimates of the Weibull coefficients for parameters *b*, *d* and *e*, and their standard error (s.e.), also the predicted hard seed (%) and standard error at the final assessment (May 2017, 926 days) for each tropical legume.

	b		d		e		Hardseed (%)	
Legume	Estimate	s.e.	Estimate	s.e.	Estimate	s.e.	Estimate	s.e.
Burgundy bean cv. B1-NSW	0.50	0.045	0.48	0.039	101	19.1	2	0.3
Butterfly pea cv. Milgarra-NSW	0.56	0.056	0.29	0.031	81	17.8	1	0.1
Butterfly pea cv. Milgarra-SQ	0.50	0.067	0.19	0.014	26	8.3	0	0.0
Fine stem stylo-NSW	0.36	0.014	0.94	0.012	61	4.7	7	0.3
Fine stem stylo-SQ	0.43	0.079	0.09	0.015	127	56.2	1	0.1
Desmanthus cv. JCU2-NSW	2.34	0.075	0.76	0.007	672	6.5	9	0.5
Desmanthus cv. JCU2-SQ	2.26	0.090	0.83	0.006	792	8.0	20	0.8
Desmanthus cv. Marc-NSW	3.06	0.546	0.88	0.005	1492	141.9	69	1.1
Desmanthus cv. Marc-SQ	0.24	0.007	0.97	0.007	1.1.E+06	29558.0	81	0.3
Caatinga stylo cv. Primar-NSW	0.60	0.027	0.85	0.019	404	18.7	16	0.6
Caatinga stylo cv. Primar-SQ	0.38	0.037	0.29	0.024	94	23.1	3	0.2
Siratro cv. Aztec-NSW	0.53	0.030	0.72	0.023	101	9.5	3	0.3
Caatinga stylo cv. Unica-SQ	0.66	0.095	0.06	0.010	129	35.3	0	0.1

13.3.3 Temperate legumes

Barrel medic, biserrula, bladder clover, yellow serradella and snail medic had the highest initial (*d*) predicted hard seed levels (>94%, $P < 0.05$)(Table 13.4). Woolly pod vetch had the lowest initial predicted hard seed value (18%) of the legumes tested ($P < 0.05$), then French serradella (55%).

All lines, with the exception of the medics and woolly pod vetch, had Weibull slope (*b*) values > 1 indicating a hard seed breakdown rate that was initially slow then increased (Fig. 13.4). Bladder clover and the serradellas had the highest Weibull slope values, ranked 1^{st}–3^{rd}, but they were not significantly different to many of the other entries. Barrel medic had the lowest Weibull slope < 1 ($b = 0.456$) and the highest scale parameter value ($e = 2717$ days) of the temperate legumes, indicating that following an initial decline in the number of hard seeds, the subsequent seed softening rate was low. This was in contrast to woolly pod vetch which had a low Weibull slope ($b = 0.524$) and the lowest scale value ($e = 188$) indicating that the initial decline in initial hard seed continued throughout the assessment period and this legume had the fastest hard seed breakdown pattern of those tested (Table 13.4, Fig. 13.4).

At the final assessment in May 2017, 11 months after the experiment commenced, barrel medic had the highest hard seed value (81%), followed by yellow serradella and snail medic (all >70%). Bladder clover had 57% hard seed. All other legumes had ≤26% hard seed with woolly pod vetch, subterranean clover cv. Dalkeith and arrowleaf clover having the lowest values (<5% hard seed) (Table 13.4).

The two subterranean clover cultivars had similar Weibull slopes (*b*) and scale (*e*) coefficients, also similar final (334 days) hard seed levels. Only their initial hard seed levels (*d*) were different with Clare having a higher proportion of hard seed than Dalkeith (82 *cf.* 66%).

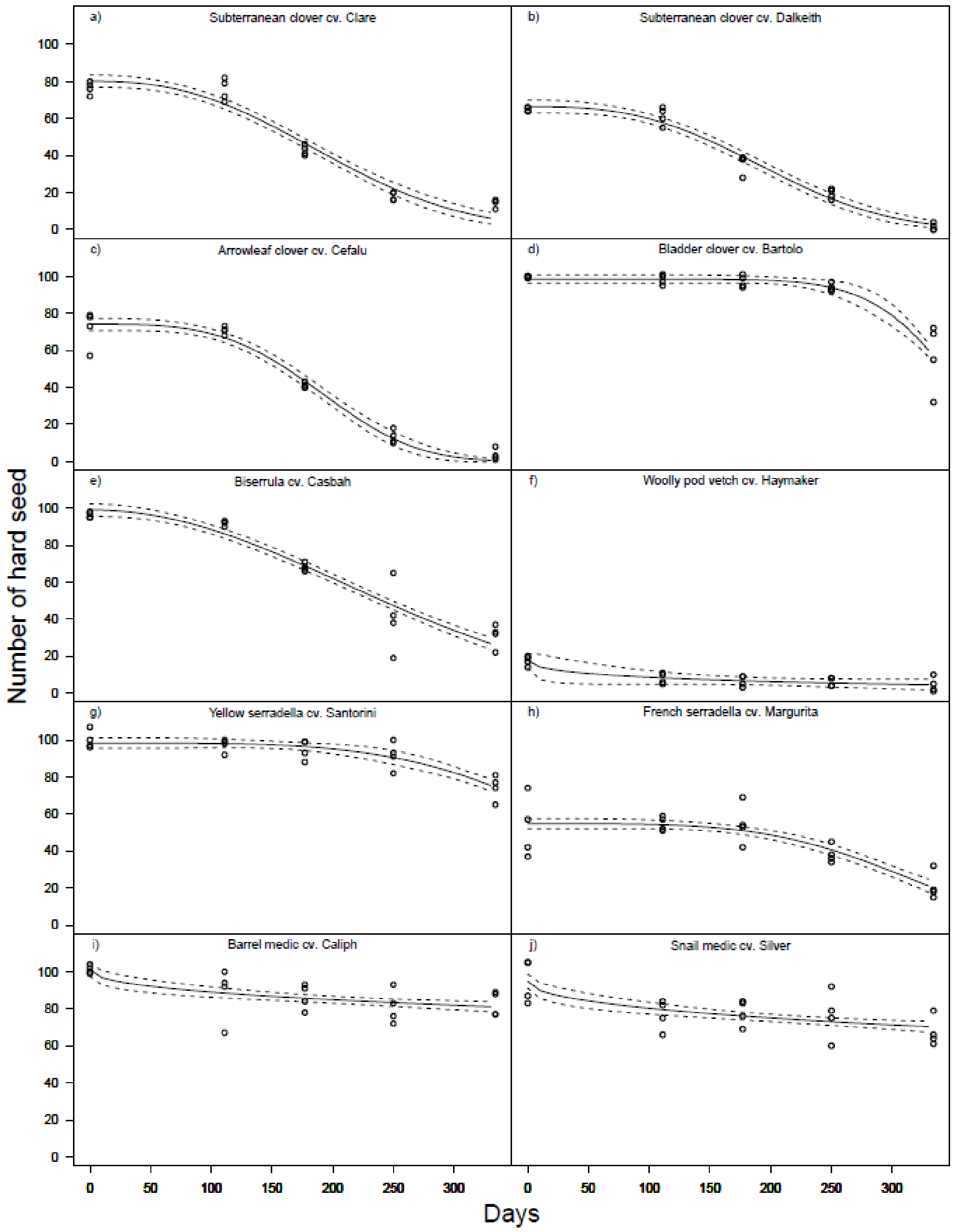

Fig. 13.4. Observed hard seed breakdown pattern of ten temperate legumes at Tamworth, November 2014–May 2017: (a) subterranean clover cv. Clare, (b) subterranean clover cv. Dalkeith, (c) arrowleaf clover, (d) bladder clover, (e) biserrula, (f) woolly pod vetch, (g) yellow serradella, (h) French serradella, (i) barrel medic and (j) snail medic. Solid and dashed lines represent the predicted hard seed ± standard error.

Table 13.4. Estimates of the Weibull coefficients for *b*, *d* and *e*, and the standard error (SE) and upper and lower confidence intervals (CI, X%) , also the predicted number of hard seeds in May 2017, the final assessment reported for each temperate legume.

		b		d		e		Hardseed (%)	
ID	Legume	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
1	Subterranean clover cv. Clare	2.51	0.366	80	3.4	226.1	10.19	6	2.9
2	Subterranean clover cv. Dalkeith	2.83	0.466	66	3.4	222.8	10.59	3	2.0
3	Arrowleaf clover cv. Cefalu	3.53	0.606	74	3.3	211.6	8.11	0	0.8
4	Bladder clover cv. Bartolo	8.35	3.395	99	2.3	359.0	11.62	57	3.8
5	Biserrula cv. Casbah	2.05	0.248	99	3.4	290.0	11.99	26	3.3
6	Woolly pod vetch cv. Haymaker	0.52	0.750	18	3.8	189.7	135.64	5	2.9
7	Yellow serradella cv. Santorini	4.16	2.326	98	2.9	454.4	80.51	74	3.8
8	French serradella cv. Margurita	4.16	1.075	55	2.6	334.2	15.04	20	3.7
9	Barrel medic cv. Caliph	0.46	0.263	101	3.8	9043.1	18412.28	81	2.7
10	Snail medic cv. Silver	0.49	0.275	95	3.8	3881.1	5926.34	70	3.0

13.4 Discussion

These studies have identified a large variation in initial hard seed levels and breakdown patterns between species of both tropical and temperate legumes in a summer dominant rainfall zone. Initial hard seed values ranged from high (>90%, e.g. desmanthus and medics) to low (<20%, e.g. butterfly pea and woolly pod vetch) with breakdown patterns ranging from slow (>2 years, e.g. desmanthus cv. Marc) to rapid (<12 months, e.g. woolly pod vetch).

13.4.1 Tropical legumes

Desmanthus seed is known to have high levels of hard seed (Hopkins and English 2004) and slow breakdown rate relative to other species (McDonald 2000; Lawrence *et al.* 2012) and this was observed in our experiment also. In a study conducted in Southern Queensland, 2 years after seed were placed in the field, species were ranked in decreasing order of hard seed: desmanthus cv. Marc > burgundy bean = siratro cv. Aztec > butterfly pea cv. Milgarra, where all species had <10% hard seed, with the exception of desmanthus (about 30%) (Lawrence *et al.* 2012). After 2.5 years, species in our study were ranked similarly, although desmanthus had significantly higher hard seed levels.

Results on initial hard seed levels and rate of hard seed breakdown are conflicting. In laboratory studies, McDonald (2000) found that burgundy bean and siratro had low initial levels of hard seed (≤40%) which declined rapidly (within 1 month) when exposed to high fluctuating temperatures. Lawrence *et al.* (2012) reported higher initial hard seed levels (76 and 58% for burgundy bean and Siratro respectively) with a slower rate of decline. Our results were intermediate with initial hard seed levels similar to Lawrence *et al.* (2012), but breakdown rates more similar to McDonald (2000).

A number of tropical legume species have been found to require temperatures >40°C for hard seed breakdown to occur (e.g. Mott *et al.* 1981; McKeon and Mott 1982; McDonald 2000), with higher temperatures resulting in faster breakdown (McDonald 2000). In our experiment, weekly average maximum soil surface temperatures were >40°C for 17–21 weeks of the 26 week period from October–March and were generally highest (>50°C) in January–February.

In a study conducted in Central Queensland, hard seed break down of several stylo species occurred during early summer which allowed for multiple germination events during the summer period. In our environment, regeneration of caatinga stylo was recorded in a study at Bingara during January–March, albeit only small numbers (maximum 2 plants/m^2; Chapter 5). These seedlings were not tagged, so their survival is not known, however none of the stylo species persisted suggesting that the seedlings did not survive winter. In this environment, frosts tend to commence in May, so seedlings of tropical species that regenerate from January need to establish quickly in order to survive winter. If this is the only time recruitment can occur, due to hard seed breakdown patterns this could partly explain the poor persistence of this species in this region. Studies are required to quantify seedling survival in late summer and winter and to determine the likelihood of soft seed (i.e. seed that softened late summer) remaining viable over winter so that it can regenerate in late spring–early summer the following season.

Initial hard seed levels of tropical legumes fell into two groups: high (<70%) and low (<50%). However, based on rate of hard seed breakdown, tropical legumes tested in this experiment fell into three categories which can affect their management and potential role in long-term or permanent pastures:

1. *Slow break down (>2 years)*. Desmanthus cv. Marc was the only entry in this category; maintaining >70% hard seed 2.5 years after the experiment commenced. Such slow hard seed breakdown means that a large seed bank is necessary to ensure that there are

sufficient numbers softening each year, but only infrequent large seed set (possibly every 3–4 years) would be required to maintain the seed bank. The frequency will become clearer once the experiment has concluded.

2. *Intermediate breakdown rates (1–2 years)*. Desmanthus cv. JCU2 was the only entry in this category as it maintained high hard seed levels (>60%) for about 12 months before the rate of hard seed breakdown increased. This pattern suggests that this cultivar may need to set seed biennially to maintain a seed bank.
3. *Rapid breakdown rate (≤1 year)*. Legume entries in this category had a range of initial hard seed values, but all declined rapidly within 1 year. Species include burgundy bean, caatinga cv. Primar SQ, fine stem stylo NSW, siratro and butterfly pea. These legumes would need to set seed annually to maintain a seed bank. Fine stem stylo SQ and caatinga stylo cvv. Primar and Unica SQ also had low hard seed levels and rapid breakdown, however their initial values were much lower than the NSW entries, suggesting potential issues with seed quality.

13.4.2 Temperate legumes

In northern NSW, medics have been reported as having higher hard seed levels than other legumes (Hagon 1974; Lodge *et al.* 1990) and this was confirmed by our findings. In a laboratory experiment subjecting a range of species to temperatures commonly experienced in a native pasture in this environment (40/25°C) naturalised burr medic and snail medic had the highest relative hard seed levels, followed by barrel medics, then subterranean clover, with woolly pod vetch having the lowest hard seed levels of the annual legumes tested (Lodge *et al.* 1990). Despite different cultivars being tested, these species rankings were similar to ours.

The majority of temperate legume hard seed breakdown studies have focused on breakdown during summer immediately following seed set (<6 months; e.g. Norman *et al.* 2006), some during the following legume growing season (up to 12 months), with only a few investigating longer term hard seed breakdown (>12 months; e.g. Taylor 1984; Loi *et al.* 1999; Norman *et al.* 2002; Taylor and Revell 2002) including one study which investigated hard seed levels after 12 years (Taylor and Ewing 1996). While the short term breakdown patterns explain the annual regeneration cycle, longer term studies are important to understand the long-term persistence of the seedbank because not all years are suitable for seedling regeneration and/or seed set (e.g. environmental conditions, grazing management).

Our study was designed primarily to determine the longer term breakdown pattern of legumes, however exhumation dates were selected to also provide some insight into the spring–summer–autumn breakdown patterns. These showed that hard seed of arrowleaf and subterranean clover broke down in spring–summer (October–February), while biserrula broke down from spring until autumn (October–May). French serradella seed broke down throughout summer and autumn (December–May), while bladder clover and yellow serradella did not commence hard seed breakdown until February. The medics did not show any obvious rapid breakdown period.

The temperate legumes in this experiment fall into three categories based on their initial hard seed levels and breakdown patterns:

1. *High initial hard seed levels (>80%) and slow breakdown (>12 months)*. Medics showed a steady decline with time. Barrel medic had higher hard seed levels than snail medic (Hagon 1974). Our results showed that after 12 months the species were similar, with only a slow decline to date; both with >80% hard seed after 12 months in the field. The results to date suggest that a large seed set every 2–3 years is potentially sufficient, but this will be confirmed as the experiment continues.

2. *Moderate to high initial hard seed levels (>50%) and breakdown (about 12 months)*. Both serradella species and bladder clover fell in this category and seed set every 1–2 years would be required to maintain a large seed bank.
3. *Rapid rate of hard seed breakdown (<12 months)*. Woolly pod vetch, subterranean clover and biserrula had rapid hard seed breakdown during the first summer period. Subterranean clover cvv. Dalkeith and Clare had moderate to high initial hard seed levels (66 and 80%, respectively), both with rapid breakdown rate; declining to <20% within 12 months of the start of the experiment. Subterranean clover recognised as having lower relative hard seed levels compared to other species such as medics and biserrula (Hagon 1974; Loi *et al.* 1999). These legumes would need to set seed almost annually.

Hard seed breakdown patterns of species vary in their response to burial, for example subterranean clover has a faster hard seed breakdown rate on the soil surface than buried (Loi *et al.* 1999) while medics breakdown faster when placed on the soil surface (Lloyd *et al.* 1997). Yellow serradella has a higher hard seed breakdown when seed is buried and not exposed to sunlight (Revell and Taylor 1998; Taylor and Revell 2002). In our experiment, temperate legumes were buried 10 mm below the seed surface. This may have increased hard seed breakdown of yellow serradella but reduced breakdown of subterranean clover and medics. In a mixed pasture seed would initially fall on the soil surface (except subterranean clover) and be at least partially covered by senesced herbage. Trampling and grazing by livestock would assist burying some seed and uncovering other seed, so practically seed could potentially be located between 0–50 mm below the soil surface. At 50 mm depth, soil temperatures would be reduced and more stable which would slow breakdown (Taylor 1984).

13.4.3 Variation in hard seed breakdown due to cultivar and seed origin

There are many examples in the literature of differences between populations of seed of temperate and tropical legumes and their hard seed breakdown patterns (e.g. Taylor 1996, Lloyd *et al.* 1997; McDonald 2000) and these can be distilled to three factors: genotype, environmental conditions during seed development and environmental conditions once seed is part of the seed bank (Taylor 2005). In our study, differences in initial hard seed levels and/or hard seed breakdown patterns between cultivars of desmanthus and caatinga stylo produced in NSW and Southern Queensland were evident, however there were few differences between the subterranean clover cultivars other than their initial hard seed levels (*d*) grown at both sites.

The location of seed increase of the tropical legumes affected hard seed levels and breakdown pattern as indicted by the standard errors for the Weibull coefficients. This is probably not surprising as environmental conditions during seed development are known to have important implications for long-term hard seed breakdown patterns (Taylor 1996) and there are multiple reports in the literature about differences due to location (e.g. Taylor and Ewing 1992; Taylor 1996; Norman *et al.* 2002).

Taylor (2005) described various factors that influence initial hard seed and the rate they breakdown; the interaction of these factors vary in different environments. An example is biserrula cv. Casbah which maintains high HS levels in Western Australia's Mediterranean environment (Loi *et al.* 1999), but had faster breakdown rates in central and southern NSW which experience an aseasonal-winter dominant rainfall distribution (Howieson and Hackney 2018). Howieson and Hackney (2018) suggested that higher humidity levels from summer rainfall may have been a contributing factor to faster hard seed breakdown and humidity has been found to increase hard seed breakdown of a range of temperate legumes (e.g. Kirchner and Andrew 1971; Fairbrother 1991) and could also be a contributing factor to faster hard seed breakdown in our environment. Previous studies conducted in summer dominant rainfall zone have noted that soil temperatures tend to be lower than those recorded in Mediterranean environment (e.g. Lloyd *et al.* 1997; Bell *et al.* 2003) due to summer rainfall which results in intermittent cloud cover and soil wetting (e.g. Lodge *et al.* 1990; Lloyd *et al.* 1997; Bell *et al.* 2003); these conditions also increase humidity.

Fine stem stylo SQ and caatinga stylo cvv. Primar and Unica SQ had lower initial hard seed levels than the NSW entries which suggest that there may have been issues with seed quality. The season that seed is produced has been considered as more important than the seed softening environment, and long mild seasons result in higher hard seed levels and slower breakdown rates than poorer seasons or those extended due to late rainfall or irrigation (e.g. Quinliven 1965; Taylor 1996). During the tropical legume seed increase period the growing seasons in both NSW and Queensland were less than optimum. In NSW irrigation was applied following sowing which may have been highly beneficial, not only for establishment but also later in the season during seed set. In contrast the Queensland seed increase area was not irrigated and was subjected to alternating months of average to well below average rainfall (Fig. 13.1).

13.4.4 Practical implications

Local experience and anecdotal evidence suggest that (a) seed banks of species such as biserrula, serradella, arrowleaf clover and burgundy bean are longer lasting than our data indicate and (b) large desmanthus recruitment suggests the hard seed break down patterns may be higher than our results indicate. These differences could be due to a range of factors including ground cover and seed bank dynamics.

13.4.4.1 Role of ground cover

Hard seed breakdown studies reported in the literature tend to be conducted on bare soil, which is not realistic of many situations they will be grown, that is, long term or permanent pastures with perennial grasses. Bare soil temperatures are higher than those with ground cover (Quinlivan 1965) and a live cover (i.e. ground cover provided by live plants) may decrease soil temperatures further which could impact hard seed breakdown (Lodge *et al.* 1990). In WA, cover provided by senesced subterranean clover herbage reduced daily soil surface temperatures by up to 20°C and slowed hard seed breakdown of subterranean clover seed compared to when the herbage was removed (Quinlivan 1965). In native pastures in this region, fluctuating temperatures of 40/25°C have been reported (Lodge *et al.* 1990).

In a laboratory study (Lodge *et al.* 1990), hard seed breakdown of snail and barrel medics and subterranean clovers was less at fluctuating temperatures 40°C/10°C and 40°C/25°C (these temperatures representing those experienced in a native pasture during summer) than extreme fluctuating temperatures of 60°C/25°C (representing a bare soil surface; Lodge *et al.* 1990). Similarly, hard seed breakdown of siratro has been reported to be less in pastures with >90% cover (Jones 1981) where soil temperatures would have been 15–30°C less than those on a bare soil (Gardener 1975). These suggest that hard seed breakdown rates may be slower in a summer growing perennial pasture than reported in our study.

13.4.4.2 Seedbank dynamics

There are three pools of seed in a seed bank: immediate – those softened and ready for immediate germination when environmental conditions are suitable; intermediate – softening commenced in preparation for germination when environmental conditions are suitable; and long term – hard seed that will not germinate in the near future. Each year a legume sets seed, a new generation of seed joins the seed bank and as the pasture ages the seed bank becomes a composite of multiple generations of seed.

Our study showed that seed of arrowleaf clover underwent rapid breakdown from October when it had 74% hard seed to 6% in May. Locally it is observed that arrowleaf can have high hard seed set one year then not been seen for several years. This could suggest that grazing management is an issue (i.e. inflorescences are grazed by livestock) and/or that there are additional factors which influence breakdown in a mixed pasture. These factors could include, but not be limited to, light, ground cover and/or humidity in a perennial pasture.

All literature consistently indicates that desmanthus is hard seeded, so the large recruitment events that are observed in the field are somewhat surprising. These observations, however, could again mean that there is an interaction between the microclimate factors (such as light, cover and humidity) in a perennial pasture. Alternatively it could indicate a large seed bank, such that, germination of a small proportion of a large seed bank could result in a significant number of regenerating seedlings (for example, 5% of 100 seeds/m^2, compared with 1000 seeds/m^2 is 5 and 50 seedlings/m^2 respectively). Seed yield and seed bank studies would address this issue.

13.4.5 Categories of hard seed break down

Above, tropical and temperate legumes were categorised based on their initial hard seed level and hard seed breakdown pattern, while there were differences, the categories could potentially be amalgamated to form a general matrix. Using three levels of initial hard seed and three breakdown patterns, a matrix similar to Table 13.5 can be formed. Initial hard seed levels based on parameter *d* (%) in the Weibull model were divided into high (>80%), intermediate (50–80%) and low (<50%). The hard seed break down were based on the Weibull parameter *e* (days) and were defined as slow (>1000), intermediate (400–1000) and rapid (<400).

Table 13.5. Categories of hard seed breakdown of temperate and tropical legumes in a summer dominant rainfall zone.

Hard seed breakdown pattern	Initial hard seed levels		
	High (I_h; >80%)	Intermediate (I_i; 50–80%)	Low (I_l; <50%)
Slow (P_s; e > 1000 days)	Desmanthus cv. Marc Barrel medic cv. Caliph Snail medic cv. Silver		
Intermediate (P_i; 400 < e < 1000 days)	Desmanthus cv. JCU2 Bladder clover cv. Bartolo Yellow serradella cv. Santorini	French serradella cv. Margurita	
Rapid (P_r; e < 400 days)	Caatinga stylo cv. Primar? Fine stem stylo Biserrula cv. Casbah	Caatinga stylo cv. Primar? Siratro cv. Aztec Subterranean clover cvv. Clare and Dalkeith	Burgundy bean cv. Milgarra Butterfly pea cv. B1 Woolly pod vetch cv. Haymaker

Using this matrix desmanthus cv. Marc and the two medics were classified as I_hP_s. Desmanthus cv. JCU had a faster hard seed breakdown rate than cv. Marc and was classified as I_hP_i, together with bladder clover and yellow serradella. French serradella had lower initial hard seed levels and was classified as I_iP_i. The remaining species had rapid hard seed breakdown patterns (P_r). Fine stem stylo and biserrula fell in the I_hP_r category while caatinga stylo cv. Primar could be either I_h or I_i. Siratro and subterranean clover were classified as I_iP_r while burgundy bean, butterfly pea and woolly pod vetch were grouped together in I_lP_r with low initial levels of hard seed.

This experiment is ongoing and with data collected over a longer period of time, it may be possible to apply a timeframe for hard seed breakdown that will be practically applicable e.g. slow intermediate and rapid being >2 years, 1–2 years and <1 year respectively.

13.5 Conclusion

This study has shown that both temperate and tropical legume species have different initial hard seed levels and hard seed breakdown patterns; ranging from species such as the medics and desmanthus that have high initial hard seed levels and a slow breakdown rate, to woolly pod vetch, burgundy bean and butterfly pea which have low initial hard seed levels with rapid breakdown rates. In addition, a matrix based on initial hard seed and breakdown pattern was proposed that can be used for both temperate and tropical legumes.

This experiment is ongoing with the final treatment for the tropical and temperate legumes to be exhumed in October 2018 (about 1440 days or 4 years after the experiment commenced) and May 2019 (about 1050 days or almost 3 years after the experiment commenced) respectively.

14 First report of Alfalfa mosaic virus on the tropical legume *Desmanthus virgatus* in Australia and the role of the cowpea aphid (*Aphis craccivora*) as the virus vector

J. van Leur, Z. Duric, J.H. George and S.P Boschma

14.1 Introduction

Desmanthus species (desmanthus) are a group of summer growing legumes adapted to neutral to alkaline soils ranging in texture from medium to heavy-clay soils in the drier subtropical environment. Desmanthus is a drought-tolerant perennial legume, commonly found in regions with annual rainfall 500–1000 mm (Cook *et al.* 2005). It is palatable to livestock with high nutritive value (Gardiner and Rangel 1994; Cook *et al.* 2005) but does not cause bloat in cattle due to presence of condensed tannins (Adjei *et al.* 1993). Desmanthus plants are defoliated by heavy frosts but regrow from plant crowns in early spring. Plants can set large quantities of seed which have high hard seed levels, and desmanthus readily recruits from seed following summer rainfall (Cook *et al.* 2005).

Desmanthus has been identified as having potential in sown and native pastures in tropical and subtropical regions in Australia, and although there are two commercial cultivars currently available and it has performed well in experiments (e.g. Pengelly and Conway 2000; Boschma *et al.* 2012), adoption has been slow and its potential has not yet been realised. A number of *Desmanthus* spp. are represented in the Australia commercial cultivars: cv. Marc (*D. virgatus*) and cv. Progardes (composite of five cultivars from three species: *D. virgatus*, *D. bicornutus* and *D. leptophyllus*). Over the last 8 years, desmanthus has also been evaluated in northern NSW (e.g. Boschma and Harris 2009) and has shown potential as a companion legume in sown tropical pastures. These findings extend the boundaries of desmanthus adaptation beyond tropical environments, thereby potentially exposing it to different abiotic (higher frequency of frost and smaller proportion of summer rainfall) and biotic (insect pests and fungal diseases) stresses.

14.2 Disease identification

Severe leaf yellowing (Fig. 14.1) and plant stunting was observed in 12 month old *D. virgatus* cv. Marc plants in experimental plots at the Tamworth Agricultural Research Institute (31^{o}08’43” S 150^{o}58’06” E) in November 2015. Symptomatic plants were randomly distributed through the plots and did not form clear foci, (i.e. have clear points of disease origin, Fig. 14.2). No similar symptoms were observed in other legume species co-located in the paddock, but *Cicer arietinum* (chickpea) grown in neighbouring paddocks showed a high incidence of severe virus symptoms in October.

Virus infection was suspected and Tissue blot immunoassay (TBIA), a reliable, fast and cost efficient methodology to detect viruses in large numbers of individual plants (Freeman *et al.* 2013) was used to test plants for the presence of viruses known to occur on legume crops in the northern Australian grain growing region.

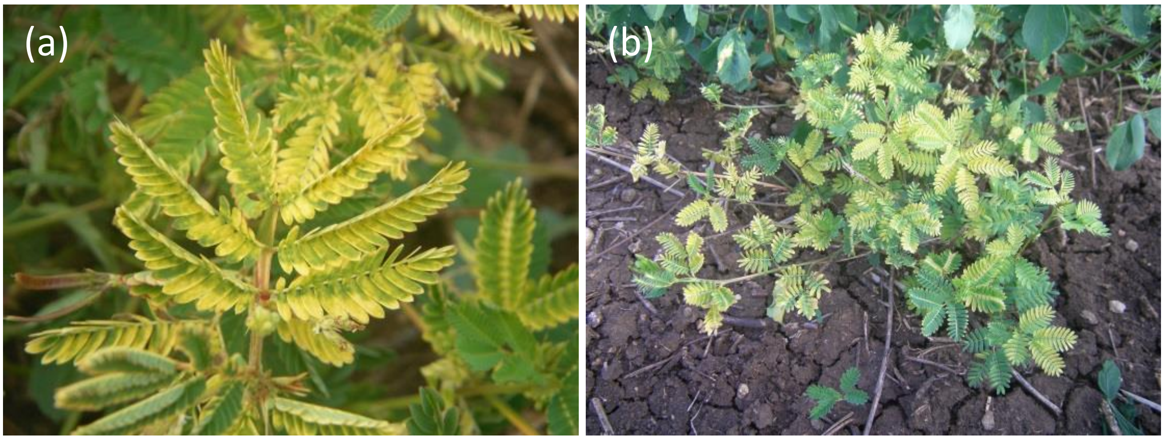

Fig. 14.1. An example of (a) typical leaf symptoms and (b) extensive yellowing and plant stunting of *Desmanthus virgatus* in an experimental plot at Tamworth, New South Wales.

Fig. 14.2. Symptomatic plants were randomly distributed through the experimental plots and did not form clear foci.

In November 2015, 18 symptomatic and 28 non-symptomatic desmanthus plants were sampled by taking 5–10 cm long shoot tips. Random samples of other, non-symptomatic, legumes trialled in the same paddock were also taken: *Medicago sativa* (lucerne), *Leucaena leucocephala* (leucaena), *Biserrula pelecinus* (biserrula) and *Trifolium vesiculosum* (arrowleaf clover). Each stem was blotted on nitrocellulose membranes (Amersham Protran, 0.45 µm pore size) using 6 replicates. The blotting position of each individual stem sample was kept the same on each duplicate membrane, so an individual plant's reaction to different antibodies could be compared. The membranes were processed with polyclonal antibodies specific for *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic* *virus* (BYMV) and *Cucumber mosaic virus* (CMV) and with a monoclonal antibody (5G4) that reacts to luteo and poleroviruses, including *Bean leafroll virus* (BLRV), *Turnip yellows virus* (TuYV), *Soybean dwarf virus* (SbDV) and *Phasey bean mild yellow virus* (PBMYV). All antibodies were obtained from DSMZ (Braunschweig, Germany). TBIA processing protocols described by Freeman *et al.* (2013) were followed. Symptomatic and non-symptomatic chickpea plants were separately sampled from a neighbouring paddock in October 2015 and analysed using the same procedure. All 18 symptomatic desmanthus plants reacted positive to AMV, but not to any of the other antibodies used. All 28 non-symptomatic desmanthus plants reacted negative to all of the antibodies tested. Five out of 10 lucerne plants, three out of 16 biserrula, and one out of 13 arrowleaf clover plants were AMV positive, but none reacted to the other antibodies. AMV was also the predominant virus in the symptomatic chickpea plants; of the 40 symptomatic chickpea plants sampled, 34 were AMV positive and 8 reacted with the general luteovirus monoclonal antibody, while only four of the 40 non-symptomatic chickpea plants sampled were AMV positive and none reacted with the luteovirus antibody. Similarly high incidences of severe symptoms related to AMV infection were found in chickpea and faba bean (*Vicia faba*) crops at several locations in northern NSW during the end of the 2015 growing season (J van Leur, pers. comm.). The high incidence of AMV was unusual compared to earlier surveys done in the same region (van Leur *et al.* 2013) and likely related to a peak in migrating aphids during September (data not presented).

14.3 Confirming virus transmission

AMV is a non-persistently transmitted virus with a very wide host range and has been reported to occur naturally in 47 species in 12 families (Hull 1969), including all pasture and crop legumes commonly grown in Australia. Lucerne is widely grown in northern NSW and can harbour very high levels of AMV infection without showing obvious symptoms (van Leur and Kumari 2011). As a perennial legume it is a likely source of AMV inoculum for other legume species. Inoculum for mechanical transmission was prepared by grinding AMV positive lucerne in a pH neutral phosphate buffer using a chilled mortar and pestle. Healthy desmanthus plants, grown in pots inside a greenhouse, were inoculated by dusting young leaves with carborundum powder and gently rubbing the inoculum into the leaves. The same inoculum was applied to 3-week old greenhouse grown faba bean plants (var. Fiesta). Inoculated plants were observed for symptom development and tested for AMV presence with TBIA. Several attempts to mechanically inoculate desmanthus with AMV did not result in symptoms or in positive TBIA tests, while faba bean plants showed typical symptoms (leaf yellowing, stem necrosis) within 3 weeks of inoculation. Failure to mechanically inoculate plants with AMV have also been reported for lucerne (Hull 1969) and *Cullen australasicum* (Nair *et al.* 2009).

Aphis craccivora Koch (Hemiptera, Aphididae) (cowpea aphid) is considered a serious pest and one of the most common species on legume crops in Australia (Kamphuis *et al.* 2012). It is a cosmopolitan species that is highly polyphagous and feeds on a wide range of pasture and crop legumes including lucerne (Blackman and Eastop 2000; Ilse 2000). Currently it is also the only aphid species reported to feed on desmanthus (Blackman and Eastop 2006). Cowpea aphids can reach high numbers on individual plants and cause damage directly by feeding. It is also a highly effective vector of over 50 virus species (Chan *et al.* 1991). The potential of cowpea aphids to transmit AMV from lucerne to desmanthus was studied in a greenhouse experiment. Morphological characters of the starting colony of laboratory raised cowpea aphid were examined under stereomicroscope and identification confirmed using descriptions in Blackman and Eastop (2000). Mass reproduction of cowpea aphids was performed on a mixture of faba bean, *Pisum sativum* (common pea) and *V. sativa* (common vetch) plants in entomological cages. The plants were regularly watered and examined for aphid colonisation. After the population reached a good density to operate, the cowpea aphids were placed on healthy desmanthus cv. Marc plants to observe whether it would adapt to the new species. The aphids colonised the plants after 2 weeks (Fig. 14.3). The same protocol was followed for *Acyrthosiphon pisum* Harris (pea aphid), however, after four trial repetitions pea aphids did not feed or multiply on desmanthus. To determine if cowpea aphid was a suitable vector for AMV transmission to healthy desmanthus plants, the aphid colony was gently placed on AMV positive lucerne plants in entomological cages. After 7 days, during which the aphids fed and multiplied, virus-free desmanthus plants were placed in the cages with the infected lucerne (Fig. 14.4) and AMV detected by TBIA in the desmanthus plants after 4 weeks.

Fig. 14.3. Developed colony of cowpea aphid on desmanthus plants.

Fig. 14.4. Uninfected desmanthus plants placed in entomological cages with the AMV infected lucerne plants

This is the first report of *D. virgatus* as a host of AMV in Australia. While its suitability as a host for cowpea aphids increases its vulnerability to AMV and other viruses, cowpea aphids are not necessarily the only vector to infect desmanthus with AMV: AMV is a non-persistent virus that can be transmitted by short probing periods of a range of aphids, not only species that are feeding on the host plant. Our field observations suggest that desmanthus productivity is greatly affected by AMV infection but warrants quantification. The potential of AMV to be seed transmitted in *D. virgatus* (Mih and Hanson 1998) will also have implications for seed multiplication and distribution. There are over 300 accessions of Desmanthus spp. held in the Australian Pastures Genebank

(Pengelly and Liu 2001). Further investigations on the susceptibility of these accessions and other commercially available *Desmanthus* spp. is warranted.

14.4 Conclusion

Severe yellowing and stunting of plant growth was observed in experimental plots of *Desmanthus virgatus* (desmanthus) at the Tamworth Agricultural Institute during the 2015–16 summer season. Both symptomatic and non-symptomatic plants were tested for the presence of a range of viruses by Tissueblot immune assay and symptomatic plants consistently reacted positive to AMV, while non-symptomatic plants were virus-free. Attempts to transmit the virus from AMV-positive lucerne plants to desmanthus by mechanical inoculation were unsuccessful; however AMV was successfully transmitted from lucerne to desmanthus by cowpea aphid. Aphid feeding studies showed that the cowpea aphid, but not pea aphid, could colonise and multiply on desmanthus. AMV could become a limiting factor for the adoption of desmanthus as a pasture legume in NSW, particularly as AMV has the potential to be seed transmitted.

15 Can the performance of tropical legumes match lucerne in mixed pastures with digit grass?

S.R. Murphy, S.P. Boschma and S. Harden

15.1 Introduction

Tropical perennial grass species can be productive, persistent and have become increasingly sown in Northern Inland NSW. These grasses are highly responsive by way of dry matter production and improvement to forage quality of nitrogen (N) fertiliser application (Boschma *et al.* 2014a, 2016). However, sole reliance on N fertiliser application on a pure grass sward can be expensive. Companion legumes are one option that can offer a N source to the grass and increase the sward forage quality.

Northern Inland NSW presents a challenging environment for temperate annual legumes to germinate, grow and set seed within a summer active perennial grass sward. The grasses will utilise all plant available soil water over summer (Murphy *et al.* 2008a), autumn is the season with lowest rainfall, and springs often become hot before temperate legumes can set seed, thereby limiting persistence of these legumes. Lucerne (*Medicago sativa*) is the most widely grown legume in our region and has general acceptance as a forage by growers, notwithstanding potential for bloat in cattle. Persistence of lucerne can also be a problem, with production declining over time as the plant population diminishes.

Recent field studies within the MLA Feedbase Improvement project examined the effects of varying sowing configuration and lucerne activity rating in an attempt to manipulate the timing of water use and herbage production (Chapter 12). That study found that herbage production and water use efficiency was higher for a winter active lucerne type compared with more winter dormant types, and that maintaining a closer sowing configuration (that is 1:1 or 3:3 sowing configuration) enhanced herbage production and water use efficiency. Persistence of lucerne within pure swards and in some of the configurations showed substantial decline, even in the 3.5 years of the experiment.

In addition, species evaluation of legumes that are possibly suitable as companions with tropical perennial grasses in our environment identified a range of options (both annual and perennial, temperate and tropical) worthy of field evaluation in mixtures (Chapters 4–6). Two perennial tropical legumes demonstrated high persistence and reasonable production: desmanthus (*Desmanthus virgatus*) and leucaena (*Leucaena leucocephala*) (Chapter 5 and 6).

A field experiment was established to compare the performance of digit grass (*Digitaria eriantha*), lucerne, desmanthus and leucaena grown as both pure swards and in mixtures of the tropical grass and each of the legumes. Data were collected on soil water dynamics, rooting depth, herbage production, and water use efficiency over 4 years (2014–2018) at a field site at Tamworth NSW Australia. The study examined two hypotheses; 1) that pure swards of desmanthus and leucaena would deliver herbage growth similar to that of lucerne, and 2) that in mixed swards the complimentary water use patterns of the tropical legumes and tropical grass would enhance water use efficiency compared with those containing lucerne.

At the time of writing, these studies are ongoing, and this chapter represents the progress to date including analyses of data to the end of 2016–17 growing year.

15.2 Methodology

15.2.1 Site description

The experiment was conducted at Tamworth Agricultural Institute (31°08’43”S 150°58’06”, 400 m above sea level) on a brown Vertosol (Isbell 1996). The annual average rainfall at Tamworth is 674 mm with 60% falling during the warm season between October–March.

The site was previously used in legume-grass experiments conducted between 2008–12 within the EverGraze Program for Northern NSW (Murphy *et al.* 2014). At the conclusion of those studies (November 2012), the site was sprayed with glyphosate (450 g/L a.i. at 1.5 L/ha) to terminate the previous experiment. The site was fallow over summer before cultivating to plant forage oat (*Avena fatua*) in autumn 2013. The forage oat was sprayed with glyphosate (450 g/L a.i. at 1.5 L/ha) before reaching maturity in August 2013. The site was fallow over summer 2013–14 and through winter 2014 to accumulate stored soil water and allow mineralisation of nitrogen. The site was cultivated in September 2014 in preparation for sowing the experimental treatments in November 2014.

An automatic weather station located at the site recorded rainfall (0.5-mm pluviometer) at 30-min intervals.

15.2.2 Neutron probe access tube installation and soil profile description

On 6 November 2008 an aluminium access tube (49 mm extern. diam. sealed at the bottom) was installed in the centre of each plot to 1.9 m depth after removing a soil core (51 mm extern. diam.) with a hydraulic push-coring machine and pouring about 1 L of a clay slurry (1 kg kaolinite: 1 L water) to fill any voids and ensure good contact between the access tube and soil (White and Ridley 1998). A cap was placed over the top of the tube to prevent rainwater from entering. The soil cores were used to calibrate the neutron moisture meter (NMM, CPN503–DR Hydroprobe, Boart Longyear Co., Martinez, CA) using method described in Murphy *et al.* (2017) and to analyse soil characteristics.

Soil water content (g/g^3) was determined by dividing each removed soil core into segments (0–0.1, 0.1–0.3, then 0.2 m intervals to 1.9 m) and retaining the samples in plastic bags, which were weighed in the field to obtain a wet weight and then again after drying at 105°C for 48 h. Soil bulk density (Mg/m^3) was calculated using the soil dry weight and volume of each soil core segment.

Three samples at each depth were air dried and analysed to determine soil pH_{Ca}, electrical conductivity ($EC_{1:5}$ dS/m) and soil particle size distribution.

The soil profile was non-saline ($EC_{1:5}$ <0.65 dS/m) with an alkaline pH trend with values being alkaline at the soil surface (pH_{Ca} 8.2) and increasing alkalinity into the lower profile, 0.9–1.9 m, (pH_{Ca} 8.8–9.1, Table 15.1). Proportion of soil particles (sand 20–2000 µm, silt 2–20 µm, clay <2 µm, gravel >2000 µm) for each layer were determined using the laser diffraction method (Malvern Mastersizer 2000) and showed that the proportion of clay was greater than for either sand or silt for all sampling layers (Table 15.1). Clay content was >39% for sampling layers below 0.3 m depth, while silt content was high at both the surface (38.3%) and in the deepest sampling layer (38%), but decreased in the middle profile (32–35%). Soil texture at the surface (0–0.1 m) was silty clay loam and trending to silty clay at depths >0.1 m.

The soil physical data were used in pedotransfer functions (McKenzie and Cresswell 2002) to predict soil water content values for the drained upper limit (c. -10 kPa) and crop lower limit (c. -1500 kPa) for each soil layer. The profile stored soil water at drained upper limit and crop lower limit were 817±1.1 and 439±0.1 mm, respectively suggesting maximum plant available water content (PAWC, mm) of 378±1.0 mm.

Table 15.1. Proportion of sand (20–2000 µm), silt (2–20 µm) and clay (<2 µm) soil particles, soil salinity and soil pH of the brown vertosol sampled in 10 layers at Tamworth Agricultural Institute.

Sampling layer (m)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Soil salinity (dS/m)	Soil pH_{Ca}	Soil bulk density (g/g^3)
0–0.1	0.1	26.4	38.3	35.3	0.18	8.2	1.19
0.1–0.3	3.2	23.1	35.0	39.0	0.19	8.6	1.34
0.3–0.5	2.6	23.8	33.0	40.7	0.22	8.7	1.40
0.5–0.7	3.2	24.2	32.0	40.7	0.26	8.7	1.45
0.7–0.9	2.2	25.0	33.0	39.7	0.29	8.7	1.46
0.9–1.1	1.3	25.4	34.0	39.3	0.32	8.8	1.48
1.1–1.3	1.2	24.2	35.0	39.7	0.33	8.8	1.48
1.3–1.5	1.6	22.4	35.3	40.3	0.38	9.1	1.47
1.5–1.7	0.5	22.1	36.0	41.3	0.48	9.1	1.51
1.7–1.9	0.4	21.3	38.0	40.3	0.62	9.0	1.48

15.2.3 Neutron probe calibration

The NMM was calibrated by determining the relationship between neutron count and values of volumetric soil water content (SWC, θ_{vol} m^3/m^3), collected when access tubes were installed and at other times with conditions of high (near drained upper limit) and low (near crop lower limit) SWC, giving a range of values (n = 140) (Lodge *et al.* 2010b). When access tubes were installed SWC was allowed to equilibrate for 24 h before taking neutron counts. Gravimetric SWC was determined on retained soil cores sampled at 0.2 m depth intervals from 0.1 m to a maximum depth of 1.9 m and converted to volumetric SWC by using bulk density values for each layer (Table 15.1).

For each soil sampling layer, values of volumetric SWC (θ_{vol}) and neutron count were plotted and logarithmic regression (Y = 131559 Ln (x) + 26398) best explained the relationship between SWC (x) and neutron count (Y, R^2 = 0.87, n = 140).

15.2.4 Experimental design and establishment

The experiment was a partially randomised block design with three replicates of nine treatments making 27 plots in total, with each plot 6 x 9 m. The nine treatments (Table 15.2) included; digit grass cv. Premier (DI), desmanthus cv. Marc (DE), lucerne cv. Venus (LU), and leucaena cv. Tarramba

(LE) sown as pure swards of each species and then as mixes of digit grass with each legume (DE-DI, LU-DI, LE-DI). The final two treatments were digit grass sown in the alleys of the leucaena treatments (i.e. >2.5 m from leucaena rows as a pure sward (LE-IR) or mixture with digit grass (LE-DI IR).

Digit grass, desmanthus and lucerne treatments were sown on 19 November 2014 into a prepared seed bed at 0.17 m row spacing using a cone seeder with press wheels. Lucerne and desmanthus seeds were inoculated with commercial Rhizobia and kept cool prior to sowing. For the mixtures, digit grass, lucerne and desmanthus seeds were sown in alternate rows at 50% of the rate for pure treatments.

Table 15.2. Description, sowing rate (kg/ha viable seed or leucaena plants/ha) and species configuration, also neutron moisture meter access tube location for treatments in the experiment at Tamworth Agricultural Institute.

Treatment	Code	Cultivar	Sowing rate (kg/ha, plants/ha)		Configuration	Description	Access tube location
			Grass	Legume			
Digit grass	DI	Premier	2	-	All rows	Digit grass sown in every drill row	Plot centre
Desmanthus	DE	Marc	-	4	All rows	Desmanthus sown in every drill row	Plot centre
Desmanthus + digit grass	DE-DI	Marc + Premier +	1	2	1:1	Desmanthus and digit grass sown in alternate rows	Plot centre
Lucerne	LU	Venus	-	2	All rows	Lucerne sown in every drill row	Plot centre
Lucerne + digit grass	LU-DI	Venus + Premier	1	1	1:1	Lucerne and digit grass sown in alternate rows	Plot centre
Leucaena	LE	Tarramba	-	3333	Twin rows 1 m apart	Leucaena seedlings transplanted at 0.5 m intervals into twin rows 1 m apart on 12 m spacing. Digit grass sown no closer than 2.5 m from leucaena.	Plot centre, between leucaena rows
Leucaena inter-row sown to digit grass	LE-IR	Premier	2	-	All rows	Digit grass sown in the inter-row between leucaena rows	6 m from centre of leucaena rows
Leucaena + digit grass	LE-DI	Tarramba + Premier	2	3333	Digit all rows, Tarramba twin rows 1 m apart	Leucaena seedlings transplanted at 0.5 m intervals into twin rows 1 m apart on 12 m spacing. Digit grass sown up to 0.5 m from leucaena.	Plot centre, between leucaena rows
Leucaena + digit grass inter-row sown to digit grass	LE-DI IR	Premier	2	-	All rows	Digit grass sown in the inter-row between leucaena + digit grass	6 m from centre of leucaena rows

Leucaena seedlings were transplanted into pre-ripped (0.5 m-deep), twin rows, 9.0 m long and 1.0 m apart along the centre axis of the plot, on 21 November 2014. For the mixture with digit grass (LE-Di), the twin rows were perpendicular to the grass sowing rows. Ripped lines were pre-irrigated using inverted soaker hoses to ensure high soil water content prior to planting seedlings at 0.5 m spacing along the rows. Total density of leucaena plants was 3333/ha. The soil surface of the pure leucaena (LE) treatment was maintained weed and digit grass free for 2.5 m on either side of the leucaena rows and covered with sugar cane mulch at 1500 kg DM/ha, while in the mixture, digit grass was sown to within 0.5 m from the leucaena rows. For the first 12 months after transplanting the LE-DI mixture, the area within the twin rows and 0.5 m on either side were kept free of digit grass seedlings by applying imazethapyr (700 g/kg a.i.) at 70 g/ha.

Heavy rainfall between 1 December 2014 and 4 January 2015 (280 mm, see Fig. 15.1) resulted in soil erosion across the site, which was most severe in 4 plots of lucerne (LU, plots 1 and 13) and lucerne-digit (LU-DI, plots 4 and 12) treatments in replicates 1 and 2. These treatment plots were resown on 5 February 2015 at the same rate as previous and were given supplementary irrigation on 6 occasions (5 mm per irrigation) through-out February to ensure germination and establishment.

15.2.4.1 Treatment plots and experimental management

The leucaena treatments, which represent an alley system of twin rows of leucaena separated by an alley of grass, were allocated specific rows of plots in the experimental structure. Within each replicate group, pure leucaena (LE) and leucaena-digit grass (LE-DI) plots were separated by a plot of digit grass to represent a 12 m wide alley of grass between the leucaena (LE-IR, see Plate 1, centre image).


LE-DI with LU-DI in foreground


Site


LE with DE-DI in foreground

Plate 15.1. The site in December 2016 to illustrate the twin row plantings of Leucaena and good growth achieved in spring and early summer.

Herbicides were applied throughout the experiment to control annual broadleaf and grass weed seedlings. In pure digit grass plots (DI), MCPA (500 g/L a.i. at 1L/ha), dicamba (500 g/L a.i. at 400 ml/ha), metsulfuron methyl (600 g/kg a.i. at 2 g/ha) were applied as required to control seedlings of broadleaf weeds. In pure plots of desmanthus (DE) and leucaena (LE), imazethapyr (700 g/kg a.i. at 70 g/ha) was applied in winter and or spring as needed as a pre-emergent herbicide. Lucerne (LU) received applications of flumetsulam (800 g/kg a.i. at 25 g/ha), 2,4-DB amine (500 g/L a.i. at 1.5 L/ha), or fluazifop-P (212 g/L a.i. at 500 mL/ha) as required to control seedlings of grass and broad leaf weeds. Digit grass-lucerne plots (LU-DI) received applications of flumetsulam (800 g/kg a.i. at 25 g/ha) and 2,4-DB amine (500 g/L a.i. at 1.5 L/ha) to control seedlings of grass and broadleaf weeds.

The entire experiment received 200 kg/ha single superphosphate (8.8% phosphorus, 11% sulfur) applied in spring each year from 2014–18. The pure digit grass treatment (DI) also received 110 kg/ha urea (46% N) applied in both spring and summer (total of 220 kg/ha, timed to occur with rainfall to minimise volatilisation). Digit grass in mixtures or in the alley between leucaena rows (LE-IR, LE-DI IR) did not receive additional N.

In the establishment year (2014–15) treatment plots were not grazed to allow seed set of digit grass and desmanthus. At the final assessment each growing season (usually in May), desmanthus was left ungrazed to allow seed set. At the beginning of the next growing season, usually in September, treatment plots were mown to remove residual frosted material and the leucaena plants trimmed to 0.3 m height with the exception of 2016 when the plants remained green throughout the uncommonly mild winter.

15.2.5 Data collected

15.2.5.1 Soil water content

Soil water content (SWC) was estimated at approximately 3-week intervals using a NMM. Neutron counts were taken on 37 occasions from 20 November 2014 to 5 July 2017. Counts were taken over a 16-s period with readings taken at the midpoint of 0.2 m soil layers from 0.1–0.3 m to a maximum depth of 1.9 m. Values of volumetric SWC for each layer were converted to stored soil water (SSW, mm) and summed to obtain values for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and the total profile (0.1–1.9 m). Soil water content continued to be measured through 2017–18 and will cease following first frosts in 2018.

15.2.5.2 Herbage production

Herbage production (kg DM/ha) was estimated on 20 occasions at approximately 6-week intervals during the pasture growing period (September–May). The first assessment being in early spring to measure growth of lucerne, which was usually the only treatment having growth through winter. Values of herbage production were accumulated for each of three growing years beginning on 1 July and ending on 30 June.

Green herbage mass was estimated by two observers placing four quadrats (0.4 x 0.4 m) along separate transects that ran along the long axis of each plot (a total of eight quadrats per plot). Quadrats were placed to avoid edge effects and total herbage production was assessed to a height of about 0.1 m using a continuous 0–5 scale (0, nil; 5, high) and the percentage of legume or grass was estimated (on a dry-weight basis). At each sampling time, 20 calibration quadrats (0.4 x 0.4 m), selected to cover the range of herbage mass and proportion of legume at the site, were harvested to 10 mm above ground level, sorted into sown legume, sown grass and other (all voluntary species), and each portion dried at 80°C for 48 h before weighing.

Scores and percentage estimates for these calibration quadrats were regressed (linear or quadratic $R^2 > 0.75$, $n = 20$) against actual herbage mass (kg DM/ha) and percentage of legume and grass to determine the herbage mass of each component in each strata per treatment plot.

Plots were grazed by wether sheep to a residual height of about 100 mm after each assessment and any residual stem >100 mm in height was removed using either a flail or rotary mower equipped with a catcher.

Leucaena growth and herbage production were assessed by recording the physical dimensions of the shrubs together with a visual standard technique (e.g. Murphy *et al.* 2017). Firstly, the overall width (m) and height (m) of the twin rows of leucaena were measured at four points along the rows. Secondly, a typical cane (e.g. length and amount of edible leaf and stem) of the leucaena plants was harvested, and using this cane as a guide, the number of similar canes was counted for four shrubs in each twin row (total of 8 plants/plot). Edible material from the reference cane (leaf and stem to 10 mm diameter) was then removed, dried at 80°C for 48 h and weighed. Edible herbage mass for the plot (kg DM/ha) was determined by multiplying the reference value by the mean count of canes per plant and the total number of plants per hectare (3333/ha).

Herbage production will continue to be assessed until the first frosts of 2018.

15.2.5.3 Plant frequency

Plant frequency (Brown 1954) was assessed twice a year; after the start and prior to the end of each growing year (c. November and May). The number of cells (0.1 x 0.1 m) containing a live plant of either digit grass or legume were counted in two fixed quadrats (1.0 x 1.0 m, total 100 cells) in each plot about 7 days after grazing and the proportion of occurrence determined. Plant frequency of leucaena in LE and LE-DI plots was not measured, but frequency of digit grass was measured in LE-DI plots. Leucaena plants in each row were counted to record any losses. Plant frequency data will be compiled and analysed at the completion of the experiment.

15.2.6 Statistical analysis

Maximum extractable water. SWC data for each treatment were plotted for each measurement layer and sampling time. Periods of 3 months or more during each growing year (September–May) when SWC declined without interruption by complete profile rewetting (i.e. significant rainfall events) were identified as they demonstrated the capacity of the treatments to extract soil water (Murphy and Lodge 2006). The difference between the high and low values of SSW (i.e. start and end date of the periods identified respectively) for each measurement layer and treatment (Neal *et al.* 2012) was calculated as the maximum extractable water (MEW, mm). Values of MEW for each depth were summed to values for the upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and full (0.1–1.9 m) soil profile layers. Analysis of variance was used to model values of MEW for each soil layer against the factors treatment, year and their interaction. Replicate effects within each year were accounted for in the model by including the interaction of year by replicate as a block term. The least significant difference (LSD) was calculated using a 5% level of significance.

Plant root depth. Changes in soil water content values between the dates identified to determine MEW were also used to estimate plant rooting depth (Murphy and Lodge 2006). On each date, the trend in SWC over depth among the three replicate values was modelled using a linear mixed model for each plant density. The model included depth as a covariate and a fixed term to model the linear trend over depth and a spline term as a random effect to model a smooth curvilinear trend (Verbyla *et al.* 1999). Terms to account for differences between the replicates were included as random effects in the model. Predicted values of SWC with depth (±s.e. of prediction) were plotted for each plant density and plant root depth identified as the greatest depth where the modelled decrease in soil water content was >0.01 m^3/m^3 (Murphy and Lodge 2006).

Soil profile refill. Soil moisture accumulation, or refill (mm), during the winter period (typically May to September) when digit grass was frosted and not growing was calculated. Using plots of SWC data for each soil layer and sampling times, periods when SWC changed from lowest to highest were identified. The differences between the values of low and high SWC for each soil layer were used to calculate change in stored soil water (SSW, mm). Values of change in SSW were summed for each part of the soil profile [i.e. upper (0.1–0.7 m), middle (0.7–1.3 m), lower (1.3–1.9 m) and full (0.1–1.9 m)]. Analysis of variance was used to determine significant differences among start and end stored soil water, change in SSW in each soil layer, and rainfall refill efficiency. The least significant difference (LSD) was calculated using a 5% level of significance.

Plant production. Trend in herbage production (kg DM/ha) and legume percentage were modelled using a linear mixed model for each treatment. The model included sample date as a covariate and a fixed term to model the linear trend over time and a spline term as a random effect to model smooth curvilinear trend. Predicted values of herbage production were accumulated for each growing year. Analysis of variance was used to model values of accumulated plant dry matter against the factors of treatment, year and their interaction. The least significant difference (LSD) was calculated using a 5% level of significance. Replicate effects within each year were accounted for in the model by including the interaction of year by replicate as a block term.

Water use efficiency (WUE, kg DM/ha/mm) for each 6-week interval during the growing year was calculated by dividing herbage production (kg DM/ha) by actual evapotranspiration (ETa, mm) (Allen *et al.* 1998). Actual evapotranspiration was determined by water balance, under the assumption that no water was lost by runoff or deep drainage. WUE of dry matter production for each growing year was calculated for each treatment in each year and for total dry matter production over 3 years. Significant differences were determined by analysis of variance and least significant difference (LSD) calculated using a 5% level of significance.

Plant frequency. Trend in plant frequency (%) was modelled using a linear mixed model for each plant density. The model included sample date as a covariate and a fixed term to model the linear trend over time and a spline term as a random effect to model smooth curvilinear trend. Predicted values of plant frequency with time (±s.e. of prediction) were plotted to determine changes for each pasture type.

15.3 Results

15.3.1 Rainfall

Seasonal rainfall totals received at the site have shown distinct periods where values were below and above average (Fig. 15.1) The experiment treatments were established in a 6 month period where rainfall totals were well above average (e.g. summer 2014–15 and autumn 2015, Fig. 15.1). In the following 2 year period, seasonal rainfall totals from winter 2015 to autumn 2016 were consistently below average, while totals from winter 2016 to autumn 2017 were consistently above average (Fig. 15.1).

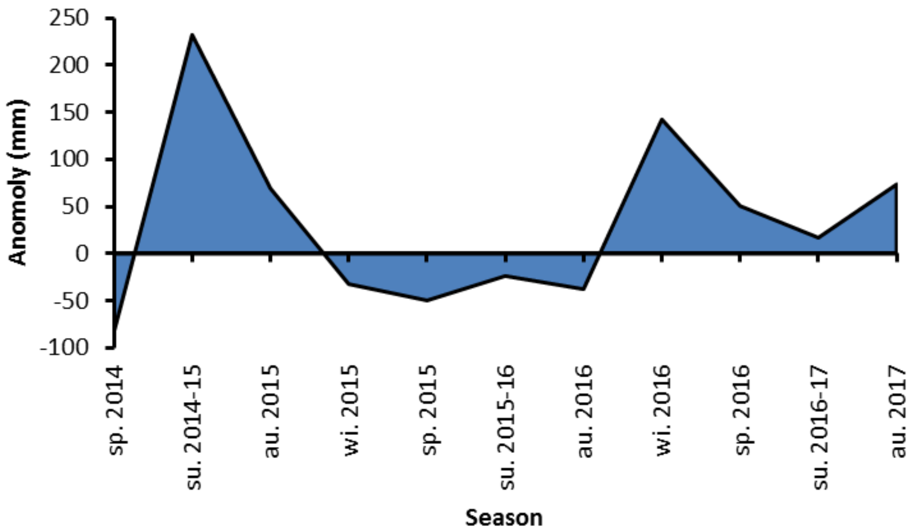

Fig. 15.1. Seasonal (su. – summer; au. – autumn; wi. – winter; sp. – spring) rainfall anomaly for the experimental site compared with the long-term average for Tamworth NSW for the period sp. 2014–au. 2017.

15.3.2 Soil water content through time

The soil water content heat maps provide a visual representation of soil water dynamics though time and depth (Fig. 15.2). The blue colours indicate wetter soil and the red colours indicate dryer soil. The patterns of declining SWC over summer (summer drying, darker reds and progressively deeper in the profile) and increasing SWC during winter (winter refill, darker blues and progressively deeper in the profile, Fig. 15.1).

During summer 2014–15 and autumn 2015, soon after treatments were sown, DE, DE-DI and LE-DI showed greater areas of red colouring than the other treatments (Fig. 15.2c, e, f). All treatments showed a distinct increase in area of red colouring, a decline in SWC, through spring 2015 to autumn 2016, which coincided with below average rainfall (Fig. 15.1). The treatments that showed the greatest area and intensity of low soil water content were DI, LU, LU-DI and LE-DI IR (Fig. 15.2).

Digit grass (Fig. 15.2a) showed the strongest drying of the soil down to 1.2 m depth (darker red colours in the upper and middle horizons), while leucaena (LE) stood out as having weaker drying, with generally higher soil water content indicated by greater area of blue and green colours (Fig. 15.2b).

Lucerne (Fig. 15.2d) and LU-DI (Fig. 15.2g) showed little extraction of soil water in 2014–15 due to the slow start of growth by these treatments. In later years, however, LU showed strong drying and extraction of soil water (i.e. more red colours) for longer period of time (September–May) compared with DE or DE-DI (Oct or Nov–May). The patterns of soil water content of digit grass sown in the leucaena inter-row (LE-IR or LE-DI IR, Fig. 15.2h, i) appear more similar to that of pure digit (DI, Fig. 15.2a) until autumn 2016, but thereafter appeared to have high soil water content.

15.3.3 Plant root depth and maximum extractable water

Plant root depth varied considerably between treatments and between years, with all treatments achieving maximum root depths in either 2015 (1.68–1.80 m) or 2016 (1.56–1.80 m, Table 15.7). Plant root depth and maximum extractable water achieved by each treatment in the 2017 growing year is represented graphically in Fig. 15.3.

A maximum root depth of 1.80 m was achieved by DE, LU, LU-DI, LE-DI, and LE-DI IR but not LE (1.68 m) or DE-DI (1.75 m).

In 2015, DE (191 mm) extracted more soil water than for five treatments (LU-DI, LU, LE-IR, LE-DI IR, and DI, 56–113 mm) ($P < 0.05$, Table 15.7). In 2016 and 2017, however, all treatments extracted similar amounts of soil water ($P > 0.05$, Table 15.7), despite the fact that all treatments extracted greater amounts of water in these latter years compared with 2015. Closer examination of the data analyses indicated that the residuals were larger for a single treatment plot in one replication for specific treatments in each year of 2016 and 2017, resulting in differences among the treatments being non-significant.

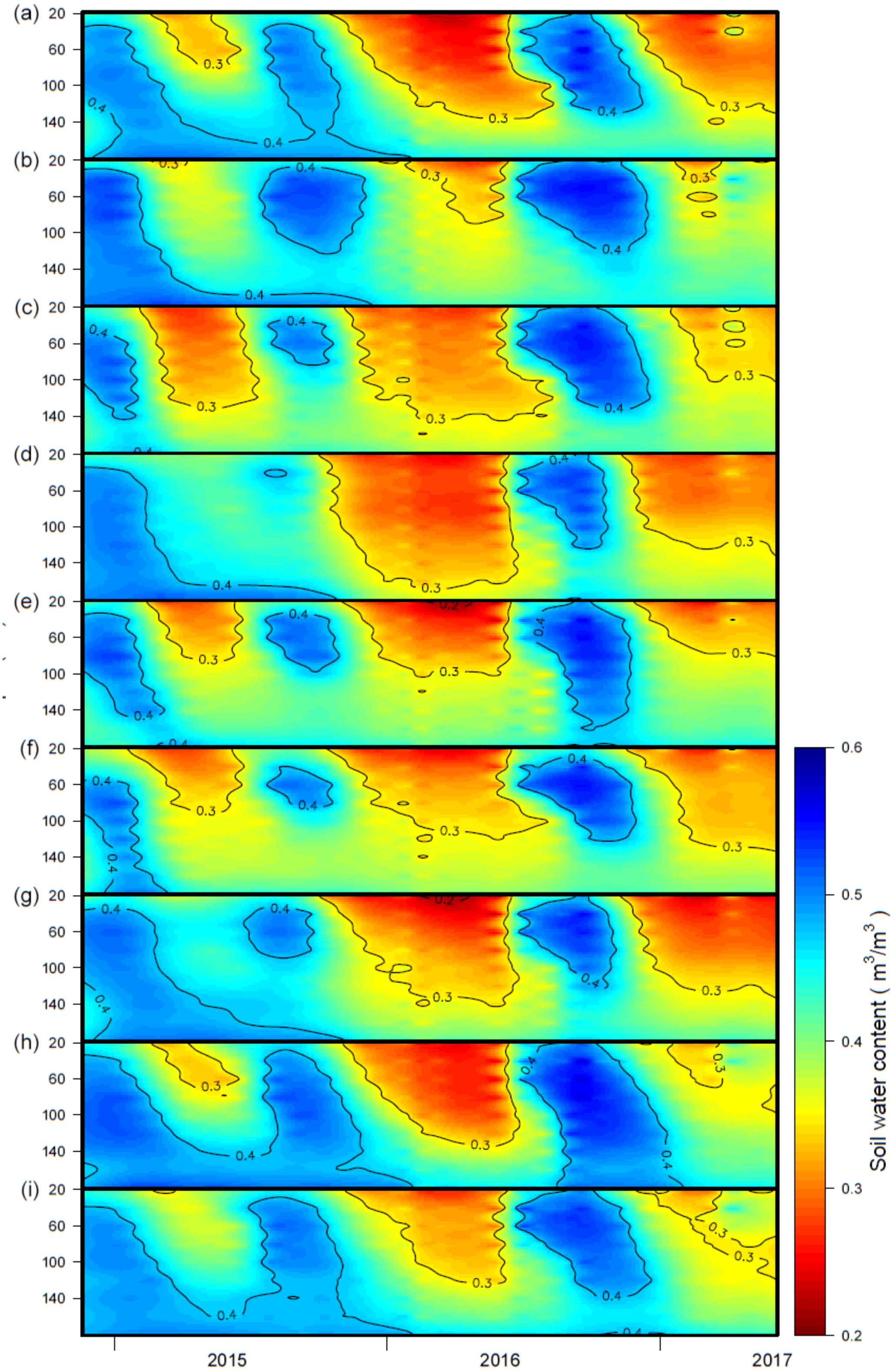

Fig. 15.2. Patterns of distribution of profile (0.2–1.8 m) soil water content (m^3/m^3) through time for (a) DI, digit grass; (b) LE, leucaena; (c) DE, desmanthus; (d) LU, lucerne; (e) LE-DI, leucaena-digit grass; (f) DE-DI, desmanthus-digit grass; (g) LU-DI, lucerne-digit grass; (h) LE-DI IR, leucaena-digit grass inter-row and (i) LE-IR, leucaena inter-row from November 2014 to July 2017.

Table 15.7. Plant root depth (m) and maximum extractable water (MEW, mm) for treatments in each of four growing years. Least significant differences (LSD; *P* = 0.05) among treatment means for each year is provided where appropriate.

Treatment	Root depth (m)			MEW (mm)		
	2015	2016	2017	2015	2016	2017
DI	1.30	**1.56**	1.46	113	247	**249**
DE	**1.80**	**1.80**	1.36	191	183	**218**
DE-DI	**1.75**	1.12	1.44	162	144	**217**
LU	0.40	**1.80**	1.71	69	221	**239**
LU-DI	1.24	**1.80**	1.47	56	242	**257**
LE	**1.68**	1.54	1.48	128	**198**	175
LE-IR	1.29	**1.69**	1.55	79	199	**202**
LE-DI	1.53	**1.80**	1.60	158	174	**226**
LE-DI IR	1.10	**1.80**	**1.80**	109	**261**	235
Mean	1.34	**1.66**	1.54	118	208	**224**
LSD	-	-	-	67.6	NS[1]	NS

[1]NS, not significant

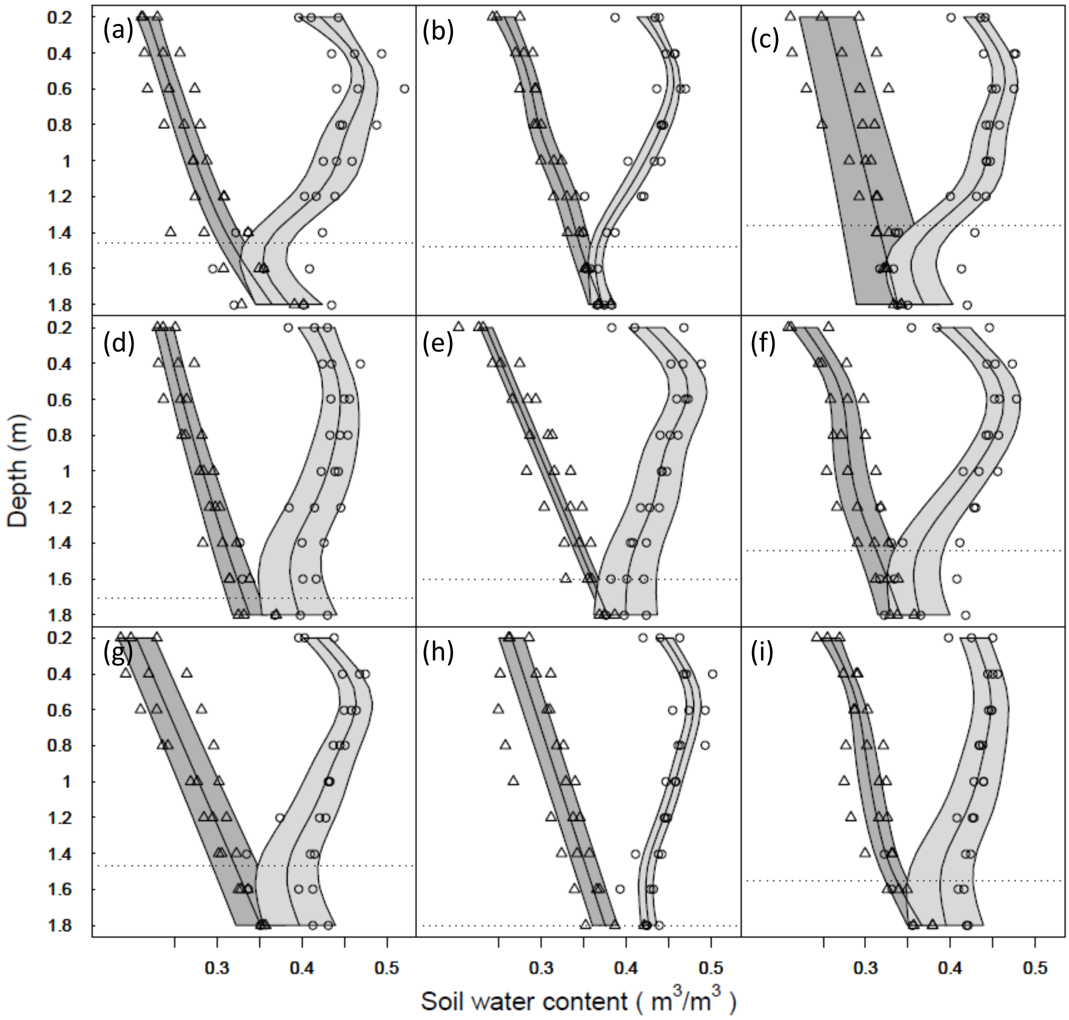

Fig. 15.3. Observed replicate values of maximum (○) and minimum (Δ) volumetric soil water content (m^3/m^3) for each treatment (a) DI, (b) LE, (c) DE, (d) LU, (e) LE-DI, (f) DE-DI, (g) LU-DI, (h) LE-DI IR, and (i) LE-IR indicating maximum extractable water (mm) in growing year 2017. Area of shading represent the predicted soil water content ± s.e. of prediction. Root depth is indicated by the horizontal dot line.

15.3.4 Rainfall refill efficiency

Change in stored soil water (mm) and rainfall refill coefficient (%) were different among the treatments for 2015 but not 2016 (Table 15.8). The mean amount of refill that occurred in 2015 (100 mm) was less than half that occurred in 2016 (254 mm, Table 15.8), but the mean rainfall refill coefficients were similar (59 *v.* 62%, Table 15.8). In 2015, LU and LU-DI treatments (22 and 33 mm) captured less winter rainfall than the other treatments, while DE (167 mm) captured more rainfall than LE-IR (87 mm, Table 15.8). Lucerne plants in the LU and LU-DI plots were still establishing through the winter of 2015 and so the soil water content was still quite high (Fig. 15.2), preventing high rainfall refill coefficients.

Table 15.8. Change in profile stored soil water (SSW, mm) and rainfall refill efficiency (%) in each winter rainfall refill period. Least significant differences (LSD; P = 0.05) are provided where appropriate.

Treatment	Change in SSW (mm)		Rainfall refill (%)	
	2015	2016	2015	2016
DI	114	268	70	67
DE	167	248	102	54
DE-DI	116	214	71	46
LU	22	280	16	70
LU-DI	33	275	24	69
LE	113	201	58	51
LE-IR	87	222	53	56
LE-DI	126	263	64	66
LE-DI IR	119	317	73	80
Mean	100	254	59	62
LSD	59.5	NS[1]	36.3	NS

[1]NS, not significant

15.3.5 Growing year herbage production, total water use and water use efficiency

15.3.5.1 Leucaena row height and width

At every sample date the mean height and width of leucaena rows in the LE treatment were greater than those in the LE-DI treatment (Fig. 15.4). The height of the row in LE was on average 0.32 m higher (range 0.1–0.61 m) than for the LE-DI rows (Fig. 15.4a). The width of the row in LE was on average 0.49 m wider (range 0.23–0.74 m) than for the LE-DI rows (Fig. 15.4b). These differences in row height and width, while not yet statistically analysed, translated into substantial differences in edible herbage mass produced by the two leucaena treatments.

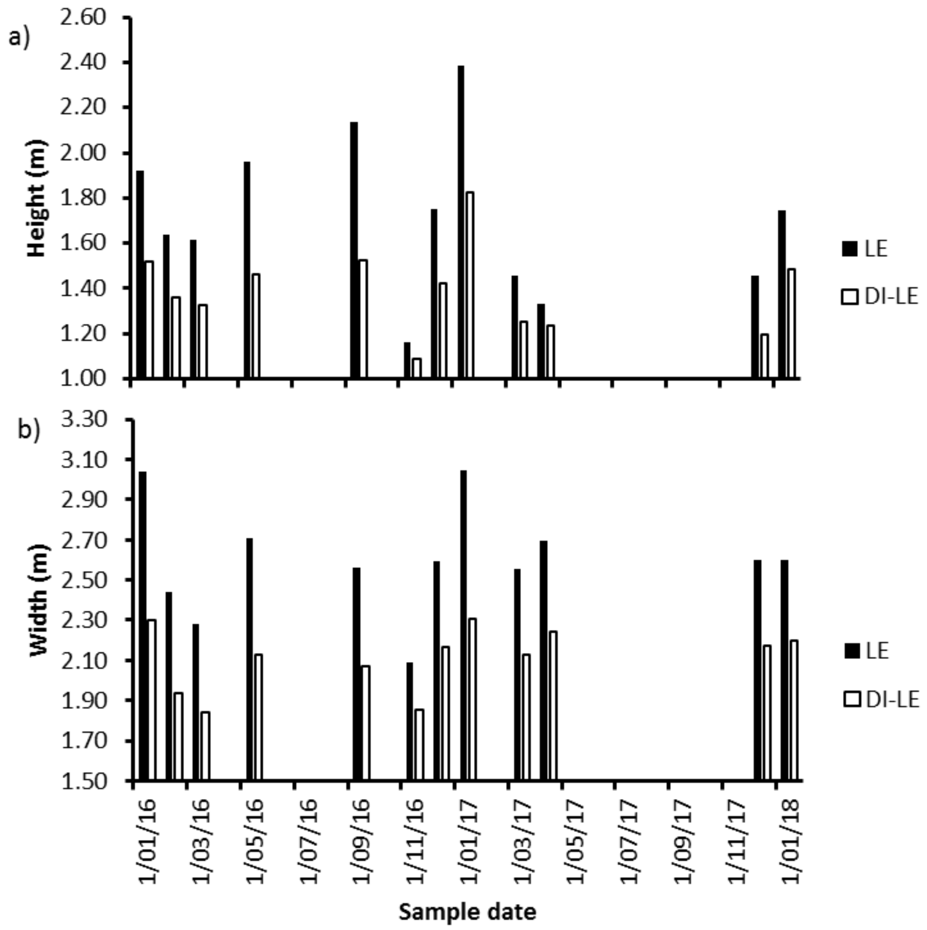

Fig. 15.4. Mean (a) height (m) and (b) width (m) of leucaena rows in pure leucaena (LE) and leucaena-digit grass mix (LE-DI) treatments on each date when herbage mass was assessed.

15.3.5.2 Herbage mass

Annual herbage mass of digit grass as a pure sward (DI) ranged 10.8–13.3 t DM/ha (Table 15.9). Typically, DI had greater herbage mass compared with the grass component in all legume mixtures (DE-DI, LU-DI and LE-DI), except in 2016 when the amount of grass in the LE-DI (10424 kg DM/ha) was similar to that of DI (13286 kg DM/ha, Table 15.9). Interestingly, while the herbage mass of grass in LE-IR (8283–9523 kg DM/ha) and DI were similar each year, herbage mass for LE-DI IR was lower in 2016 and 2017 (9732 and 7823 kg DM/ha, respectively, Table 15.9).

Legume herbage mass differed among the treatments each year, but the best performing legume also differed each year. In 2015, legume herbage mass for DE (14370 kg DM/ha, Table 15.9) was greatest, while in 2016, DE, LU and LU-DI were similar (8185–10669 kg DM/ha) but greater than LE (4731 kg DM/ha) and the lowest was LE-DI (1355 kg DM/ha, Table 15.9). In 2017, legume herbage mass for LU (16913 kg DM/ha) was greatest and LE-DI (2638 kg DM/ha) was lower than all other treatments, respectively. Legume herbage mass for LE (4731 and 10326 kg DM/ha) was greater than for LE-DI (1355 and 2638 kg DM/ha) in both 2016 and 2017 (Table 15.9).

Over the three years of data presented, 2015–2017, it was interesting that the proportion of legume in total herbage mass increased in LU-DI (59 to 74%, mean 69%), decreased in DE-DI (71 to 27%, mean 47%) and was low each year in LE-DI (12–23%, mean 17%, Table 15.10).

Table 15.9. Digit grass, legume and total herbage mass (kg DM/ha), also total water use (mm) and water use efficiency (kg DM/ha/mm) for treatments in each of four growing years. Least significant differences (LSD; *P* = 0.05) are provided where appropriate.

Treatment	Herbage mass (kg DM/ha)									Total water use (mm)			Water use efficiency (kg DM/ha/mm)		
	Grass			Legume			Total								
	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
DI	10819	13286	12041	-	-	-	10819	13286	12041	731	694	855	14.8	19.1	14.1
DE	-	-	-	14370	8487	8245	14370	8487	8245	819	603	848	17.6	14.1	9.7
DE-DI	3928	4172	6754	9841	2913	2547	13769	7085	9301	766	614	850	17.9	11.5	10.9
LU	-	-	-	2842	10669	16913	2842	10669	16913	734	641	886	3.8	16.5	19.1
LU-DI	2258	2774	4145	3277	8185	11951	5534	10959	16096	682	675	904	7.9	16.2	17.8
LE	-	-	-	na[2]	4731	10326	na	4731	10326	767	653	845	na	7.3	12.4
LE-IR	8283	12325	9523	-	-	-	8283	12325	9523	724	649	856	11.4	19.5	11.1
LE-DI	4348	10424	8641	na	1355	2638	4348	11779	11279	774	595	827	5.5	19.8	13.7
LE-DI IR	10825	9732	7823	-	-	-	10825	9732	7823	760	670	834	14.2	14.7	9.4
LSD	4612.3	4659.8	3201.5	5944.9	2470.9	2426.3	4787.1	4185.0	2937.3	NS[2]	NS	NS	5.57	6.45	3.62

[1]na, not assessed; [2]NS, not significant

Table 15.10. Proportion (%) of legume of total herbage mass each year for each mixture.

Treatment		2015	2016	2017	Average
Leucaena-digit grass	LE-DI	-[1]	12	23	17
Desmanthus-digit grass	DE-DI	71	41	27	47
Lucerne-digit grass	LU-DI	59	75	74	69

[1]-, not assessed

15.3.5.3 Total water use

Despite some reasonable ranges of values, total water use was not different among the treatments in each growing year (Table 15.9) and mean values of total water use across the treatments varied by >100 mm from year to year (2015, 751 mm; 2016, 644 mm; 2017, 856 mm). Therefore, these water use data suggest that differences in total herbage mass rather than total water use *per se*, led to differences in water use efficiency.

15.3.5.4 Water use efficiency

Digit grass as a pure sward (DI) delivered annual water use efficiency ranging 14.1–19.1 kg DM/ha/mm (Table 15.9). The WUE for LU and LE increased each year from 3.8 to 19.1 and 7.3 to 12.4 kg DM/ha/mm respectively, while that for DE decreased from 17.6 to 9.7 kg DM/ha/mm (Table 15.9). The only occasion that LU had a lower WUE than either DE or LE was in 2015, when both DE and DE-DI (17.6 and 17.9 kg DM/ha/mm) had higher WUE than for LU or LU-DI (3.8 and 7.9 kg DM/ha/mm, respectively). In 2016, WUE of both LU and DE (16.5 and 14.1 kg DM/ha/mm, respectively) were higher than LE (7.3 kg DM/ha/mm), but WUE of DE-DI (11.5 kg DM/ha/mm) was less than that of LE-DI (19.8 kg DM/ha/mm), with the latter result due to herbage mass of digit grass, rather than leucaena (Table 15.9). In 2017, LU and LU-DI (19.1 and 17.8 kg DM/ha/mm, respectively) had higher WUE than the other legumes and their mixtures.

With one season of data yet to be finalised, it appears that the WUE of DE is declining (17.6–9.7 kg DM/ha/mm) and WUE of LE is increasing (7.3–12.4 kg DM/ha/mm).

15.4 Discussion

15.4.1 Discussion against hypotheses

The study examined two hypotheses; 1) that pure swards of desmanthus and leucaena would contribute herbage growth similar to that of lucerne (FALSE), and 2) that in mixed swards the complimentary water use patterns of the tropical legumes and tropical grass would enhance water use efficiency compared with those containing lucerne (FALSE).

15.4.1.1 Desmanthus and leucaena did to provide similar herbage to lucerne

Data from the experiment to date suggests that neither desmanthus nor leucaena were able to match the productivity of lucerne in this environment. The establishment year is the only exception, where desmanthus in either pure sward (DE) or in mixture with digit grass (DE-DI) generated high herbage mass, which easily exceeded that of lucerne (LU) or lucerne mixtures (LU-DI). However, during this season, growth of lucerne and lucerne mixture treatments were delayed due to plots in replicate 1 and 2 being resown in February 2015. In subsequent years the yield of lucerne increased, while that of desmanthus decreased.

In late-spring 2015, desmanthus plants first showed leaf yellowing and subsequent testing confirmed that the plants were infected with alfalfa mosaic virus (AMV). With infection most likely from an outbreak of aphids in September. Over the following years desmanthus plants showed severe yellowing and stunting. It is not possible to quantify or determine statistically the decline in herbage mass production of desmanthus due to AMV as the experiment was not set up to test this, however, growth of desmanthus was lower in subsequent years. Desmanthus has been reported as a host of AMV (Mih and Hanson 1998) however this is the first report of infection in Australia (Chapter 14).

Two important findings have emerged from the herbage mass data for leucaena. Firstly, that herbage production from the pure leucaena was less than for lucerne and secondly, that the productivity of leucaena was severely penalised when grown in mixture with digit grass.

While leucaena herbage mass was not assessed in the establishment year (2015), plants grew to about 1.8 m, flowered and set seed, indicating that considerable herbage mass was generated (Plate 2). In subsequent years, with 6 weekly grazing regime and removal of herbage material, the leucaena plants did not flower. Being susceptible to frost, leucaena plants recommenced growth each season from the plant crown. Production of new canes delayed accumulation of sufficient herbage mass and hence grazing until about December. This was also noted at experiments conducted at Bingara, Manilla and Trangie (Chapter 6).

Plate 15.2. Leucaena cv. Tarramba plants showing substantial height with flowers and seed pods in April 2015, some 5 months after planting.

The data from the LE-DI treatment clearly showed that leucaena herbage mass was severely restricted when growing in mixture with digit grass. Total herbage mass of leucaena in LE-DI was typically just 25% of that in LE, further exacerbating the underperformance compared with LU based treatments. This showed that competition from digit grass growing close (i.e. 0.5 m) to the leucaena plants during the establishment year severely affected their establishment and subsequent production. In contrast, leaving a grass-free band (e.g. 2.5 m) limited the competition effect of the grass, thus confirming similar findings from Queensland (Dalzell *et al.* 2006).

The dominance of lucerne within LU-DI mixtures was demonstrated by there being no difference in total herbage mass between LU-DI and LU throughout the experiment to date. However, as the experiment progressed, the dominance of lucerne increased with the proportion of lucerne in the mixture rising to 74% (69% overall). In contrast, the dominance of desmanthus declined throughout the experiment as the proportion of desmanthus in the mixture fell to just 27% (47% overall), while leucaena was the least competitive averaging just 17% overall in its mixture.

15.4.1.2 Complimentary water use patterns did not always lead to high WUE (AMV on DE, slow starts by LE)

The second hypothesis that complimentary water use patterns between tropical legumes and digit grass would lead to higher WUE was not supported by the data. There are, however, a number of issues that complicate analysis of this hypothesis: lack of significant differences in total water use of the treatments due to differences in initial stored soil water; reestablishment of plots; and impact of AMV on desmanthus.

Total water use by the treatments did not differ in any year to date. Given that within each year values of total water use varied by considerable amounts (2015, 137 mm; 2016, 99 mm, 2017, 77 mm) it was surprising that these differences were not statistically significant. By contrast, in the lucerne variety mixtures experiment (Chapter 12), least significant differences in total water use among treatments were as little as 25.6 mm. Closer examination of the residuals associated with specific replicates of some treatments in the current experiment revealed the reason for the lack of significant differences. Unfortunately, differences in stored soil water among the replicates at the beginning of the experiment resulted in large error terms and lack of significance. This will be further examined at the conclusion of the experiment to ascertain whether covariates can be used to account for these differences. Once this is done, we will be able to revisit whether this hypothesis was upheld.

The water use data and herbage mass data for desmanthus, appear to have been severely impacted by AMV, which has made it more difficult to examine this hypothesis. As discussed above, we were not able to quantify the degree that production was diminished, but it appeared that the decline in productivity occurred following infection with AMV.

15.4.2 Practical implications for industry

The findings from this experiment again suggest that lucerne as a legume in mixed swards is 'hard to beat' in this environment. Both desmanthus and leucaena provided useful contributions of legume herbage mass in specific seasons and under specific conditions, but both underperformed compared with lucerne across the three years of the experiment.

From the perspective of the companion legume fixing and providing sufficient nitrogen to the system, only lucerne exceeded the amount considered a minimum to sustain grass production (i.e. ≥4 t DM/ha per year to make available ≥50 kg N/ha; Boschma *et al.* 2014b). At the conclusion of the experiment, total values across all years will be quantified to further examine this finding.

Desmanthus showed great potential, particularly in the first and early stage of the second growing seasons, prior to infection with AMV. AMV has a broad range of hosts (Latham and Jones 2001), including lucerne and is transmitted by aphids (Garran and Gibbs 1982). Lucene is also capable of harbouring high levels of AMV infection without showing either leaf symptoms or loss of productivity (van Leur and Kumari 2011) and was found to have 50% positive AMV infection when desmanthus was first tested (Chapter 14). Growing both lucerne and desmanthus in close proximity on the experimental site may certainly have contributed to cross-infection. Desmanthus infection with AMV also demonstrates that pushing the boundaries of adaption by a pasture species has potential to expose them to new pests and diseases.

Leucaena production appears to fall short of what we need in this environment to make a meaningful contribution to a grazing system. The impact of frost on leucaena herbage production warrants closer examination. In three of the four winters experienced during the experiment, leucaena plants were totally frosted. This requires plants to regrow from basal buds and re-establish. In Queensland, it is recommended to not graze plants until they are more than 1.5–2.0 m tall, otherwise recovery after grazing will be slow, overall production can be limited, and the plant frame will be weak (Dalzell *et al.* 2006). For our frost prone plants, first grazing generally occurred in late November or early December, about 8–10 weeks after last frost. Given that optimal time for regrowth is about 8–12 weeks, depending on soil moisture and temperature, leucaena in our environment might be grazed just 2–3 times per season, thereby limiting its overall contribution to the grazing system. It is important to note however, that during extended periods of low rainfall when the other species had ceased growth, leucaena continued to grow. The ability to provide quality forage during dry periods is an advantage.

15.4.3 Unanswered questions?

Following completion of the experiment, unanswered questions will become clearer. However, at this stage the following appear likely:

1. Lucerne again dominated overall herbage production when grown in mixture with digit grass, which reinforces the findings from the lucerne variety mixtures experiment reported in Chapter 12. This finding suggests the need to do further studies to determine the optimal proportion of lucerne within mixtures with digit grass.
2. Initial production and seed set from desmanthus was impressive, but the impact of AMV on subsequent production was substantial. While plants survived, and at times did not appear symptomatic, production was severely reduced in the latter part of 2016 and into 2017–18. Further studies are required to determine the variation in susceptibility between lines of the *D. virgatus* and other *Desmanthus* spp, also heritability of AMV through subsequent generations and quantify the impact on production.
3. Production from leucaena, whether in pure or mixed swards, was less than that of lucerne and desmanthus (before infection with AMV). Water use data and herbage production suggest that recovery from frosting severely limits overall production, as the plants are required to re-establish a canopy each growing season. Further work to determine the minimum temperature thresholds where leucaena is viable is warranted.

15.5 Conclusion

This experiment has shown that lucerne in mixed swards with digit grass is highly productive in this environment. While both desmanthus and leucaena provided useful contributions of legume herbage mass in mixtures with digit grass in specific seasons and under specific conditions, both underperformed compared with lucerne. This experiment will cease in autumn 2018 and further analysis of total water use, herbage mass and water use efficiency following final data collection.

16 Differences in soil water dynamics between temperate and tropical pastures for Central Western NSW

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16.1 Introduction

Globally, shifts in rainfall patterns represent a major impact of climate change. In the winter dominant rainfall environments of South Eastern Australia a warming of up to 3.38°C, and annual rainfall reductions by up to 28% have been predicted (Cullen *et al.* 2009). While the effect of future climate change on pasture production is somewhat uncertain, a high degree of regional variation is predicted (Howden *et al.* 2008; McKeon *et al.* 2009). As key determinants of pasture growth are seasonal patterns and variability in rainfall, adaptation strategies that offer greater flexibility in pasture growth and production, identification of forage species adapted to local environments are required to support sustainable management of livestock production systems under climate change.

Areas of low to medium rainfall (400–600 mm), with limited seasonality of growth such as the mixed farming region of Central Western New South Wales (NSW) offer an opportunity to examine pasture persistence and growth patterns under suboptimal environmental conditions. In this region, winter rainfall is more effective because rainfall intensity and evapotranspiration rates are low compared to summer when rainfall which commonly falls in high intensity storms and temperatures are high (Hobbs and Jackson 1977). Sown pastures in this area have traditionally consisted of temperate annual and perennial species with *Medicago sativa* (lucerne) the most widely sown perennial legume (Lodge *et al.* 1991). Temperate annual legumes such as *Trifolium subterraneum* (subterranean clover) and *Medicago* spp. (medics) are sown on the neutral and alkaline soils respectively (Lodge *et al.* 1991). However, over past decades, tropical grasses have been sown in Northern Inland NSW due to a range of desirable agronomic and environmental attributes, but only limited adoption in the Central West. Maintaining the productivity in these tropical pastures requires good fertility, in particular nitrogen (Boschma *et al.* 2014a) and a niche for productive, sustainable legumes may offer cost effective alternatives to inorganic nitrogen (e.g. Peck *et al.* 2011, 2012b). Tropical legumes provide a new suite of legumes that have a complementary growth pattern with tropical grasses compared to temperate legumes that may benefit pasture productivity. However, while tropical grasses have been successfully sown in Central Western NSW, there is an information gap in understanding the growth, productivity and persistence of companion legumes species and the complementarity of tropical or temperate legumes to tropical grass pastures in medium rainfall environments of Central Western NSW.

Water use and water use efficiency (WUE) are key indicators used to evaluate plant responses in low rainfall environments (Steiner and Hatfield 2008) and information on seasonal soil water dynamics, rooting depths, peak growth periods and WUE are fundamental traits to underpin recommendations to producers and to identify diverse forage options in medium rainfall environments. This study aimed to compare soil water dynamics, rooting depths, peak growth periods and WUE for new emerging tropical legumes and a perennial tropical grass with currently used temperate legume species.

16.2 Methodology

16.2.1 Site description

This study was part of a larger multi-site study which examined the use of legumes as companion species within tropical perennial grass pastures (Chapters 4 and 5). The study site was located at Trangie Agricultural Research Centre in Central Western NSW, Australia (31°59’45” S, 147°56’18” E; elevation 220 m, Fig. 16.1). Mean annual rainfall at the study site is 493 mm (87 years of records) with no distinct seasonality. Mean monthly maxima ranges from 33.3^{0}C in January to 15.4^{0}C in July and minima from 18.5^{0}C in January to 3.2^{0}C in July. Frosts are common in winter.

The soil type is categorised as a brown Chromosol soil (Isbell 1996) with surface soil (0–0.1 m depth) having a clay loam texture with weak pedal structure and sub-soil (0.1–1.0 m) having a sandy clay texture and a massive pedal structure. Soil chemical properties derived from nineteen samples collected from the general site area in March 2013 to a depth of 0.2 m (Table 16.1) indicate that the near surface soil has pH in the lower end of ideal pH range for plant growth, is non saline, non-sodic and of low fertility. The site was on flat ground with moderate drainage.

Table 16.1. The range of soil structural and chemical characteristics of field study site derived from 19 soil samples (0–0.1 m and 0.1–0.2 m).

Depth (m)	pH ($CaCl_2$)	EC (dS/m)	Phosphorus (Colwell) (mg/kg)	Total Nitrogen (%)
0.0–0.1	5.2–5.6	0.037–0.070	7.8–15	0.02–0.11
0.1–0.2	5.2–5.7	0.021–0.062	7.6–19	0.061–0.09

The site had not been cropped or fertilised for a number of decades prior to commencing the WUE experiment but had been subject to grazing by domestic stock. A combination of glyphosate (450 g/L a.i. at 500 ml/ha) and 2,4-D amine, (625 g/L a.i at 500 ml/ha) and cultivation was used for weed management prior to the commencement of the experiment.

16.2.2 Experimental design and treatments

Six pasture treatments including two cultivars of tropical legume, desmanthus (*Desmanthus virgatus* cvv. JCU2 (www.progardes.com.au) and Marc); three temperate legumes, lucerne (*Medicago sativa* cv. Venus), woolly pod vetch (*Vicia villosa* cv. Haymaker) and barrel medic (*M. truncatula* cv. Caliph); and tropical digit grass (*Digitaria eriantha* cv. Premier) and a control (no plants or fallow) were planted in a modified randomised complete block design with three replications (Fig. 16.1). Desmanthus cvv. JCU2 and Marc were selected as summer-growing perennial legumes adapted for neutral to alkaline, medium and heavy-clay soils in the drier (mean annual rainfall, 550 mm) subtropical environment. Lucerne cv. Venus, has a 'semi-dormant' winter growth pattern and was selected as a deep rooted perennial legume widely used in the local area. Woolly pod vetch cv. Haymaker and barrel medic cv. Caliph are both winter growing annual legumes, the latter has historically been a widely used pasture species in Central Western NSW. Digit grass is a tufted perennial summer-growing grass best suited to medium and light textured soils and is also sown within Central Western NSW. All legumes were transplanted as spaced plants, while digit grass had persisted in an established plot at the Trangie Agricultural Research Centre, sown sixteen years prior to the commencement of the experiment and was located about 100 m from the legume treatments.

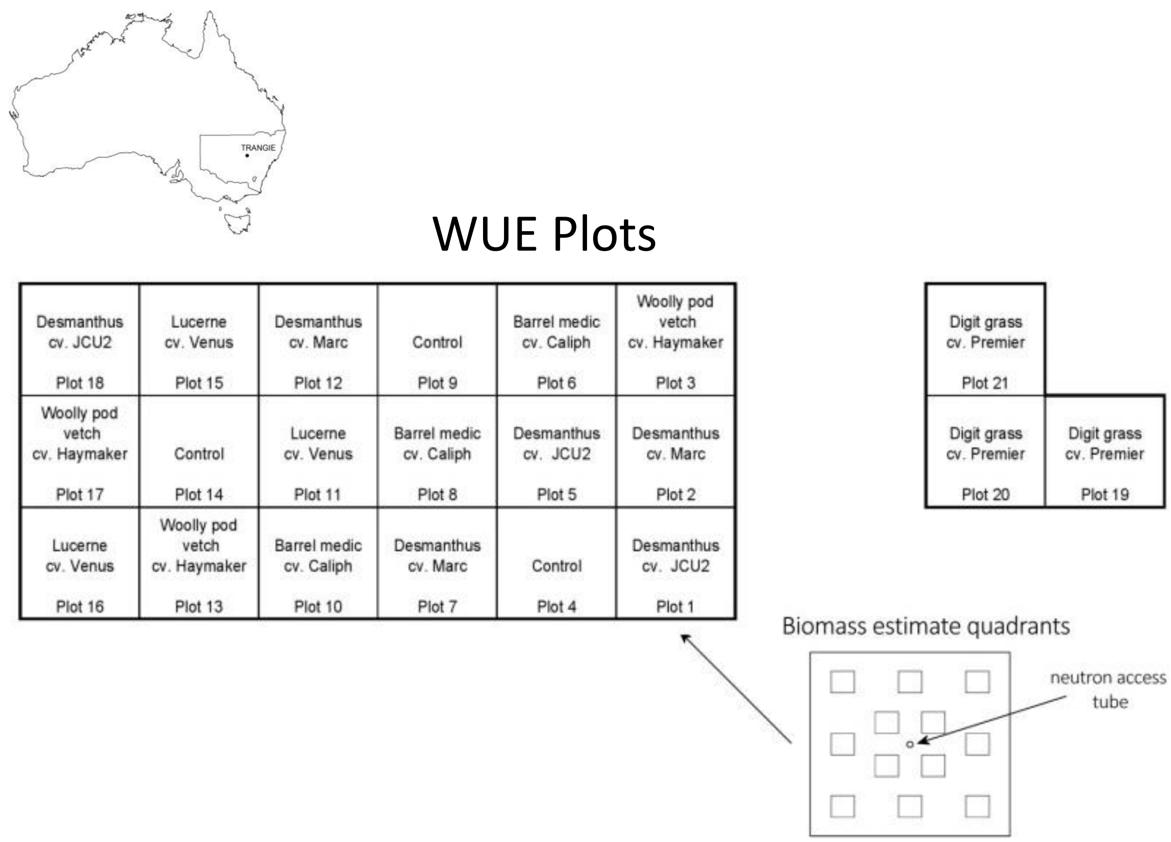

Fig. 16.1. The location of the study site in Central Western NSW and experimental site plan showing plot treatments, location of neutron access tubes and the position of 12 biomass estimate quadrants within each plot.

In 2014, seed of the two desmanthus cultivars (JCU2 and Marc) were germinated in the glasshouse and transplanted as 6 week old seedlings in November 2014 into replicated pure sward plots as spaced plants (9 plants/m^2; Fig. 16.1). In the following autumn (2015) all temperate legumes were transplanted as spaced plants following the same procedure. The plots were irrigated as required to ensure establishment of the experiment.

16.2.3 Herbage mass

Herbage mass (kg DM/ha) was assessed at approximately 6 week intervals in each plot from June 2015 until April 2017 using a modified Ranked Set Sampling method of McIntyre (1952) which has been previously described by El-Shaarawi and Esterby (1999). Here, 8 (0.50 x 0.50 m^2) quadrats were randomly located within the perimeter of each plot (two on each side of the plot) and 4 permanently marked 0.50 x 0.50 m^2 quadrats in close proximity to an access tube located in the center of each plot (Fig. 16.1). Each of the 12 quadrats were visually scored using a continuous scale (0–5), scores from one plot were independent of scores on another plot. A photographic record for each plot, at each assessment date was also made. Only one quadrat representing the median (score = 3) was cut (to 10 mm) from the random quadrats around the plot perimeter. The 4 permanent quadrats were not cut. Cut samples were dried in a dehydrator at 80^{0}C for 48 hours, weighed and plot herbage mass (kg DM/ha) determined. Plant dry matter was not harvested or removed following each assessment of herbage mass.

16.2.4 Soil water content

A neutron moisture meter (NMM, CPN 503DR Hydroprobe; Boart Longyear co., Martines, CA, USA) was used to measure SWC at approximately 3-week intervals from late winter 2014 to early autumn 2017. A single aluminium access tube (50 mm outside diameter, 48 mm inside diameter) was installed, 6 weeks prior to the first transplant date, in the centre of each plot to a depth of 1.2 m to capture the likely plant root depth. Access tubes were installed using a hydraulic push-coring machine and pouring about 1 L of a clay slurry (1 kg kaolinite: 1 L water) to fill voids and ensure good contact between the access tube and soil (White and Ridley, 1998). A cap was placed over the top of the tube to prevent rainwater entering.

To study soil water dynamics, NMM measurements were taken and values converted to volumetric soil water content (SWC, θ_{vol} m^3/m^3) following a calibration for red-brown earths at the study site (R^2 = 0.909; McKenzie *et al.* 1990). Counts were taken over a 16-s period at 0.1 m intervals down the soil profile with readings representing soil layers 0.1 m thick to a depth of 1.25 m (i.e. 0.05–0.15; 0.15–0.25; …1.15–1.25 m). Values of volumetric SWC for each layer were converted to stored soil water (SSW, mm) for each 0.1 m increment and full profile (0.05–1.25 m).

In August 2016 a scheduled NMM reading was not taken due to equipment failure. To obtain an estimate of SWC to coincide with the pasture herbage mass assessment done on 30 August 2016, linear interpolation was used between values of SWC for each profile depth on the preceding and following sample dates (i.e. SWC = (43*(SWC on 18 July 2016) + 28*(SWC on 28 September 2016))/71).

16.2.5 Statistical analysis

All analyses were conducted in GENSTAT Release 19.1 (VSN International, Hemel Hempstead, UK). Analysis of variance of a completely randomised experiment was used to determine significant differences among start and end stored soil water, change in SSW in each soil layer, herbage mass, WUE and rainfall refill efficiency. The least significant difference (LSD) was calculated using a 5% level of significance.

Maximum extractable soil water. Periods were identified where total profile (0–1.2 m) SSW declined from high to low without interruption by complete profile rewetting (i.e. significant rainfall events) as such periods demonstrate the capacity of the treatments to extract soil water and so represent plant root depth (Murphy and Lodge 2006). Maximum extractable soil water (MEW, mm) was defined as the difference between the high and low values of SSW (i.e. start and end date of the periods identified, respectively) within these periods with values determined for the upper (0.05–0.75 m), lower (0.75–1.25 m) and full soil profile (0.05–1.25 m). Plant root depth was estimated by analysing the values of soil water content (SWC) at each 0.10 m depth at the start and end of the period used to determine MEW (Murphy and Lodge 2006).

Plant root depth. For the dates identified to determine MEW, changes in SWC values were also used to estimate plant rooting depth. For each treatment the trend in SWC over depth among the three replicate values was modelled using a linear mixed model for each treatment. The smoothing function of depth was a spline with 3 df. (Verbyla *et al.* 1999). Predicted root depth was calculated as the greatest depth at which the two curves +/- standard error did not overlap (Murphy and Lodge 2006).

Herbage mass. A variance components analysis was undertaken using REML repeated measures for herbage mass and analysed for species/cultivar and date effects. Two separate analyses were undertaken for tropical (i.e. desmanthus and digit grass) and temperate species (temperate legumes).

Water use efficiency. Standing herbage mass was determined for each sample date within each of three growing years beginning on 1 July and ending on 30 June (vis. 2014–15, 2015–16, 2016–17). Within each growing year, changes in soil water content (SWC) were used to identify a distinct growth period, with the start and end being determined by regeneration (and/or high SWC) and peak standing dry matter (and/or low SWC), respectively. For lucerne, which can grow year-round in this region, growth periods were selected based on continuous growth. The dates and length of each growth period for the treatments are given in Table 16.2. WUE (kg DM/ha/mm) of peak standing dry matter was calculated by dividing herbage mass (kg DM/ha) at that time by actual evapotranspiration (mm, Allen *et al.* 2008) from the start of the growth period. Actual evapotranspiration was determined by water balance with the assumption that no water was lost by runoff or deep drainage.

Table 16.2. The dates and duration (weeks) of growth periods for treatments during each of the three experimental years.

Treatment	2015			2016			2017		
	Start date	End date	Duration (wks)	Start date	End date	Duration (wks)	Start date	End date	Duration (wks)
Digit grass	-	-	-	17Sep15	22Apr16	31	27Sep16	4Apr17	27
Desmanthus cv. JCU2	18Nov14	29Jun15	32	1Oct15	22Apr16	29	27Sep16	4Apr17	27
Desmanthus cv. Marc	18Nov14	29Jun15	32	1Oct15	22Apr16	29	27Sep16	4Apr17	27
Lucerne	27Apr15	2Nov15	27	16May16	3Aug16	11	27Sep16	8Nov16	6
Barrel medic	23Apr15	1Oct15	23	16May16	4Oct16	20	-	-	-
Woolly pod vetch	27Apr15	1Oct15	22	16May16	4Oct16	20	-	-	-

Soil profile refill. Soil moisture accumulation, or refill (mm) of the tropical species (digit grass and desmanthus) was calculated during the cool season period (typically March to September) when tropical species were frosted and not growing, similarly refill of lucerne was calculated during a single period of inactivity (March–September 2016). Refill periods were identified by interpreting plots of profile SSW through time where SSW continuously increased from low to high. Soil water refill (mm) was calculated as the difference between the values of low and high SSW for each soil layer for the upper (0.05–0.75 m), lower (0.75–1.25 m) and total (0.05–1.25 m) soil profile.

16.3 Results

16.3.1 Rainfall

Rainfall over the experimental periods was highly variable with generally warmer temperatures, higher than long-term average rainfall experienced over the warmer seasons (Fig. 16.2). Monthly rainfall in both winter and spring of 2016 were considerably above average (Fig. 16.2).

16.3.2 Maximum extractable soil water and plant rooting depth

Significant differences in MEW values occurred among treatments for the growing periods in each year of the experiment (Table 16.3). In all cases, there was greater extraction in the upper profile (0.05–0.75 m) than the lower profile (0.75–1.25 m). During the 2014–15 growing season, only the two desmanthus treatments were assessed; cv. Marc (69.0 mm) extracted more water than cv. JCU2 (61.2 mm) ($P < 0.05$). During 2015–16, digit grass (89 mm) extracted more water ($P < 0.05$) than all other treatments apart from desmanthus cv. Marc (73.6 mm). Woolly pod vetch extracted the least water (59.0 mm), but was similar to the remainder of the treatments. In 2016–17, digit grass (136.9

mm) extracted more water (*P* < 0.05) than the other perennial species (i.e. desmanthus and lucerne; 83.5–86.0 mm), which in turn extracted more water (*P* < 0.05) than the annual legumes (51.7–53.1 mm).

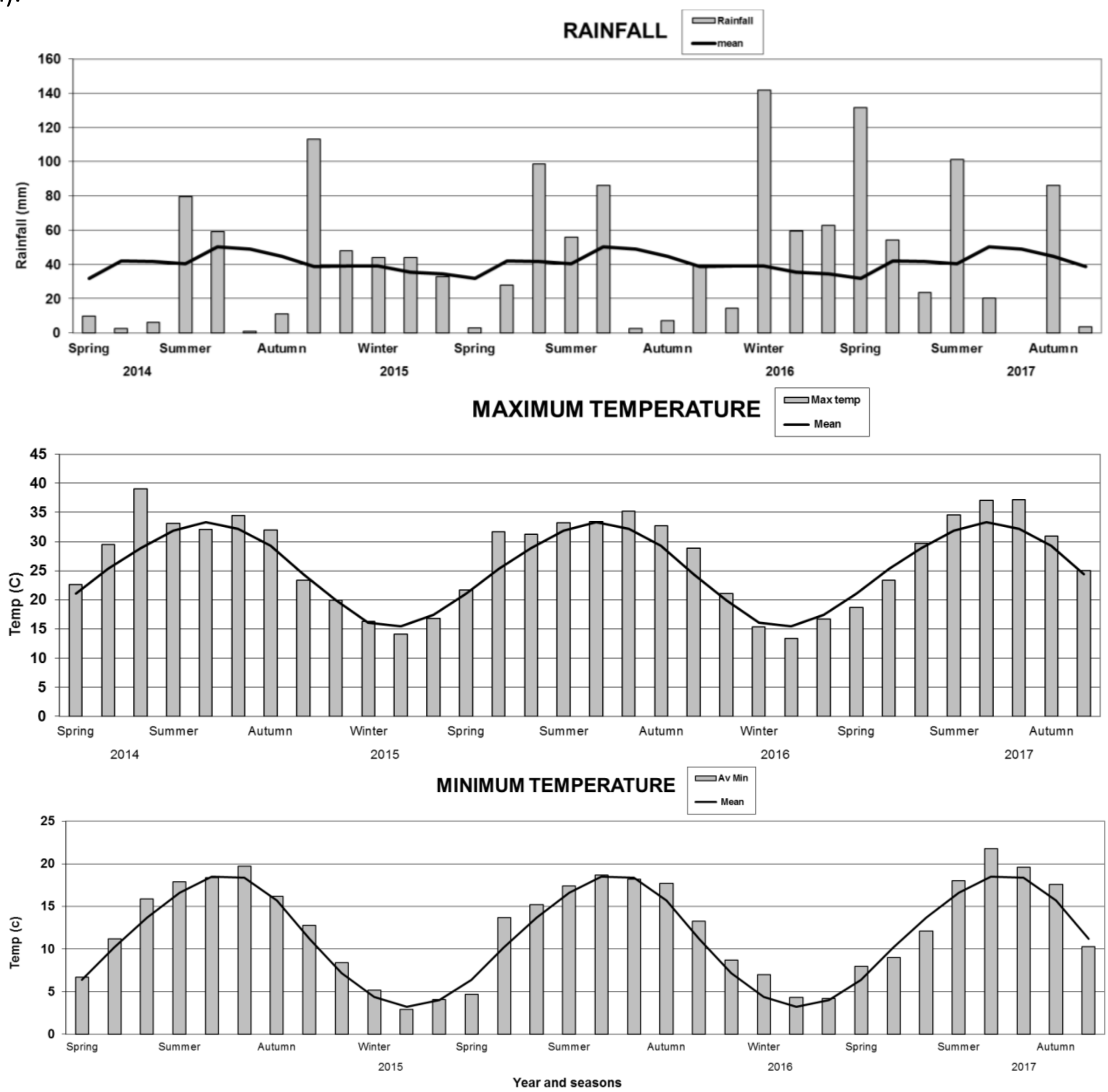

Fig. 16.2. Rainfall (mm), maximum and minimum temperature (°C) at the study location from spring 2013 (prior to commencement of experiment) and for the three experiment growth periods (2015, 2016, 2017). Long-term mean and actual rainfall (Rainfall), long-term mean and actual maximum and minimum temperature are given for seasons within each year.

In all years of the experiment, rooting depth of the perennial species was >1.2 m, while rooting depth for the annual legumes was shallower; <1.0 m for both species with the exception of barrel medic which had a rooting depth of 1.15 m in 2016.

16.3.3 Herbage mass and water use efficiency

Values of herbage mass over time are given in Fig. 16.3. Here, for the tropical species, desmanthus (cv. JCU2) consistently had the highest herbage mass compared to digit grass and within the temperate species, woolly pod vetch tended to have highest values. In each year of the experiment, the duration varied for the growth periods used to calculate WUE. In both years that annual legumes were assessed, the start date was clear as it was the time the legumes regenerated and the final date was when herbage mass was peak. The growth period for the perennial species was more difficult to determine. For the tropical species, the growth period started when growth recommenced in spring and finished when herbage mass was peak. Growth periods identified for lucerne were shorter than the other treatments (11 and 6 weeks in 2015–16 and 2016–17 respectively).

Table 16.3. Maximum extractable water (mm) in the upper (0.05–0.75 m), lower (0.75–1.25 m) and full soil profile (0–1.25 m) and estimated root depths (m) for treatments over three experimental years.

Treatment	Max SSW (mm)	Min SSW (mm)	Maximum extractable water (mm)			Plant root depth (m)
			0.05-0.75 m	0.75-1.25 m	0.05-1.25 m	
			2014–15			
Digit grass	-	-	-	-	-	-
Desmanthus cv. JCU2	358.8	297.6	43.1	18.7	61.2	>1.20
Desmanthus cv. Marc	363.2	294.2	47.8	22.4	69.0	>1.20
Lucerne	-	-	-	-	-	-
Barrel medic	-	-	-	-	-	-
Woolly pod vetch	-	-	-	-	-	-
LSD	*NS*[1]	*NS*	*3.39*	*1.40*	*4.64*	*NA*[12]
			2015–16			
Digit grass	373.0	284.0	64.8	27.4	89.0	>1.20
Desmanthus cv. JCU2	372.1	301.5	50.5	20.7	70.6	>1.20
Desmanthus cv. Marc	375.1	301.5	53.4	21.2	73.6	>1.20
Lucerne	362.9	293.2	49.7	20.1	69.7	>1.20
Barrel medic	372.3	301.9	56.9	13.5	70.3	1.15
Woolly pod vetch	373.3	314.3	48.2	11.0	59.0	0.92
LSD	*NS*	*11.53*	*7.62*	*4.76*	*17.40*	*NA*
			2016–17			
Digit grass	427.9	291.1	86.2	53.6	136.9	>1.20
Desmanthus cv. JCU2	386.1	300.6	56.0	34.2	85.4	>1.20
Desmanthus cv. Marc	383.7	297.7	57.7	35.9	86.0	>1.20
Lucerne	376.2	292.7	58.0	32.7	83.5	>1.20
Barrel medic	370.7	317.7	44.6	9.1	53.1	0.92
Woolly pod vetch	374.3	322.6	41.6	12.1	51.7	0.85
LSD	*16.58*	*15.93*	*17.39*	*12.13*	*8.41*	NA

[1]NS – not significant; [2]NA – not applicable

Total water use across treatments during the growth periods in each year of the experiment significantly varied. In two of the three years, lucerne had the lowest total water use (197 and 119 mm, 2015–16 and 2016–17, respectively) and in 2014–15 it had lower water use than desmanthus cv. Marc ($P < 0.05$), but more than the annual legumes (293 *v.* 354 and 355, and 293 *v.* 225 and 223 mm, respectively) (Table 16.4).

For growth periods during 2014–15, the perennial legumes had higher water use than the annual legumes ($P < 0.05$), but in 2015–16 the reverse occurred. In the earlier year, however, the amount of rainfall that occurred during the growth period of annual legumes was half that in the corresponding growth period of the second year (144 *v.* 297 mm).

In 2014–15 the perennial legumes achieved lower herbage mass than the annual legumes (<2100 *v.* >4000 kg DM/ha; $P < 0.05$), despite having higher water use, resulting in significantly lower water use efficiency; c. 25% of the WUE of the annual legume treatments ($P < 0.05$; Table 16.4). Data values for 2015–16 were less straightforward. The annual legumes had higher total water use ($P < 0.05$), but herbage mass for the annual legumes was similar to both desmanthus cultivars, however desmanthus cv. JCU2 had more herbage mass than cv. Marc ($P < 0.05$). This resulted in WUE for desmanthus cv. Marc and the annual legumes being equal (8.9–9.1 kg DM/ha/mm), while desmanthus cv. JCU2 (13.0 kg DM/ha/mm) had higher WUE than both desmanthus cv. Marc and woolly pod vetch (8.9–9.1 kg DM/ha/mm).

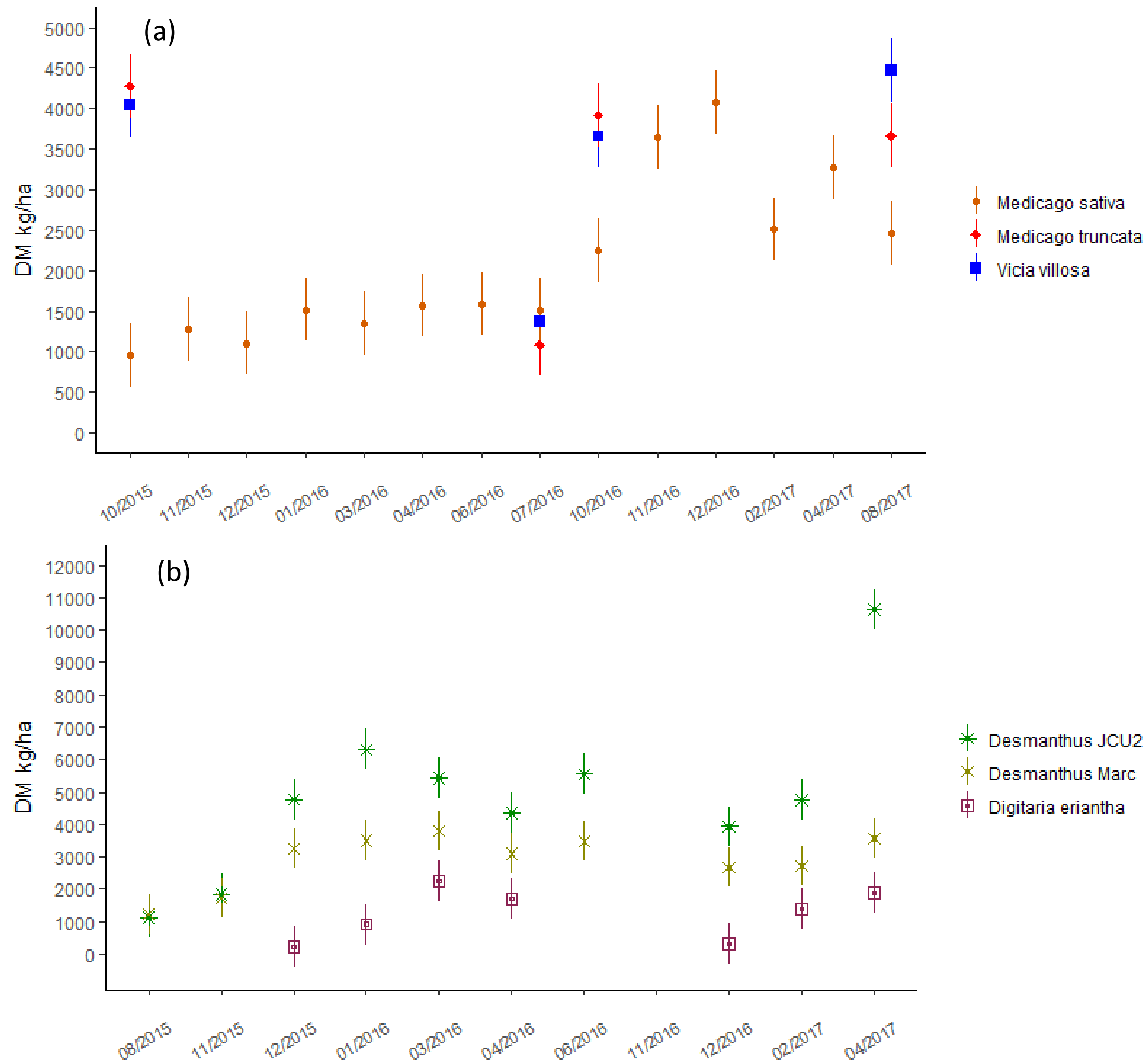

Fig. 16.3. Herbage mass (DM kg/ha) recorded for (a) temperate and (b) tropical species at each sample date over the experimental period.

Digit grass had low herbage mass in the second and third years (i.e. 1694 and 1878 kg DM/ha in 2015–16 and 2016–17 respectively), resulting in it having the lowest WUE in 2015–16 (4.6 kg DM/ha/mm) and also low but similar WUE to desmanthus cv. Marc in 2016–17 (5.2 *v.* 10.0 kg DM/ha/mm).

16.3.4 Rainfall refill efficiency

These data show that digit grass achieved higher change in SSW for the full profile (0.05–1.25 m) during the refill period in both years and subsequently had refill efficiency values of around 30% (Table 16.5). However, it was only in 2016–17 that digit grass had higher refill efficiency than the perennial legumes (29.7 *v.* 17.69–18.7%, $P < 0.05$).

Table 16.4. Start and end stored soil water (SSW), rainfall, total water used (mm), herbage mass production (kg DM/ha) and water use efficiency (WUE, kg/DM/ha/mm) of defined growth periods (see Table 16.2) for treatments during each of the three experimental years.

Treatment	Start SSW (mm)	End SSW (mm)	Rainfall (mm)	Total Water Used (mm)	Herbage mass (kg DM/ha)	WUE (kg DM/ha/mm)
			2014–15			
Digit grass	-	-	-	-	-	-
Desmanthus cv. JCU2	358.8	365.9	362.0	354.8	1861	5.3
Desmanthus cv. Marc	363.0	371.2	362.0	353.8	2022	5.7
Lucerne	365.9	324.5	251.2	292.7	1271	4.4
Barrel medic	359.6	308.1	173.6	225.2	4279	19.0
Woolly pod vetch	371.2	321.9	173.6	223.9	4040	18.1
LSD	*NS*[1]	*18.29*	-	*17.70*	*742.0*	*2.96*
			2015–16			
Digit grass	367.3	284.2	283.0	366.1	1694	4.6
Desmanthus cv. JCU2	359.5	306.6	283.0	335.9	4356	13.0
Desmanthus cv. Marc	361.2	303.8	283.0	340.4	3093	9.1
Lucerne	309.6	363.4	250.40	196.6	1572	8.0
Barrel medic	340.3	359.8	416.20	396.6	3909	9.8
Woolly pod vetch	353.9	358.5	416.20	411.7	3655	8.9
LSD	*20.10*	*9.98*	-	*16.07*	*1208.6*	*3.46*
			2016–17			
Digit grass	384.3	322.8	296.6	358.1	1878	5.2
Desmanthus cv. JCU2	376.4	320.3	296.6	352.7	10622	30.1
Desmanthus cv. Marc	377.5	316.4	296.6	357.7	3565	10.0
Lucerne	368.2	314.1	65.2	119.3	3649	30.7
Barrel medic	-	-	-	-	-	-
Woolly pod vetch	-	-	-	-	-	-
LSD	*6.46*	*NS*	-	*21.95*	*3003.4*	*9.82*

[1]NS – not significant

Table 16.5. Stored soil water (SSW, mm) at the start and end of the soil refill period together with changes for upper (0.05–0.75 m), lower (0.75–1.25 m) and total soil profile (0.05–1.25 m) and rainfall refill efficiency (%) for treatments during each of three experimental years.

Treatment	Start SSW (mm)	End SSW (mm)	Change in SSW (mm)			Rainfall (mm)	Rainfall refill efficiency (%)
			0.05–0.75 m	0.75–1.25 m	0.05–1.25 m		
				2016			
Digit grass	277.2	372.1	67.9	27.0	94.9	288.2	32.9
Desmanthus cv. JCU2	297.7	372.1	53.7	20.7	74.4	288.2	25.8
Desmanthus cv. Marc	294.2	374.6	56.1	24.3	80.4	288.2	27.9
Lucerne	-	-	-	-	-	-	-
LSD	*9.47*	*NS*[1]	*5.28*	*NS*	*12.40*		*7.85*
				2017			
Digit grass	384.3	284.2	68.5	31.9	100.1	460.6	29.7
Desmanthus cv. JCU2	376.4	306.6	51.6	19.3	69.8	460.6	18.6
Desmanthus cv. Marc	377.5	303.8	52.9	21.0	73.6	460.6	18.7
Lucerne	368.2	292.4	56.6	19.2	75.8	465.4	17.9
LSD	*6.27*	*7.90*	*3.50*	*2.36*	*4.85*		*1.27*

[1]NS – not significant

16.4 Discussion

This experiment has shown that:

1. Lucerne is capable of growing year-round in this environment.
2. Perennial species can access soil water >1.2 m depth, while temperate annual legumes accessed soil water to a depth of about 1 m.
3. For the tropical species, soil water replenishment can occur over winter, the non-growing season.

16.4.1 Lucerne grows year round

Lucerne grows year round in this environment, and has potential to be able to respond to rainfall year round, albeit growth rates would be low during the winter months. Lucerne is competitive and its ability to regrow each year makes opportunities of soil water replenishment more difficult. Therefore, growth periods would be determined by occurrence of rainfall of sufficient magnitude to replenish plant available SSW.

Once established, lucerne is recognised as a strong and rapid user of stored soil water (e.g. Lolicato 2000; Chapter 12, 15). The rapid use of stored soil water and subsequent herbage mass growth would have implications for the timing of grazing management in order to obtain maximum benefit of feed quality. Ideally, grazing value would be maximised before lucerne becomes subject to water stress and drops its leaf.

16.4.2 Rooting depth of species

Perennial species accessed soil water below 1.2 m, the depth of the access tube, while temperate species commonly rooted up to 1.15 m. In two studies conducted at Tamworth in northern NSW (Chapter 12, 15), similar treatments demonstrated extraction to greater depths; digit grass (1.30–1.56 m), lucerne (1.71–1.80 m) and desmanthus (1.36–1.80 m) compared with 1.2 m at Trangie. In a different study, also conducted at Tamworth, temperate annual legume (subterranean clover) was found to have a rooting depth of 1.2–1.4 m and extracted 119–203 mm of soil water on a brown Vertosol having at least 2.0 m of soil depth (Murphy, unpublished data, EverGraze).

The majority of soil water extraction by plant roots occurred in the upper layers of the profile during both experiments, but further extraction occurred to much greater depths, indicating the importance of this source of SSW for these species when accounting for total extraction. This variation in rooting depth values between experiments is a function of a range of factors including soil water holding capacities of the different soil types, initial levels of soil water content and soil nitrate (full profile), together with the difference in maximum measurement depth (vis. depth of access tubes).

16.4.3 Maximum extractable soil water values

MEW values determined for digit grass and lucerne in this study were only a fraction of those from other recent experiments (e.g. Murphy *et al.* 2017, 2018; Chapter 12, 15). For studies done on a similar soil type at Tamworth, extraction by digit grass (129–175 mm) and lucerne (213–242 mm, Murphy *et al.* 2018), were substantially greater than those that were observed at Trangie for the same species (89–137 mm and 70–84 mm, respectively). For similar studies done at Tamworth on a brown Vertosol type with a greater water holding capacity, highest values of MEW for digit grass were 167 mm (2013–14, Chapter 12) and 249 mm (2017, Chapter 15). Similarly, highest values of MEW for desmanthus cv. Marc (218 mm) and lucerne cv. Venus (239 mm), were more than double the values determined at Trangie (86 and 84 mm, respectively). A large part of the difference in values between the sites is likely due to the maximum depth that NMM measures were performed, with shorter access tubes at Trangie. Moreover, studies done to evaluate plant available water capacity of wheat on a chromosol at Trangie Agricultural Research Centre, indicate plant roots extracted 141 mm of water to a depth of 1.6 m (ApSoil No. 683, Dalgliesh *et al.* 2012), which indicate that our values obtained here are likely an underestimate, particularly for the perennial species.

16.4.4 Soil water refill

Soil water replenishment occurred in all treatments in this aseasonal rainfall environment, although the values were lower than those reported in a similar study conducted at Tamworth (Chapter 15). At Trangie, the change in stored soil water values were smaller to those recoded at Tamworth, which is partly a reflection of the soil water holding capacity and rainfall received during the refill periods, but the refill efficiencies were also lower at Trangie. For example, at Tamworth the lowest refill efficiency for desmanthus was 54% while at Trangie, the maximum efficiency was 28%. Rainfall refill efficiency of digit grass at Tamworth was 67–70% compared to a maximum of 33% at Trangie.

16.4.5 Comparison of WUE with related study conducted at Tamworth

The key difference between the experiment at Trangie and those conducted at Tamworth is that treatments in the latter experiments were grazed up to 7 times a year (i.e. every 6 weeks during the growing season; Chapter 15). In the Trangie experiment, treatments were not grazed, meaning that growth periods were more difficult to define and there was a large amount of carryover dry matter between assessments. The carryover dry matter, which masks the actual growth that took place, also limits initiation of new growth through shading, ties up nutrients in the dry matter and leads to senescence. Therefore, in the current study we quantified WUE for growth periods over which we could determine that there was a net increase in herbage mass. This in and of itself makes it difficult to compare values of WUE with those obtained in other studies, where WUE was quantified either by season of the year, total growing season or an annual basis (e.g. Murphy *et al.* 2018; Chapter 12, 15).

Despite this difficulty, comparison of mean WUE values show that the values from Trangie were considerably lower for digit grass (4.9 *v.* 16.0 kg DM/ha/mm) and desmanthus cv. Marc (8.3 *v.* 13.8 kg DM/ha/mm) but similar for lucerne cv. Venus (14.4 *v.* 13.3 kg DM/ha/mm, Chapter 15). Interestingly the range in WUE values at Trangie was greater than at Tamworth for both lucerne (4.4–30.7 *v.* 3.8–19.1 kg DM/ha/mm) and desmanthus cvv. (5.3–30.1 *v.* 9.7–17.6 kg DM/h/mm), but smaller for digit grass (4.6–5.2 *v.* 14.1–19.1 kg DM/ha/mm) over the same growing years.

The WUE of digit grass at Tamworth was 3–4 times higher than at Trangie. This difference is possibly due to the age and fertiliser history of the grass stand at Trangie (16 years) compared to the newly established and fertilised stand that was regularly grazed at Tamworth. The large variation in values of WUE possibly reflects the more variable nature of rainfall in the Trangie region and or the underestimation of MEW.

16.4.6 Tropical legumes

Tropical legume species evaluation experiments were sown twice at Trangie and failed to establishment both times (Chapter 5). Interestingly the few plants that did establish were desmanthus and burgundy bean (*Macroptilium bracteatum*) (I Toole, pers. comm.). In the current study, desmanthus was irrigated to ensure establishment, however once established, both species were productive, especially cv. JCU2. This and other studies conducted at Trangie show the potential of tropical pastures, however establishment is a major issue and further work (e.g. desktop and field studies) needs to be conducted to develop and test strategies to minimise risk of establishment failure. These strategies may include role of ground cover and sowing time.

The high productivity of desmanthus cv. JCU2 in this experiment is in part due to the woodiness of cultivar when it is not grazed. We suspect that the significantly higher WUE values recorded for JCU2 in the latter two years of the experiment (c. 13 and 30 kg DM/ha/mm) is in part due to the accumulation of the woody material.

16.4.7 Challenges interpreting these data

There were a number of challenges to this experiment that have implications for interpretation of the results:

1. Implications of access tubed being too shallow. The depth of the access tubes in this study were too shallow for the perennial species as demonstrated by their rooting depth exceeding 1.20 m. Unfortunately, this has implications for both the calculation of maximum extractable water (all but one treatment being < 100 mm), total water use and so WUE. When estimates of total water use are lower than reality, then the resulting values of WUE will be higher than reality, particularly for the perennial species whose rooting depth was indicated to be exceeding the depth of measurements. Be that as it may, all treatments were subjected to the same approach, so comparisons within the experiment are still fair.
2. Plots were not defoliated throughout the course of the experiment. Plant dry matter was not cut and removed after each assessment of herbage mass, thus making it impossible to determine the true growth from one assessment to the next. Further, the actual herbage accumulation was likely from periods other than near peak growth rate (i.e. later in the sigmoidal growth curve rather than earlier during active regrowth). Therefore, the values of herbage mass for each growth period were probably an underestimate of potential, which also impacts on the resultant WUE values. Further analysis of the data to determine net growth and subsequent WUE between each assessment period could be conducted, which may be more representative of actual growth. However since plant dry matter was not cut and removed (i.e. representative of grazing), the results will likely still be confounded.
3. Established plots of digit grass. The digit grass plots used in this study were mature swards located a short distance from the remainder of the treatment plots. Aside from not meeting the fundamental assumptions of a randomised complete block statistical analysis, an old stand of digit grass that has not been adequately fertilised is likely to have lower productivity than a newly established stand. Mature tropical grass stands of tropical grass as the productivity and soil water dynamics of mature and establishing stands can be different. In Queensland, fertility rundown has been identified as a major limitation of tropical pastures in Queensland (Peck *et al.* 2011). Therefore, it is not that surprising this mature stand of digit grass had both low yield and WUE.

16.5 Conclusions

This experiment has shown that in this medium rainfall environment lucerne is capable of growing year-round, perennial species of both grass and legume can access soil water to depths of >1.2 m within 12 months of establishment, and temperate annual legumes generally access water down to about 1 m. The experiment has also shown that soil water replenishment can occur during the season species are not growing, even in this aseasonal rainfall environment. The shallow depth of the access tubes and nil grazing imposed on the experiment, however, limit interpretation of the data and applicability of the results.

17 General discussion

S.P Boschma, S.R. Murphy and C.A. Harris

17.1 Tropical legumes as productive persistent companion legumes in mixed pastures

Evaluation studies showed that:

1. Desmanthus has potential as a companion legume in tropical grass pastures in Northern Inland NSW. Although desmanthus is not as productive as lucerne (Chapter 5), it increased the productivity of the tropical grass pasture and was persistent (Chapter 5), had high levels of hard seed with a slow rate of breakdown (Chapter 13), and following good summer rainfall readily regenerated from seed in mixed pasture (Chapter 5).
2. Leucaena is persistent once established (Chapter 6), but also had lower productivity than lucerne (Chapter 15).
3. Other tropical species (e.g. stylos, burgundy bean and Wynn cassia) did not persist in mixed pastures (Chapter 5) and had varying seed quality and hard seed breakdown patterns (Chapter 13).
4. Establishment of tropical grasses and legumes is an issue in the Central West (Chapters 4–6) and was addressed with further studies (Chapter 8) and are discussed in more detail in Section 17.3.

17.1.1 Desmanthus

Desmanthus has shown considerable potential in this project which supports the findings from earlier studies (Boschma and Harris, unpub. data) and the few published evaluations that have been conducted (e.g. Moylan and Crocker 2000; Boschma *et al.* 2012).

Desmanthus is not as productive as lucerne, however, it made a useful contribution and increased herbage mass by 40% compared to a digit pasture with a poor legume. Advantages of desmanthus as a companion legume compared with lucerne is that it does not appear to directly compete with digit grass, is non-bloating, sets large quantities of seed (Cook *et al.* 2005) and has a high proportion of hard seed with a slow breakdown rate in this environment (Chapter 13). While desmanthus is a short term perennial and is reliant on recruitment for long term persistence, it has the potential to remain a longstanding component of a pasture if a seed bank is maintained. In contrast, lucerne does not commonly recruit, fails to persist more than 3–5 year and eventually would need to be resown.

Desmanthus is commonly recommended for heavier more alkaline soils (e.g. Cook *et al.* 2005), so the Chromosol soils at the Bingara, Manilla and Trangie sites (pH_{Ca} 5.0–6.1) were less than optimum and may have negatively impacted their productivity. Despite being on marginal soils, desmanthus was clearly productive and persistent.

Component studies were conducted to determine the optimum depth and time of the year to sow desmanthus for successful establishment and winter survival, and also the seed softening pattern to better understand aspects of seed bank ecology. These studies showed that on a Chromosol soil in this environment, desmanthus is best sown at 5–15 mm depth from November until late January (Chapter 9). When sown within this window, seedlings survived summer and the following winter, while those which emerged from late-summer until autumn had poor survival. Not surprisingly, establishment was most effective when soil moisture was non-limiting, so to give a new pasture the best chance of establishment, sowing into a moist soil profile with increased likelihood of follow-up rainfall is ideal. Strategies that help retain a moist soil surface after rainfall may be beneficial, for example, sowing into a paddock that has cover from cereal stubble. In addition, sowing earlier in the sowing window (e.g. November) maximises the chances of receiving pasture germinating-rainfall events over the summer months (Lodge and Harden 2009). For example, long term median (50-percentile) monthly rainfall at Tamworth for November, December and January increases with values of 64, 73 and 74 mm respectively (Harris *et al.* 2014) with 7–8 rain days in each month (Lodge and Harden 2009).

Desmanthus had the highest levels of hard seed of the legumes tested and the lowest rate of hard seed break down after 2.5 years in the field (Chapter 13). There were also differences between cultivars, with cv. JCU2 (Progardes is a composite of five individual cultivars; JCU 1–5; www.progardes.com.au) having high initial hard seed levels similar to cv. Marc, but a faster rate of breakdown after about 10 months in the field (Chapter 13). Our study used similar protocols to others published in the literature; that is, they were conducted on bare soil, however, this is not realistic of a tropical perennial grass pasture growing in in northern NSW. Surface temperatures of bare soil are higher than those with ground cover (Quinlivan 1965; Lodge and Harden 2009) and it is likely that soil temperature in a tropical grass pasture with associated shading by the plant canopy and litter on the surface would be lower still (e.g. Lodge *et al.* 1990), which may impact hard seed break down rates. In a laboratory study (Lodge *et al.* 1990), hard seed breakdown of snail and barrel medics and subterranean clovers was lower at temperatures representing those experienced in a native pasture in Northern Inland NSW during summer (i.e. fluctuating temperatures of 40°C/10°C and 40°C/25°C) than temperatures representing a bare soil surface (i.e. fluctuating temperatures of 60°C/25°) which suggests that hard seed breakdown rates may be slower in a summer growing perennial pasture than reported in our study. Conversely, humidity is likely to be higher in a tropical grass pasture and and humidity has been found to increase hard seed breakdown of a range of temperate legumes (e.g. Kirchner and Andrew 1971; Fairbrother 1991). Recruitment recorded at Bingara and Manilla (Chapter 5) and anecdotal evidence indicate that seedling regeneration can be high.

An unexpected finding during this project was the detection of AMV in desmanthus cv. Marc at Tamworth. This is the first report of desmanthus as host of AMV in Australia and it joins a lengthy list of susceptible pasture, forage and crop legumes that act as hosts (e.g. Hull 1969; Latham and Jones 2001). Lucerne is also susceptible, but does not show symptoms or productivity losses, unlike desmanthus whose productivity was severely affected, but the extent has not been quantified. Further studies were conducted to determine the causal agent and these are discussed in Section 17.4.

Based on results from studies conducted during this project, NSW DPI have confidence to recommend desmanthus as a companion legume in Northern Inland NSW, however, last year a problem with the current commercial rhizobia strain was identified that will potentially limit the adoption of desmanthus in this environment. In 2017, the Australian Inoculants Research Group (AIRG) identified that the mother culture of the commercial rhizobia strain CB3126 used to inoculate both desmanthus and leucaena was not performing typically and not producing nodules on seedlings of either species. They did not release the strain in accordance with the National Code of Practice for legume inoculants. AIRG commenced sourcing new accessions of strain CB3126 from their archives, however every attempt to resurrect the strain was unsuccessful as it was either unviable or ineffective. The final ampule of strain CB3126 from the freeze-dried collection held at the Centre for Rhizobium Studies at Murdoch University has now been tested and found to also be ineffective. This means that for the coming season there is no effective rhizobium available for inoculating desmanthus, making it two years that rhizobia may not be available. This has serious implications for seed sellers, producers and the red meat industry. Due to acute concern for the industry and limited time frame before the tropical growing season recommences scientists from NSW DPI, James Cook

University and Queensland Department of Agriculture and Fisheries have joined forces with AIRG to find a short term solution. The team will collect soils and roots from old productive stands of desmanthus or leucaena that were previously inoculated by CB3126. AIRG will isolate rhizobia from these and confirm strain identification by DNA testing. If the rhizobium is CB3126, and is found effective, the strain will be issued for multiplication (e.g. New Edge Microbial) and distribution to industry. If successful, this will provide a short term solution, but there is a critical need to find replacement rhizobia for desmanthus and possibly also leucaena and this should be a priority for future research.

17.1.2 Leucaena

Studies conducted over the last five years have shown that leucaena is not as productive as lucerne and has a shorter growing season than other species (Chapter 15), however, it is persistent (Chapter 6) and was the only species that produced green forage during extended periods of low rainfall (Chapter 15). Results suggest that it may have potential in this environment and warrants further investigation, although the primary limitation of leucaena in NSW is its weed potential.

Leucaena was established in all experiments conducted during this project as transplanted seedlings (as opposed to sown seed), and all cultivars established well in Northern Inland NSW (>98%; Chapter 6 and 15). Establishment was lower in the Central West (44–68%; Chapter 6), however, this may have been partly due to the smaller seedling size, low overnight temperatures at the time of transplanting and below average rainfall during the growing season. But in general, especially in Northern Inland NSW, once established leucaena was highly persistent; leucaena in our studies had high persistence at Bingara, Manilla and Tamworth with >70% of plants surviving throughout the respective experiments. Similarly, there tended to be few losses at Trangie after the establishment period. Leucaena has poor cold tolerance (Cooksley *et al.* 1988), and although plant growth ceased over winter at all experimental sites (with the exception of 2016 winter), it did not appear to impact plant survival. This supports Queensland experiences where they have been persistent for >30 years (Radrizzani *et al.* 2010).

Leucaena has a wide adaptation and occurs naturally on infertile soils (Cook *et al.* 2005), but to realise the production potential of leucaena it is best sown on deep, fertile alkaline soils (Dalzell *et al.* 2006). The soils on which our evaluations were conducted were less than optimal (pH_{Ca} 5.0–6.1) and productivity may have been less than their potential in this environment. Despite this, our studies showed that of the commercial cultivars tested cvv. Wondergraze and Cunningham were the most productive (Chapter 6). Where leucaena was grown on deeper and more fertile soil (e.g. Vertosol at Tamworth, Chapter 15), the preliminary data indicate production levels were well below that of lucerne. In addition, the lower productivity and shorter growing season of leucaena in this environment was due to frost killing all stems resulting in regrowth from crowns each year. Re-establishing this woody framework each year took 2–3 months which delayed the first graze. The delay could potentially be minimised with consideration of slope (location of the frost line) and aspect (north *cf*. south facing), that is, if leucaena was be planted above the frost line on a north facing slope, the level of frosting could possibly be reduced.

One of the challenges identified by research conducted during this project is establishment of tropical grass in the alleys between leucaena hedge rows. The presence of grass in the alleys has both production and environmental benefits. Cattle weight gains have been reported to be higher on leucaena-grass than pure-leucaena pastures (Esdale and Middleton 1997). The grass also utilises N produced by leucaena and provides ground cover protecting the soil from erosion and controlling weeds. In our evaluation study (Chapter 6), the recommended method of establishment in Queensland was used, that is, establish leucaena in the first year and the tropical grass in the second year (Dalzell *et al.* 2006). This is to reduce competition between the species as leucaena is a weak seedling and slow to establish (Lambert 2013). This technique, however, was not successful in NSW and considered by local producers to be too long for a pasture to be unproductive.

Establishing the pasture over a two year period means that two summers of adequate rainfall are required, which increases the risk of establishment failure. Moreover, in the second year, the established leucaena will have a developed root system (Radrizzani *et al.* 2010; Chapter 15) that can use soil moisture in the alley between the hedge rows making it increasingly difficult to establish the grass (Chapter 6). This was noted at Manilla where there were obvious cracks on the soil surface across the 6 m alley between leucaena hedge rows (Chapter 6). If grass establishment fails in the second summer, a third year would be required to establish the grass.

Discussion with producers indicated that two years of lost productivity while the pasture is establishing was too long and would impede local adoption. Leucaena pastures are costly to establish (Dalzell *et al.* 2006), so the sooner the pasture can be grazed, the sooner there is a return on investment. This is even more important in this environment as the growing season of leucaena is less than Queensland (i.e. 5–6 months in northern NSW *cf.* ≥8 months in Central Queensland meaning that the time to recover the cost of establishing the pasture in NSW will be longer than in Central Queensland. We propose that a possible solution is to sow the grass and leucaena in the same season, but with a bare ground buffer between the grass and legume, which will reduce competition between the species, minimise the risk of establishment failure, and minimise the time that paddock is out of production.

The soil water dynamic study conducted at Tamworth (Chapter 15) established two leucaena-based treatments; with digit grass sown either 1 or 2.5 m from the leucaena hedge rows. Where grass was sown 1 m from the leucaena rows, the leucaena plants were smaller (i.e. height and width) and were consistently less productive (i.e. yielding 25% of those with greater bare soil buffer) compared with leucaena having grass established 2.5 m away. In that study, the larger bare ground buffer was maintained throughout the experiment for the purpose of the treatment being imposed, however, practically, this bare ground 'buffer' may only need to be maintained in the establishment year as grasses like digit grass recruit and the gap can be easily filled the following season. Grass recruitment between the hedge rows was observed in previous (SP Boschma, pers. comm.) and current experiments at Manilla and Tamworth (Chapter 6, 15).

The two widths of bare buffer used in the Tamworth water study where grass and leucaena were established simultaneously clearly showed the effect on establishment of the leucaena in the first year and consequences for ongoing productivity. Minimising competition during the establishment year is essential for long term high productivity; while persistence was not affected, there was an ongoing loss of productivity suggesting that if growth of the leucaena plants is inhibited by competition in the first growing season, it appears to have ongoing repercussions for the longer term productivity of the pasture.

One of the advantages of leucaena may be its ability to grow and maintain green forage when all other perennial pasture species have ceased growth due to depleted soil water reserves. On-going data collection from the study at Tamworth (Chapter 15) showed that despite rainfall well below average during the 2017–18 growing season [Tamworth received 283 mm (September2017–April 2018), 59% of LTA], leucaena was green throughout the entire summer producing about 4500 kg DM/ha while both lucerne and desmanthus produced about 4400 kg DM/ha each (SR Murphy, data in prep.). Although the total amount of green herbage mass produced by the different legumes was similar, the timing of the production was vastly different. The bulk of production from the leucaena occurred after mid-December, when both lucerne and desmanthus had stopped producing due to lack of stored soil water. Stored soil water data also showed that leucaena had higher reserves than any of the other forages throughout the entire summer to the end of autumn. Therefore, the prospect that leucaena can provide green forage late in a dry season would be a highly valuable resource to complement dry standing feed.

As described for desmanthus above, the commercial rhizobia strain CB3126 used to inoculate leucaena and desmanthus was not available last summer. Fortunately leucaena has an alternative commercial strain, CB3060, which AIRG found to be effective and released late last season. Historically, CB3060 has been reported as difficult to maintain in sufficient numbers in peat to be commercially available (Anon. 2005), so while this strain has solved the issue short term, there is a need for wider testing of suitable rhizobia strains for leucaena.

At this stage, leucaena will not be recommended in NSW, primarily because it has weed potential (Walton 2003a, b). However, this is an issue for multiple States in Australia and was the impetus for development of a sterile leucaena breeding program (Anon. 2017). Further, our production data suggest that cold tolerance would also be beneficial in this environment. Cold tolerance could assist over wintering which may enhance production earlier in the growing season and so spread production over a longer period. We look forward to evaluating lines in future to assess both timing of and total herbage production and persistence of these sterile and cold tolerant lines.

17.1.3 Other legumes

Fine stem stylo, and particularly caatinga stylo are commonly sown legumes in southern Queensland, so it was somewhat surprising that they were not persistent in our study. Similar to previous studies conducted in Northern Inland NSW, the legume and grass established well but the legume failed to persist (Boschma and Harris, unpublished). Reasons for the poor persistence could be poor frost tolerance (aspect and location in the landscape), poor seed quality, low levels of initial hard seed or fast hard seed breakdown rates, and/or poor nodulation. Caatinga stylo has specific rhizobia requirements and an assessment conducted at the Manilla evaluation site found no nodules on the stylo species, which indicated ineffective nodulation (Chapter 5). Issues with inoculation and rhizobia survival, which have been previously reported (e.g. McInnes and Date 2004; Johnson *et al.* 2015), suggest that further studies are required to firstly identify and then address the key limiting factors.

Burgundy bean had an annual growth habit and failed to persist in mixtures with tropical grass (Chapter 5), which supports anecdotal and unpublished findings of others in southern Queensland and northern NSW. Interestingly, it was noted that initial establishment and seedling vigour of burgundy bean was excellent, to the point where it seemed to have a negative impact on digit grass establishment. In subsequent years, however, seedling survival was comparatively poor, which may be due to limitations of soil moisture and nutrients, shading from the tropical grass or combinations of these factors. Burgundy bean is considered to be drought tolerant, with greater tolerance of cool temperature than many tropical legumes (Cook *et al.* 2005), but while not effective in a mixed pasture, it does have potential as a short term ley in subtropical grain systems (Bell *et al.* 2012).

Round-leaf cassia was included in the evaluation study to test the boundaries of the species. It is known to be adapted to soils that are free draining, acid to neutral pH, and with low-medium fertility in high rainfall environments (900–1500 mm), but is also reported as having an annual habit, regenerating each spring from seed in lower rainfall environments (>600 mm) (Cook *et al.* 2005). In this study, however, round-leaf cassia failed to persist or regenerate at both sites in this experiment and is not a suitable companion legume for tropical perennial grasses for Northern Inland NSW.

17.1.4 Future work

Some areas for further study include:

1. *New rhizobia for desmanthus and leucaena*. Recent failure of CB3126 commercial rhizobia strain for inoculating desmanthus and leucaena failed in 2017. Efforts are currently being made to resurrect the strain, but there is urgent need to evaluate other strains which may be more effective, persistent and resilient.
2. *Agronomy and management package for desmanthus*. This project addressed several components of an agronomy package to support producers and their advisors manage desmanthus, but there are a number of outstanding gaps in our knowledge. These include: herbicides (no herbicides are currently recommended for desmanthus in NSW), grazing management to maintain an effective seedbank (how frequently do we need to let desmanthus set seed?), seedling survival from late summer and autumn through winter, timing of seed softening and the likelihood of that soft seed remaining viable over winter, establishing desmanthus into an established grass pasture (a large proportion of the 450,000 ha of tropical pastures sown in NSW do not have a productive legume component), and the relative competitiveness of seedling tropical legumes and tropical grasses.
3. *Cultivar development for desmanthus and stylo species*. Our studies identified a number of issues with growing theses species in our environment, particularly AMV susceptibility and cold tolerance. Evaluation of material with higher seedling vigour and both AMV and greater frost tolerance are desirable for both species and warrants investigation. A broad range of germplasm collected across the globe from environments and latitudes similar to southern Queensland and northern NSW, including Central and South America and Mexico (Pengelly and Liu 2001) is held in the Australian Pastures Genebank. Neither, the impact of AMV on productivity by desmanthus, nor the susceptibility of other commercial cultivars is known. Our observations suggest desmanthus experienced a substantial decline in productivity after becoming infected with AMV, but the decline could not be quantified within the bounds of the experiment.
4. *Field survey of nodulation of tropical legumes*. The primary reason legumes are incorporated into grazing systems is for N fixation, however, poor nodulation is an ongoing issue. A field survey should be conducted in Queensland (current area of adaptation) and northern NSW (emerging area) to determine the nodulation status of sown pastures of different ages and to further understand requirements to maintain N fixation.
5. *Sterile, cold tolerant leucaena*. It is unlikely that leucaena will be recommended to growers in NSW unless there is a sterile variety that avoids the weed risk potential. Lines with improved cold tolerance are desirable and sterility is essential.
6. *Long term persistence and economics of leucaena*. The high cost of establishment and shorter growing season, plus competition with cropping enterprises for deep fertile soils may make leucaena an unviable option in some or all regions in NSW. An economic evaluation is warranted to determine where and under what assumptions leucaena is viable in NSW.
7. *Leucaena agronomy and management package*. If leucaena was to be recommended in NSW, a package to support producers and their advisors would be essential. This project has made advances towards components of such a package, but additional studies are required to address optimum sowing time and depth, alley width for different soil types, grazing management, and critically, animal husbandry to maintain rumen flora. Enquiries from advisors and producers suggest that there is interest in leucaena in NSW. If this interest increases, producers who are ‘early-adopters’ will establish leucaena pastures without NSW DPI recommendation. To provide relevant information to guide these producers and their advisors a greater understanding of leucaena agronomy and management is required.

8. *Pest and disease survey of pasture in NSW and Southern Queensland*. The susceptibility of many pastures, forages and crop species to AMV and the role of aphids as vectors prompt a need to determine the frequency of this disease. Despite the growing need to firstly identify and quantify the diseases and pests that are preying on pastures and secondly to quantify the productivity losses they are causing, there is a deficiency within the grazing industries of pest and disease expertise.

17.2 Understanding and improving temperate legumes in mixed pastures with tropical perennial grasses

From the studies conducted during the project we found that:

1. Barrel medic and woolly pod vetch were the superior temperate annual legumes (by herbage production and persistence) at all three sites, but their ranking varied with latitude and annual rainfall received. At the northern most site, which received the highest rainfall during the experimental period (average 563 mm/year (2013–2015); Bingara) woolly pod vetch was the best performing legume, followed by barrel and snail medic. At the other two sites with lower rainfall (average 382 mm/year (2013–2015); Manilla and Trangie) barrel medic was the best performing temperate annual legume. At Manilla, snail medic also performed well, followed by woolly pod vetch, while at Trangie, the southern-most site woolly pod vetch was ranked second and biserrula third.
2. Sowing temperate annual legumes in autumn (either before or after the tropical grass) resulted in more productive legumes than sowing podded hard seeded legumes in spring with the tropical grass. Studies investigating the hard seed breakdown patterns of a range of legumes highlighted the initially high levels of hard seed in the medics and the subsequent slow breakdown pattern compared to the other legumes. The study also highlighted the large differences in the rate of hard seed breakdown of legumes in this summer dominant rainfall environment compared to those in southern NSW and Western Australia.
3. Studies of digit grass pastures with differing plant density showed that density of 4–9 plants/m^2 allows initial resources of stored soil water and nutrients to be utilised over a 2 year period from sowing, which offers a beneficial window to aid establishment of temperate legumes.
4. Lucerne was the most productive perennial legume (temperate or tropical) evaluated in this project, however its competitiveness in a mixture was evident, especially at the Manilla site and in variety and configuration trials at Tamworth. Sowing configuration studies found that productivity of a lucerne-digit grass mixture was greatest when the lucerne and grass were sown in alternating bands ≤0.5 m (1:1 and 3:3 alternate row configuration), while separating the species by sowing each in bands of 1 m reduced productivity.

17.2.1 Temperate annual legumes

The core premise of investigating temperate annual legumes is that a substantial legume component (15–50%; Kemp 1991) improves quality of forage for grazing livestock and provides nitrogen fixation for use by the perennial grass component. However, for temperate annual legumes to fulfil these roles, they must be both productive and persistent, which means achieving minimum levels of annual dry matter production, and setting adequate seed with sufficient levels of hard seed to maintain a seedbank.

The consistent high performance of barrel medic and woolly pod vetch (in particular cvv. Jester and Haymaker, respectively) across the three sites shows their broad environmental adaptive range. The long term persistence of temperate annual legumes is dependent on using adapted species and cultivars that are productive and set seed, and then maintaining a large seed bank with high levels of hard seed. High seedling vigour together with deep rootedness also support reliable regeneration and production. However, management will continue to be a key to persistence, but a shift in livestock from predominantly sheep to cattle (Lodge 2011) will assist this.

Two key components of persistence of annual legumes are large seed set and successful regeneration. The first was difficult to achieve in the first year at the two northern sites (Bingara and Manilla) due to low rainfall in the growing season. These studies were conducted during a series of challenging years with below average rainfall. In the establishment year, untimely and low rainfall severely affected establishment and consequently seed set. Despite a management protocol that encouraged seed set in each year of the experiment, seedling regeneration remained low, most likely due to the low plant population and minimal seed set in the first year. Establishment failures occur in this environment (Lodge 1991) and the risk is higher in a sown tropical grass pasture as stored soil water levels are low/negligible in autumn (Murphy *et al.* 2018), leaving the regenerating legume dependent on autumn-winter rainfall whose probability is declining in Northern Inland NSW.

High seed production and/or high proportions of hard seed in annual legumes are required for long term persistence because regeneration and seed set is not guaranteed each year. If a legume fails to set seed in the first year it is likely to struggle to persist long term. Practically, if a legume fails in the first year, resowing is likely the best option to quickly build the seed bank.

A recent survey conducted in southern and central NSW reported over 90% of legume pastures were ineffectively nodulated (Hackney *et al.* 2017a,b). While soil acidity and fertility status were contributing factors, other factors such as high summer temperatures and low rainfall can also reduce nodulation and rhizobia persistence (Slattery *et al.* 2001; Hackney *et al.* 2017a,b). Our environment typically experiences hot summers and extended periods of low rainfall (not unlike the conditions our experiments were conducted in). All legumes were inoculated in this project but nodulation was not tested and may be a contributing factor in the persistence of some legumes.

There was no benefit to sowing podded (hard seeded) legumes in spring in this environment. This contrasts with finding in the Northern Agricultural Region of Western Australia (Sanford *et al.* 2017) and southern NSW (Howieson and Hackney 2018). On the South Coast of Western Australia, however, there was no consistent benefit of sowing serradella in summer compared with autumn (Sanford *et al.* 2017, 2018) and so it was concluded that serradella could be successfully established at either time (Sanford *et al.* 2017).

The temperate annual legume hard seed breakdown experiment conducted in this project was the first study in Northern Inland NSW to compare the hard seed breakdown pattern of the newer hard seeded legumes with traditional legumes. The study confirmed the high hard seed levels of barrel and snail medic ranking them as having higher hard seed levels than the newer hard seeded legumes, such as French and yellow serradella and bladder clover, which in turn had higher hard seed levels than woolly pod vetch, subterranean clover and biserrula. A precautionary note, however, as mentioned in Section 17.1.1, this hard seed breakdown experiment was conducted on bare soil, which is not representative of the conditions in a sown tropical grass pasture. The higher and greater range of temperature and soil water content conditions experienced on a bare soil may mean that the hard seed break down of these legumes in a pasture may in fact be slower, or conversely, the pasture may increase humidity which may also impact hard seed breakdown (Kirchner and Andrew 1971; Fairbrother 1991).

17.2.2 Lucerne

Lucerne is the most productive and persistent legume in Northern Inland NSW (Boschma *et al.* 2011), however, its competitive effect on digit grass was evident at both Manilla and Tamworth

(Chapter 5, 12, 15) and to a lesser extent at Bingara (Chapter 5) as demonstrated by water stress and reduced grass productivity. Separating the species by sowing lucerne and grass in alternating bands ≤0.5 m (i.e. 1:1 and 3:3 alternating rows, Chapter 12) had the highest productivity, while separating the species further (1 m wide alternate bands; 6:6 configuration) reduced overall productivity of the mixture. This was possibly because the grass was unable to utilise N from the legume as the root zones were separated, and/or because the intra-specific competition of lucerne was too high at these plant densities and restricted herbage production.

The sowing configuration studies at Tamworth (Chapter 12) showed that sowing configurations containing highly winter active lucerne (cv. Pegasis) were more productive and contributed more legume herbage mass than configurations containing the other cultivars (Venus or Q31). Sowing in 1 m wide bands (6:6 rows) contributed less legume herbage mass than either alternating rows or narrow bands ≤0.5 m wide (3:3 rows). In mixtures, the highly winter active type clearly dominated herbage production over the grass and this was largely due to the lucerne recommencing growth earlier in spring, extracting reserves of stored soil water, shading the grass and so suppressing growth of the grass. Therefore, optimum production of a mixture in narrow bands is likely to be a delicate balance between winter activity rating and plant density of the lucerne.

Due to the dry conditions experienced when many experiments were being established, irrigation was applied where necessary and possible. This was advantageous for lucerne and was possibly a reason for the high plant densities experienced in several experiments (e.g. Chapter 12, 15). The high plant densities of lucerne favoured its extraction of soil water and nutrients to the detriment of the grass. Although the main role of lucerne in a mixed pasture is to fix sufficient N to maintain productivity of the grass, we suggest that lucerne plant densities, and the proportion of legume herbage mass in our treatments were in fact too high. Therefore, reducing the lucerne plant density could potentially reduce its competitiveness with the companion grass. A replacement series experiment using seedling lucerne and tropical grasses found that lucerne was able to produce similar quantities of herbage at plant population proportions ≥25% (Boschma *et al.* 2010). If this remains true for established stands, lucerne established at lower plant densities could still be productive, making a highly valuable contribution to the pasture while being less competitive with the grass for resources. A study that investigates the productivity of mixed pastures with varying plant densities and proportions of lucerne warrants further investigation.

17.2.3 Future research

Future research:

1. *Nodulation survey in Northern NSW*. In the summer dominant rainfall zone of northern NSW and southern Queensland, there are no data on the effectiveness of nodulation in pasture legumes (pure or mixed grass-legume pastures). Findings in central and southern NSW that >90% of legume pastures were ineffectively nodulated (Hackney *et al.* 2017a,b) has significant ramifications for pasture productivity and subsequent livestock and crop productivity. A similar survey of nodulation in legume pastures (temperate and tropical) in the summer dominant rainfall zone would highlight and quantify any similar issues.
2. *Establishing legumes into established pastures*. The studies of temperate and tropical legumes conducted in this project established legumes into newly sown grass pastures, which have higher reserves of soil water and nutrient resources to aid legume establishment (Boschma *et al.* 2018b). However, there are large areas of established tropical pastures which have either no or poor legume population, either because they were not initially sown, or the legumes have not persisted. Our studies have shown that soil water is likely to be the key limiting factor preventing reestablishment of legumes in an existing tropical grass pasture and so strategies to establish, or reestablish legumes into these pastures warrants investigation.

3. *Determine optimum plant density of lucerne in a tropical grass pasture*. The findings from our current studies suggested the lucerne plant densities, and the proportion of legume herbage mass produced were too high, which resulted in the legume being >65% of the total herbage produced. The studies done here have shown that lucerne is highly competitive against digit grass in a mixture, but closer configuration (1:1 and 3:3 cf. 6:6) increased productivity, while an overall grass density of 4–9 plants/m^2 was optimum (Chapter 10). Therefore, to determine a target density that producers can aim for there is need to find the optimum proportion and /or density of lucerne in a mixed tropical grass pasture that provides the multiple benefits of optimum herbage production, desired proportion of legume and high water use efficiency.

17.3 Potential of tropical species in Central West NSW

The Central West of NSW is a challenging environment for establishing tropical perennial grasses. On cracking clay soils in a semi-arid region the main factors impeding establishment were unreliable rainfall and rapid drying of soil surface (Leslie 1965). This is true for Chromosol soils as well. Studies conducted at Trangie during this project found that for the period tested (October–June), sowing in October–November resulted in greater seedling emergence (with or without irrigation) than sowing from February into autumn and winter, and winter survival was greater when the pasture was sown in spring.

At Walgett in North-West NSW, seedlings of tropical grasses (purple pigeon (*Setaria incrassata*), curly Mitchell grass (*Astrebla lappacea*), Bambatsi panic and buffel grass) sown monthly for a 12 month period emerged sporadically during the growing season, but most germinated on rainfall received December–January (Campbell *et al.* 1995; Bowman 1990). Bowman (1990) concluded that sowing in late summer was the optimum time because although temperatures were high, the likelihood of summer storms was also high. Trangie has similar annual rainfall as Walgett, but is located 220 km south with less summer dominance. The lower summer rainfall and variable nature of rainfall in the Central West suggests that sowing early in the sowing window (e.g. October) would provide more opportunities for pasture establishment; these sporadic events potentially accumulating over the growing season resulting in a productive pasture.

In a controlled environment study, Egan *et al.* (2017; Chapter 7) found that 50% of optimum emergence occurred at temperatures ranging from 12 to 18^{o}C; 12^{o}C for Rhodes grass (cv. Katambora), 13–14^{o}C for creeping bluegrass (cv. Bisset) and digit grass (cv. Premier), 16^{o}C for panic grass and forest bluegrass and 18^{o}C for Bambatsi panic. The low temperature tolerance of Rhodes grass explains its ability to emerge at a wide range of temperatures, while the high temperature of Bambatsi panic (in addition to large seed size and high seedling vigour) explains its good emergence during summer.

Establishment was greater when irrigation was applied. This is not surprising, but highlights that by employing strategies that support maintaining a moist soil surface for an extended period of time will have a positive impact on pasture establishment (Lodge and Harden 2009). Such strategies could include a fallow to build soil moisture reserves prior to sowing, and sowing the tropical pasture into a cereal stubble, which may help reduce soil surface temperatures and evaporation. Stubble did not improve emergence of surface sown grasses (Campbell *et al.* 1995), but it may be more effective when seed is placed an optimum depth of 10–25 mm below the soil surface (Lodge and Harden 2009).

Studies conducted at Tamworth of sowing time and depth for a range of tropical grass species have recommended the strategy of sowing into dry soil in late November–early December, as soon as temperatures are favourable (Lodge and Harden 2009). This approach maximises the chance of receiving 1–2 rainfall events of 20–25 mm required to establish the tropical pasture. As indicated by the study conducted at Trangie (Chapter 8), the sowing window opens earlier (i.e. at least October) in locations like the Central West, where soil temperatures rise earlier than at Tamworth. At Trangie the probability of receiving ≥25 mm in October or November is 0.61 and 0.55, while the probability of receiving ≥50 mm is 0.34 and 0.35, respectively (Smith and Cooper (1996). The effectiveness of such rainfall events would be enhanced by strategies that store and hold moisture, like stubble retention and soil surface roughness.

The optimum plant density of a species is known to vary with climate (rainfall and temperature), soil fertility, plant available water and cultivar (Palmer and Wynn-Williams, 1976) with the optimum density for maximum herbage production likely to be lower in a lower rainfall environment than a higher rainfall environment (Dear *et al.* 2007). In northern NSW the optimum plant density to achieve high herbage production and soil water dynamics was 4–9 plants/m^2; the lower plant density was similar to that for the 400–600 mm zone in southern WA (Nichols *et al.*, unpublished data). Therefore, we suggest that the optimum plant density of tropical grasses in Central West of NSW, with its lower rainfall and higher temperature regime may be 4–6 plants/m^2.

Studies conducted at Trangie in the 1980s found tropical species to be highly productive when irrigated, but poor when grown dryland (Muldoon 1986). Studies conducted during this project also found productivity was low during dry periods (e.g. rainfall September 2017–March 2018 was 88% of LTA; Chapter 8), but they were productive when above average rainfall was received (Chapter 16). Results also suggest that although N can increase productivity (e.g. Boschma *et al.* 2014a, 2016), tactical application would be advisable in this environment because rainfall is lower and more variable.

While findings from the soil water studies done at Trangie were limited by total depth of measurement (i.e. <1.20 m), similar themes emerged in the data that support findings in the more intensively measured studies at Tamworth. For example, digit grass is highly effective at utilising stored soil water in the upper zone of the profile (0–1.25 m) and often extracted significantly more water than the perennial legumes. This has implications for establishment of companion annual temperate legumes, as previously discussed. Further, there was evidence that despite aseasonal low-medium rainfall, stored soil water can accumulate during the non-growing season of the tropical species (i.e. during winter) with digit grass and desmanthus cultivars capturing about 20–30% of winter rainfall. While the relative efficiencies are not as high as recorded at Tamworth [i.e. 49–68% for digit grass (Chapter 12, 15) and 78% for desmanthus cv. Marc (Chapter 15)], the data highlight the importance of the process of capturing winter rainfall to supplement spring growth by the tropical species.

Warmer winter temperatures in the Central West provide the opportunity for lucerne, subject to soil water availability, to grow virtually year round and it had rapid water use during growth periods, especially during warmer months of the year. The capacity to grow year round has implications for choosing cultivars with higher winter activity rating to achieve high water use efficiency (Pembleton *et al.* 2011). However, this will come with the trade-off that lucerne cultivars with higher winter activity rating are generally considered to be less persistent than more dormant types (McDonald *et al.* 2003).

Long term success of pasture species is closely linked to the plant's ability to access soil moisture deeper in the soil profile, where root depth is not restricted by subsoil constraints. At Trangie, the perennial species accessed water >1.2 m deep while barrel medic and woolly pod vetch accessed water to about 1 m (Chapter 16). These values were generally shallower, for a range of reasons previously discussed, than those recorded at Tamworth. However, the fact that the perennial species at Trangie extracted soil water to at least 1.20 m deep is encouraging.

This project has supported anecdotal evidence that tropical pastures have potential in this low-medium rainfall environment, but establishment is a significant issue. Desktop and field studies are required to better understand sowing time and develop strategies that maximise stored soil water storage both prior to sowing and on the soil surface during germination and initial emergence (i.e. first 14 days).

17.3.1 Future research

Some areas for future study include:

1. *Rainfall probabilities* determined by Smith and Cooper (1996) were conducted on weather data from 1887–1996; a similar study conducted using weather data that includes values from the last 2 decades may provide insights into the current and changing rainfall distribution. Such an analysis may indicate preferred establishment windows worthy of field testing.
2. *Optimum sowing time and seedling survival*. Seedling emergent studies conducted during this project were conducted over a 9 month period, commencing October. In this environment, August or September may also be suitable months for some species. Seedling survival data following establishment would also highlight when seedlings are lost.
3. *Strategies to increase establishment success*. Strategies during paddock preparation that increase accumulation of stored soil water prior to sowing and then maintaining a moist soil surface for extended periods of time after sowing may benefit pasture establishment. For example, to build soil moisture reserves before sowing a tropical pasture how soon should the preceding cover crop be removed? Further, what type of stubble (cereal v. legume, standing v. flat) and how much ground cover is required to aid rather than impede establishment?

17.4 Desmanthus – a new host for Alfalfa Mosaic Virus in Australia

Severe yellowing of plant growth was observed in experimental plots of desmanthus cv. Marc at Tamworth during the 2015–16 summer season. Symptomatic plants were infected with AMV, while non-symptomatic plants were virus-free. Attempts to mechanically transmit the virus to desmanthus were unsuccessful, however, transmission was successful by cowpea aphid. Additional aphid feeding studies showed that the cowpea aphid could colonise and multiply on desmanthus, while the pea aphid could not. During summer 2017–18, seed was collected from symptomatic and non-symptomatic plants so that seed transmission rates could be determined. Rates of 2.21% have been reported (Mih and Hanson 1998) and while this may seem low it is still a source of inoculum in a commercial stand, which introduces substantial risk that productivity declines will be encountered.

AMV could become a limiting factor for the adoption of desmanthus as a pasture legume in NSW, but desmanthus is only one of a large number of crop, pasture and forage legume species grown in Northern Inland NSW that are susceptible [for example, lucerne, chickpea, subterranean clover, biserrula, French and yellow serradella and white clover (*T. repens*) (e.g. Latham and Jones 2001)]. A survey of AMV in lucerne in northern NSW during summer 2009–2010, found incidence in virtually all commercial stands tested, with high incidence (up to 91%) in stands ≥3 years old (van Leur and Kumari 2011). With AMV susceptible winter and summer cropping and pasture legumes sown in this region, each provides an inoculum reservoir for the other and provides refuge for virus vectors. Therefore, it is highly likely that plantings of AMV susceptible desmanthus, regardless of where it is sown in northern NSW, will readily become infected with AMV.

Future research in this area could include:

1. In the first incidence, studies are required to quantify the susceptibility of other commercial cultivars of desmanthus available in Australia and the productivity loss due to AMV.
2. Any programs developing desmanthus for commercial release in southern Qld and northern NSW should also include an AMV screening component.
3. There is a need to conduct a comprehensive study to determine the extent of AMV infection of summer and winter pasture and crop species. In fact a broader pest and disease survey is warranted to better understand the pests and diseases that are present and their incidence. Their individual and/or collective impact, with plants possibly only showing mild or nil symptoms, may be having a significant detrimental impact on pasture productivity and therefore red meat production.

17.5 Working with producer groups in producer-directed participatory research was mutually beneficial

The method which MLA were planning to engage with producers in the Feedbase Program was modified after this project was contracted and resulted in an open call for producer groups in southern Australia to apply to conduct participatory research and development (R&D) addressing issues in their area that related to one of the Feedbase Program projects. Groups from across southern Australia applied to conduct research related to tropical pastures and six groups from three states were successful. The group names and their location, also the National project team members who supported the groups are listed in Table 1.

Table 1. Producers groups who were successful working in the Participatory R&D program with the National Tropical Pasture project team, and the team members who supported the groups.

Producer group		Supporting Project team members
Name	Location	
Dungog-Gresford Land and Beef group	Dungog and Gresford, NSW	Neil Griffiths, Suzanne Boschma
Cooks Myalls Landcare Group	Parkes, NSW	Suzanne Boschma, Trudie Atkinson
Borah Farmers Group[1]	Manilla, NSW	Suzanne Boschma
A Sheep	Esperance, WA	Ronald Masters (DPIRD)
Fitzgerald Biosphere group	South coast of WA	Paul Sanford, Eric Dobbe (DPIRD)
Southern Farming Systems and BetterBeef Network Group	Gippsland, Victoria	Paul Sanford (DPIRD)

[1]Withdrew before contracting

The project team member supporting the group assisted with defining treatments to address the questions/hypotheses, experimental design and layout, data collection methodology and protocols, statistical analyses and report writing. The amount of assistance provided by the project team varied with the group. For example, the Southern Farming Systems and BetterBeef Network Group in Gippsland had previous experience and technical expertise, and required less support than the Cooks Myalls Landcare Group at Parkes who lost the support of their technical officer halfway through the project (NSW DPI project team members supported the group by collecting data in line with their protocol and drafting milestone and final reports).

There were mixed feelings about the success of the participatory research program, but on the whole this collaborative approach was a positive experience for both the project team participants and the producer groups. The research team had the opportunity to work closely with producers, and producers could directly engage with the research team. A common comment from the groups was that research was harder and more intense than they had anticipated, that is, quality data need to be collected in a timely manner (not always easy to achieve when it was required during peak work periods of the year such as sowing or harvest) and can be time consuming (a general observation is not sufficient).

This extension method supported small groups of producers, not producers in the wider red meat industry, however, the project team used every opportunity to promote their research. Over the course of the project the NSW DPI team participated in over 60 activities ranging from field days, to MLA supported Pasture Updates and conferences attended by a total of about 2500 people.

17.6 Successful project integration and collaboration between NSW DPI and DAFWA

Throughout the project, collaboration and communication between the research teams in NSW and WA was positive and supportive, and both teams were committed to conducting quality research addressing issues of the red meat industry. Even after the WA State Government made the decision to withdraw from R&D resulting in early conclusion of research in WA, the WA team remained committed to their research and delivered quality outcomes for MLA and red meat industry (Sanford *et al.* 2017, 2018).

To support collaboration and exchange of ideas within the project, a national team meeting was held annually in a different location each year, alternating between states. These meetings consisted of a field tour (visiting research sites and meeting with producers to better understand the environment and issues) and a team meeting that provided an opportunity for the team to report and review their progress and refine plans for the next 12 months. Part of the success of this collaboration was the provision of funds, separate to the research budget that facilitated this style of collaboration and communication between the teams. This gave team members the capacity to attend without imposing on research funds.

18 Conclusions and recommendations

Research conducted during this project has made significant advances in our knowledge of temperate and tropical companion legumes for tropical grass pastures in Northern Inland and Central West NSW. This new and increased knowledge of the agronomy and ecology of these species will make quality contributions to agronomy packages for producers and their advisors and will improve both forage supply and quality of the feedbase for the red meat industry. Some key outcomes from this project include:

1. *Confidence in desmanthus as a companion legume in Northern Inland NSW*. NSW DPI are recommending desmanthus to producers because it is productive, does not appear to directly compete with digit grass, is non-bloating, sets large quantities of seed and has a high proportion of hard seed with a slow breakdown rate in this environment. While the susceptibility of all commercial cultivars of desmanthus to AMV is unknown and needs to be urgently addressed, development of the tropical grass-desmanthus pastures agronomy package has progressed with new knowledge developed in this project.
2. *Leucaena is persistent in Northern Inland NSW*. Good preparation is important for successful establishment and once established plants are highly persistent in Northern Inland NSW and moderately persistent in Central West NSW. Leucaena is not as productive as lucerne and provides forage over a shorter growing season that can be grazed from December to May, however, it was the only species that produced green forage during extended periods of low rainfall. Leucaena will not be recommended by NSW DPI until sterile lines are available as it has potential to be an environmental weed.

3. *Temperate legumes can be effective companion legumes in tropical grass pastures*. Sowing temperate legumes in autumn, either before or after the tropical grass, achieves highest legume productivity. Increased knowledge of optimum sowing time to build a seed bank, species seed softening patterns, water use patterns and optimum grass density all contribute to agronomic packages for red meat producers and their advisors.
4. *A tropical grass pasture with a plant density of 4–9 plants/m^2 is optimum for herbage production and water use efficiency.* Digit grass pasture with these densities achieves both production and sustainability goals and allows sufficient carryover resources to establish a legume over the initial 2 years of the pasture.
5. *Lucerne is productive in mixed pastures with tropical grasses*. Lucerne was the most productive legume assessed in this project, but highly competitive with the tropical grass, potentially reducing overall productivity of the pasture. Sowing configurations that keep the grass and legume in narrower bands increased productivity but the optimum plant density/proportion is not understood and potentially also limits long term production and persistence of the pasture.
6. *Central West has a low aseasonal rainfall which increases risk of tropical pasture failure*. The high risk of establishment failure was evident and preliminary studies conducted in latter stages of the project have provided insights into sowing times, seedling survival and productivity.

Areas of future research for each activity were outlined in the general discussion (Chapter 17), and have been condensed into the following recommended top priorities in decreasing order of urgency:

1. *Effective rhizobia for desmanthus and leucaena*. Scientists are currently working with AIRG to find rhizobia strain CB3126 so that the red meat industry can inoculate desmanthus seed this coming growing season. If their efforts are successful, the earliest inoculant will be available is possibly December 2018–January 2019, but is only a short term solution. There is a critical need to find a new rhizobia strain for desmanthus and leucaena and this should be a priority for immediate research.
2. *Alfalfa Mosaic Virus in desmanthus*. AMV could become a limiting factor for the adoption of desmanthus as a pasture legume in NSW. In the first instance the current cultivars of desmanthus should be screened for their susceptibility to AMV and the relative productivity losses quantified. Any current and future desmanthus breeding and development programs should incorporate an AMV screening component.
3. *Packages for companion legumes*. There are a number of outstanding gaps in our knowledge to support establishment and management of companion legumes in tropical pastures. These include: establishing legumes into an established grass pasture (many of the 450,000 ha of sown tropical pastures in NSW do not have a productive legume component), herbicides (no herbicides are currently recommended for desmanthus in NSW), grazing management to maintain an effective seedbank (how frequently do we need to let desmanthus set seed?), seedling survival from late summer and autumn through winter, timing of seed softening and the likelihood of that soft seed remaining viable over winter, the relative competitiveness of seedling tropical legumes and tropical grasses, and the optimum proportion of lucerne in a mixed pasture.
4. *Improving establishment of tropical pastures in Central West NSW*. Desktop and field studies are required to better understand sowing time and seedling survival, and develop strategies that maximise stored soil water prior to sowing and surface soil moisture during germination and emergence.
5. *Impact of diseases and pests on legume productivity*. AMV is only one of a range of potential diseases that affect crop, pasture and forage legume species grown in Northern Inland NSW and Southern Queensland. A comprehensive survey of summer and winter growing crop, pasture and forage species needs to be conducted to determine the diseases that are

present, the level of infection and associated productivity loss. Insect vectors should also be considered in this survey.

6. *Tropical legume development* – desmanthus, stylo and leucaena. These three legumes are important legumes in Queensland and also have potential in NSW. Our studies identified a number of issues with growing these species in our environment, primarily AMV susceptibility (desmanthus only), cold tolerance and vigour. A broad range of germplasm of *Desmanthus* and *Stylosanthes* collected from environments and latitudes similar to southern Queensland and northern NSW is held in the Australian Pastures Genebank. Desirable lines (identified through a desk-top study) of these species will need to be seed increased for evaluation in replicated experiments across target regions in Queensland and NSW. This evaluation would increase the knowledge of adaptation of current cultivars and germplasm and identify superior line(s) of these species for cultivar development.

19 Key messages

Tropical legumes

1. Companion legumes are important for increasing production and quality of tropical pastures. While temperate annual legumes have traditionally been used in Northern Inland NSW, when grown with tropical grasses soil water content is low at the end of summer reducing the reliability of establishment. Tropical legumes have similar growth patterns to tropical grasses; this allows soil water to replenish over the winter period while they are inactive (even in the Central West where rainfall is lower and distribution is aseasonal). The accumulated stored soil water can then support pasture growth during early spring when probability of rainfall is still relatively low.
2. Desmanthus is a productive and persistent companion legume to tropical grass pastures in Northern Inland NSW. However, desmanthus cv. Marc (and possibly other cultivars) is susceptible to infection by AMV, but the impact on its productivity is not known. AMV could limit adoption of desmanthus as a pasture legume in NSW.
3. Leucaena, a perennial shrub or tree legume widely grown in Central Queensland Australia, is persistent in Northern Inland and Central West NSW once it is established. While it is not as productive as lucerne, it can be grazed December–May, and even in dry conditions it provided desirable green leaf when other perennial species had ceased growth. At this stage NSW DPI will not recommend leucaena due to its weed potential.
4. Hard seed breakdown patterns of tropical legumes vary between species which has implications for their use and management in pasture systems. Soft seeded species (e.g. burgundy bean and butterfly pea) need to be managed to set seed more frequently than the hard seeded types (e.g. desmanthus).
5. Tropical grass and legume species show production potential in Central West NSW. Establishment is challenging so sowing in spring and strategies that store soil moisture and provide cover are recommended as areas for further work.

Temperate annual legumes

1. Barrel medic is widely adapted and was productive and persistent in both Northern Inland and Central West NSW. Woolly pod vetch was also productive and persistent. These species are suitable as companion legumes to tropical grass pastures.
2. The persistence of temperate annual legumes is associated with their ability to regenerate in late summer-autumn and their level of hardseededness; high hard seed levels providing insurance against years that are unsuitable for establishment. Hard seed breakdown patterns in the summer dominant rainfall environment vary with species and some species, had different hard seed breakdown patterns than when in Mediterranean environments. Soft seeded species (e.g. subterranean clover and woolly pod vetch) need to be managed to

allow more frequent seed set than the hard seeded species (e.g. medics) to ensure that the seed bank is maintained.

3. For successful establishment and higher legume productivity, sow temperate legumes in autumn, either before or after the tropical grass. Sowing podded hard seeded legumes with the grass in spring, results in lower legume productivity.

Lucerne

1. Lucerne was the most productive legume tested, but is highly competitive and dominated water use and herbage production, especially in early spring utilising stored soil water reserves accumulated in winter. While this results in high productivity of lucerne in spring, there is little tropical grass growth until further rainfall is received.
2. Competitiveness of lucerne can be manipulated by changing the sowing configuration of lucerne and tropical grass in a mixture. Sowing lucerne-grass mixtures in narrow bands (≤0.5 m bands) will increase overall productivity compared with wider bands (1 m).
3. Lucerne on a red Chromosol in Central West NSW has the potential to grow year round and is a vigorous water user.

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